accordingly be postulated that injury to cardiomyocyte membranes in vitamin E deficiency, accompanied by lowering of the electrical stability of the heart, is realized not only through activation of LPO, but also on accounts of the effect of arrhythmogenic factors such as fatty acids and lysophosphatides [7, 9]. This hypothesis requires experimental verification.

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SARCOLEMMAL DAMAGE AS A PATHOGENETIC FACTOR OF PITUITRIN-ISOPRENALINE-INDUCED MYOCARDIAL ISCHEMIA AND ITS CORRECTION BY AN ANTIOXIDANT (DIBUNOL)

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KEY WORDS: biomembranes; antioxidant; dibunol; catecholamine-induced myocardial necrosis.

Correlation between disturbance of functional and structural integrity of the sarcolemma and myocardial function has recently been reported. It has been shown, in particular, that in ischemic and hypoxic myocardial damage the state of the sarcolemma determines whether or not the reversible intracellular changes becomes irreversible [6, 8]. Sarcolemmal damage has been shown to be one of the principal factors in the development of acute heart failure [5]. By the use of peroxidase and ferritin as intravital tracers, disturbance of the structure of the cardiomyocyte sarcolemma has been demonstrated under the influence of toxic doses of catecholamines [11, 12]. An important role in maintenance of normal membrane function is played by lipid peroxidation (LPO). By auto-oxidation of catecholamines, free radicals are formed [13], which oxidize polyunsaturated fatty acids of membrane phospholipids. Intensification of LPO has been described in isoprenaline-induced myocardial necrosis, and prophylactic administration of the bioantioxidant tocopherol has been observed against the action of toxic doses of isoprenaline [14, 15].

The aim of this investigation was to study the state of the myocardial and erythrocyte membranes in pituitrin-isoprenaline-induced myocardial ischemia (PIMI), the role of lipid peroxidation in injury to these membranes, and the membrane-protective action of the synthetic antioxidant dibunol (2,6-di-tert-butyl-4-methylphenol).

EXPERIMENTAL METHOD

Experiments were carried out on 43 non-bred albino rats aged 24-30 months and weighing 350-500 g. Pituitrin (15 U/kg) and isoprenaline (100 mg/kg) were injected intraperitoneally in two doses with an interval of 24 h. The state of the membrane was evaluated 24 h after the second injection of isoprenaline and pituitrin, by determining passive penetration of sulfacetamide sodium into the myocardium (the ratio of the sulfacetamide sodium concentra-

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TABLE 1. Effect of Dibunol on Peroxide and Mechanical Hemolysis of Erythrocytes, Myocardial Membrane Permeability, and Heart Rate (HR) in Animals with PIMI (M ± m)

Series of experiments	Peroxide hemolysis, %	Mechanical hemolysis, %	Coefficient of penetration of sulfacetamide sodium into myocardium, %	HR, beats/min
Intact animals	3,2 ± 0,1	39,2 ± 0,3	42,7 ± 1,1	336 ± 10
PIMI	9,1 ± 0,9*	72,0 ± 1,9*	49,1 ± 0,6*	412 ± 12*
PIMI + dibunol (30 mg/kg)	5,0 ± 0,3**	53,5 ± 2,3**	51,6 ± 1,2	343 ± 6**
PIMI + dibunol (120 mg/kg)	3,7 ± 0,3**	39,1 ± 1,6**	41,2 ± 1,8**	332 ± 10**

Note. *) P< 0.05 compared with control, **) compared with PIMI.

tions in the tissue and blood serum, expressed as a percentage [3]) and the mechanical resistance of the erythrocytes (the percentage hemolysis after exposure to standard mechanical trauma [2]). The LPO level was determined by the crossed erythrocyte hemolysis test [1, 7].

Defects in cardiomyocyte membranes were visualized by electron-microscopic examination, using colloidal lanthanum as the label. The procedure was based on the method in [10] with certain modifications [4]. Ultrathin sections were cut on the OM-U3 ultramicrotome (Reichert, Austria). The JEM-100 B electron microscope (Japan) was used.

Dibunol (30 and 120 mg/kg) was given by mouth for 7 days before the experiment.

The experimental results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Increased permeability of the myocardial membranes for sulfacetamide solution and a higher level of mechanical hemolysis of erythrocytes compared with the control were found in PIMI. Increased permeability of the cardiomyocyte and erythrocyte membranes was combined with a higher level of lipid peroxidation as shown by the peroxide hemolysis test (Table 1).

Electron-microscopic investigations revealed loci of disturbance of sarcolemmal permeability for colloidal lanthanum in the myocardium of animals with PIMI (Fig. 2). Contracture and lysis of myofibrils and swelling and fragmentation of mitochondrial cristae were conspicuous in the necrotic foci. Cardiomyocytes located at a distance from the necrotic foci, like cardiomyocytes of intact rats, contained no lanthanum in their sarcoplasmic matrix (Fig. 1).

Analysis of the results of this investigation and data in the literature [9, 11, 12] suggests that injury to the sarcolemma is the key stage of catecholamine-induced myocardial necrosis.

Preliminary administration of dibunol before toxic doses of isoprenaline and putuitrin substantially lowered HR (Table 1) and prevented death of the animals from the action of toxic doses of isoprenaline and pituitrin. In particular, mortality in the PIMI model was 31%, whereas in the experiments with preliminary administration of dibunol in either dose not one animal died. These changes developed against the background of a reduction of myocardial membrane permeability and of mechanical and peroxide hemolysis of erythrocytes. Dibunol in a dose of 120 mg/kg was most effective: In these experiments the degree of peroxide and mechanical erythrocyte hemolysis and the coefficient of penetration of sulfacetamide sodium differed only a little from the control values (Table 1).

In experiments with prophylactic administration of dibunol in a dose of 120 mg/kg into animals with PIMI, by contrast with the control experiments with PIMI, electron-microscopic investigations revealed only single cardiomyocytes with intracellular colloidal lanthanum. The intracellular ultrastructure under these circumstances also remained intact: The marked swelling and fragmentation of the mitochondrial cristae and contracture of myofibrils were absent, and no foci of myofibrillary lysis were observed.

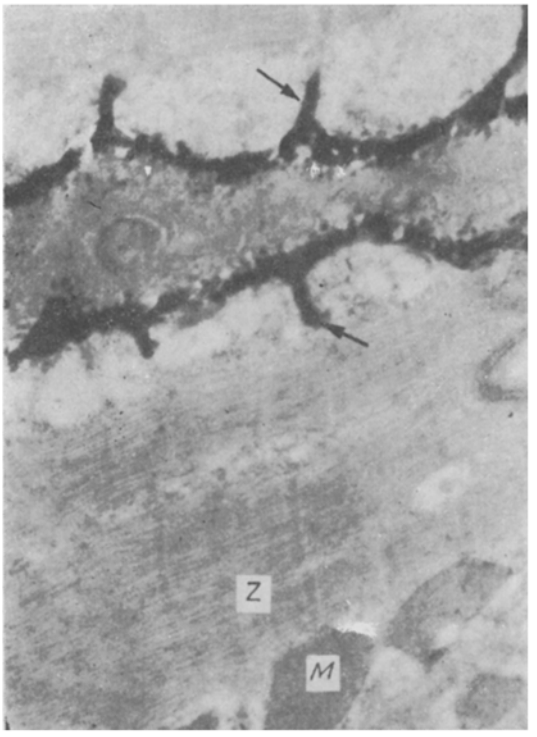

Fig. 1

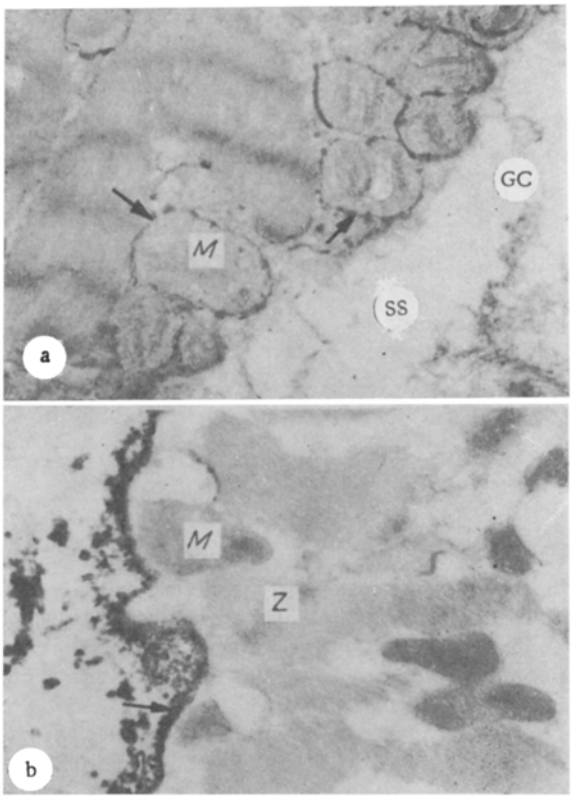

Fig. 2

Fig. 1. Myocardium of control rat. Fixation with lanthanum, unstained preparation, tracer localized on outer side of lateral sarcolemma (arrow). M) Mitrochondria; Z) Z-bands. 15,000×.

Fig. 2. Myocardium of rat 48 h after one injection of pituitrin and isoprenaline. a) Cardiomyocyte without dibunol treatment; b) cardiomyocyte after dibunol treatment. SS) Subsarcolemmal swelling; GC) glycocalyx. Remainder of legend as in Fig. 1.

Thus the protective effect of the antioxidant, dibunol, in a model of PIMI is evidence of intensification of LPO in the phospholipid membranes of the cardiomyocytes which is probbably one of the main causes of damage to the sarcolemma as a result of the action of these agents.

Inhibition of peroxide-induced destruction of the sarcolemma in animals with PIMI by means of dibunol led to protection of the myocardium, expressed as normalization of the cardiac rhythm and prevention of the animals' death. The results indicate that the state of the sarcolemma is the decisive factor in the pathogenesis of catecholamine-induced ischemic myocardial damage.

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CHANGES IN ENERGY METABOLISM AND CONTRACTILITY OF THE HEART DURING THE DEVELOPMENT OF FOCAL MYOCARDIAL NECROSIS

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KEY WORDS: heart; necrosis; glycogen; mitochondria; contractility; ^{99m}Tc pyrophosphate.

After the end of emotiogenic stimulation a quite long period of activation of the adrenergic and pituitary-adrenal system is observed, characterized by release of catecholamines and glucocorticoids and also by marked eosinopenia. At the culmination of the stress response, reflected in a fall in the corticosterone concentration and a peak of the eosinophil count taking place after eosinopenia, all these changes reach a maximum. Later processes of recovery begin to develop [2, 6, 7, 10]. If the blood eosinophil count is used as the criterion of functional activity of the pituitary-adrenal system, the temporal parameters of the phases of development of the lesion and of recovery of the changes taking place in the heart during during emotional stress can be determined [8]. One of the most important pathogenetic components of stress-induced heart damage is the action of high catecholamine concentrations [9], and there are accordingly good grounds for suggesting that in response to injection of cardiotoxic doses of adrenalin the same pattern of development of heart damage will be exhibited as during stress.

The aim of this investigation was to study changes in the energy metabolism and contractility of the heart at different times after injection of adrenalin, paying attention to the time course of changes in the biorhythm of the eosinophil count and corticosterone level in the blood and also the phases of development of injury to and restoration of the structure of the heart [5].

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on 210 male albino rats weighing 180-220 g, with an initial blood eosinophil count of 220-340 cells/μl, at 9 a.m. Adrenalin, in a dose of 7.5 mg/kg, was injected subcutaneously into the animals once. The energy metabolism of the heart was assessed by determining the glycogen concentration in heart muscle by Khoreishi's method, and also by studying oxidative and phosphorylating functions of the mitochondria by the method described previously [7]. The state of the mitochondria was judged by the following parameters: RC_c — Chance's respiratory control; ADP/t — the velocity of oxidative phosphorylation (in μmoles ADP/mg protein/min). To assess the degree of cardiac damage on the basis of the level of accumulation of ^{99m}Tc pyrophosphate (^{99m}Tc-PP) the reagent Pirfotech-99M was used, and eluate from a technetium-99M generator from the "Medradiopreparat Factory was added to it. The resulting complex, in a volume of 0.3 ml and with activity of $11.1 \cdot 10^3$-$29.6 \cdot 10^3$ Mgq (dose monitored on a CRC^R-5 Radioisotope Calibrator, from Nuclear Chicago, USA), was injected into the caudal vein of the rats. The accuracy and completeness of injection of the preparation into the blood stream was verified visually on an LFOV gamma-camera with Scintiw computer (from Searle, The Netherlands). Radiometry of the heart was undertaken on an NK-150 well-type scintillation counter (Hungary). Accumulation of ^{99m}Tc-PP by the heart muscle was expressed as an index of radionuclide uptake,

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