MORPHOLOGY AND PATHOMORPHOLOGY

Electron Microscopic Characteristic of Major Types of Acute Damage to Cardiomyocytes

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The major (independent) types of acute damage to cardiomyocytes: myofibrillar contractures, intracellular myocytolysis, primary crumpy degradation, and cytolysis are characterized ultrastructurally. It is shown that dystrophic and necrobiotic changes in the myocardium are mosaic, implying that prior to electron microscopic examination cardiomyocytes should be studied in polarized light.

Key Words: *acute damage to cardiomyocytes; myofibrillar contracture; intracellular myocytolysis; primary clumpy degradation of myofibrils; cytolysis; electron microscopy*

Investigation of patho- and morphogenesis of the damage to cardiomyocytes is crucial for cardiological diagnosis and prognosis. Pathomorphological diagnosis of acute myocardial damage is based on the presence of necrotic, inflammatory, and sclerotic alterations [9,11,15] which reflect the outcome of the damage to cardiomyocytes (CM). With the aid of histochemical enzyme and luminescence techniques necrobiotic processes can be identified in autopsy material 3-4 h after their beginning [1]. A great progress in the diagnosis of acute damage to the myocardium has been achieved with the use of polarizing microscopy. This method allows one not only to identify the early damage to CM, but also to classify individual types of this damage that lay the basement for alterative myocardial insufficiency [2,3,6,8]. More insight into structural mechanisms underlying acute myocardial damage can be provided by electron microscopy. It should be noted, however, that damaged CM are often studied without considering the data obtained by polarizing and electron microscopy.

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Our aim was to examine damaged CM under an electron microscope after identification in polarized light.

MATERIALS AND METHODS

Acute myocardial damage was produced with cardiotoxic doses of epinephrine and isopropylnorepinephrine, cobalt chloride, functional overload, and ventricular fibrillations induced by electrical current [1]. Myocardial infarction was provoked by coronary occlusion [2]. The myocardium was investigated 5 min—48 h after application of damaging factor. Myocardium of people died from acute cardiac failure, acute coronary insufficiency, myocardial infarction, and noncardiological diseases was also studied [1,2].

Specimens (left papillary muscle) for electron microscopy [7] were fixed in 4% paraformaldehyde for 2 h at 4°C, postfixed in 1-2% OsO_4 solution for 2 h, and embedded in Epon-Araldite. When necessary, the same specimens were embedded in styrene—butyl methacrylate, since semithin styrene-methacrylate sections can be more efficiently investigated in polarized light.

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Myocardium specimens in the embedding mixture were oriented so that to prepare transversal and longitudinal sections. From each block we first prepared semithin (1 μ) sections which were stained with toluidine blue or azure II and examined under a light microscope to determine the orientation of muscular fibers. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Semithin and ultrathin sections were cut in LKB III and Tesla ultratomes. The sections were examined in JEM-100B and Tesla BS-500 electron microscopes at accelerating voltage 60 kV and magnification range 5000-50,000.

RESULTS

The following major types of myocardial damage were identified in autopsy material by polarizing microscopy: segmental and subsegmental contractures, intracellular myocytolysis, primary clumpy degradation of myofibrils, and cytolysis [3]. Electron microscopic investigation of acute myocardial damage of various genesis allowed us to describe typical ultrastructural changes characteristic of each type.

Contractures were identified in polarized light by increased anisotropy of A bands and varying height

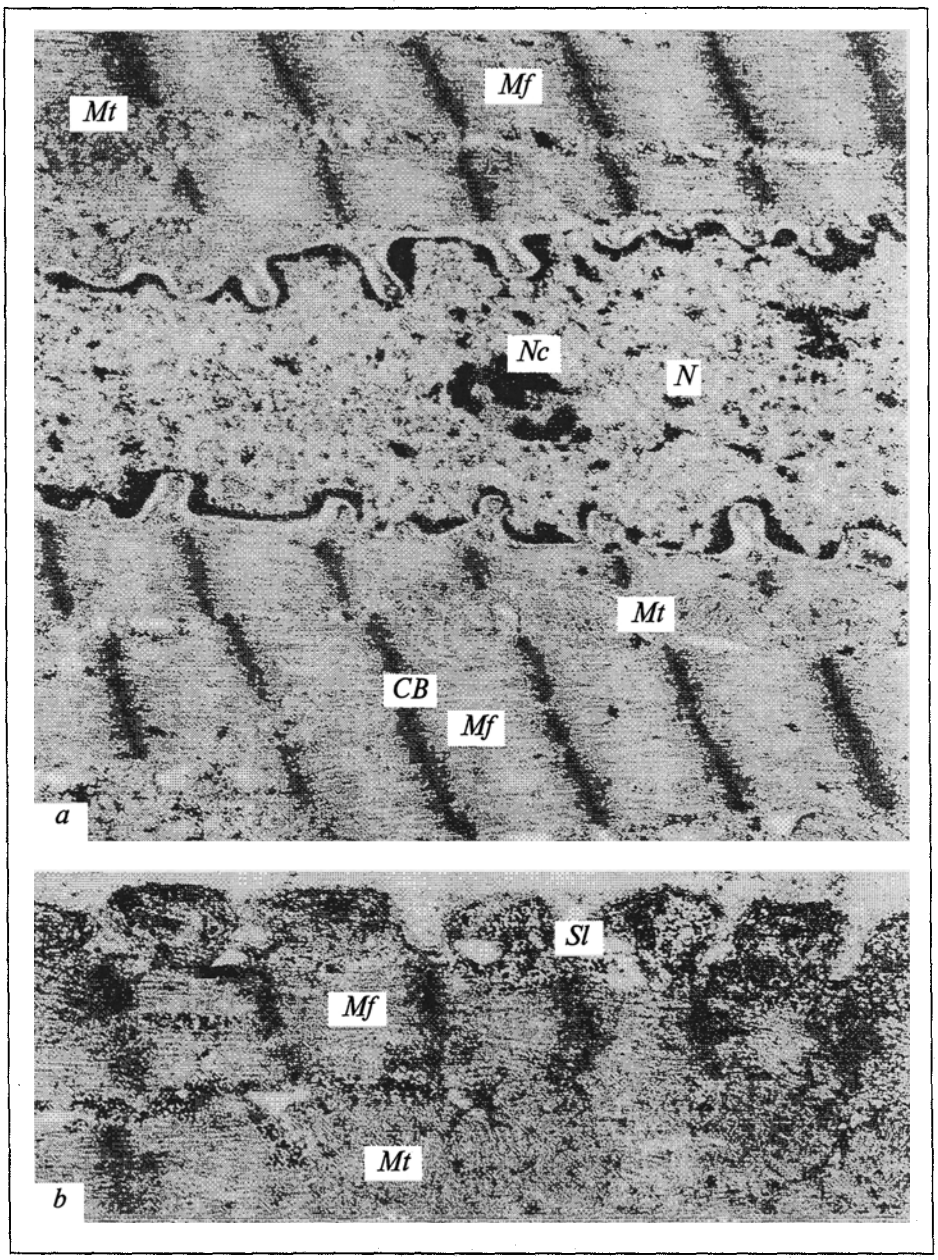

Fig. 1. The second degree myofibrillar contracture in rat myocardium 1 h after administration of epinephrine; ×14,000. *a*) wide contraction bands (*CB*) in myofibrils (*Mf*) and shortening of sarcomeres. Deformation and compression of the nucleus (*N*) and mitochondria (*Mt*). *Nc*) nucleolus. *b*) festooned sarcolemma (*Sl*).

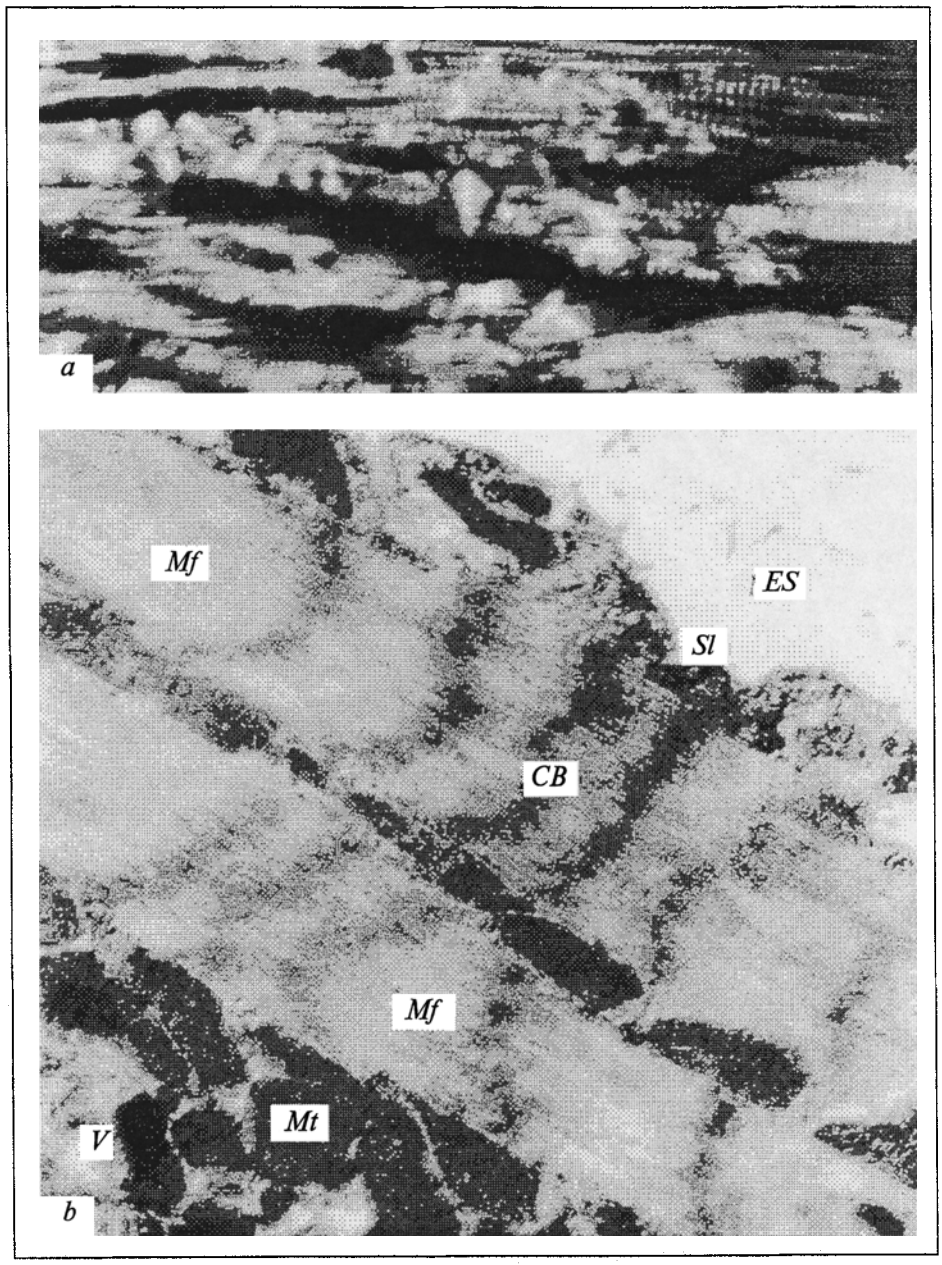

Fig. 2. Subsegmental myofibrillar contracture in subendocardial layer of rabbit myocardium. Epinephrine-induced fibrillation. *a*) in polarized light, ×1000; *b*) cardiomyocyte ultrastructure: hypercontracture of several sarcomeres. *Mf*) myofibrils; *CB*) contraction bands; *Mt*) mitochondria, *V*) vacuole; *Sl*) sarcolemma, *ES*) extracellular space, ×26,000.

of isotropic disks. Total contraction of myofibrils is referred to as a segmental contracture, while contraction of individual sarcomeres is regarded as subsegmental contracture.

In the first degree segmental contracture, the density of myofilament packing in the disks is slightly increased with strict longitudinal orientation of myofibrils. In the second degree contracture, isotropic disks are short or are absent; wide dark Z bands are seen at Z lines (Fig. 1). In the third degree contracture, Z bands become homogeneous, and M bands appear. Strong contraction of myofibrils leads to collapse of the sarcotubular system and compression and deformation of the nucleus and mitochondria. Cardiomyocytes become shorter; their sarcolemma forms festoons. The structure of intracellular organelles is preserved even at the stage of coagulation necrosis.

Subsegmental contractures were observed predominantly in the subendocardial layer of the myocardium. They were characterized by hypercontracture of adjacent sarcomeres of CM (Fig. 2).

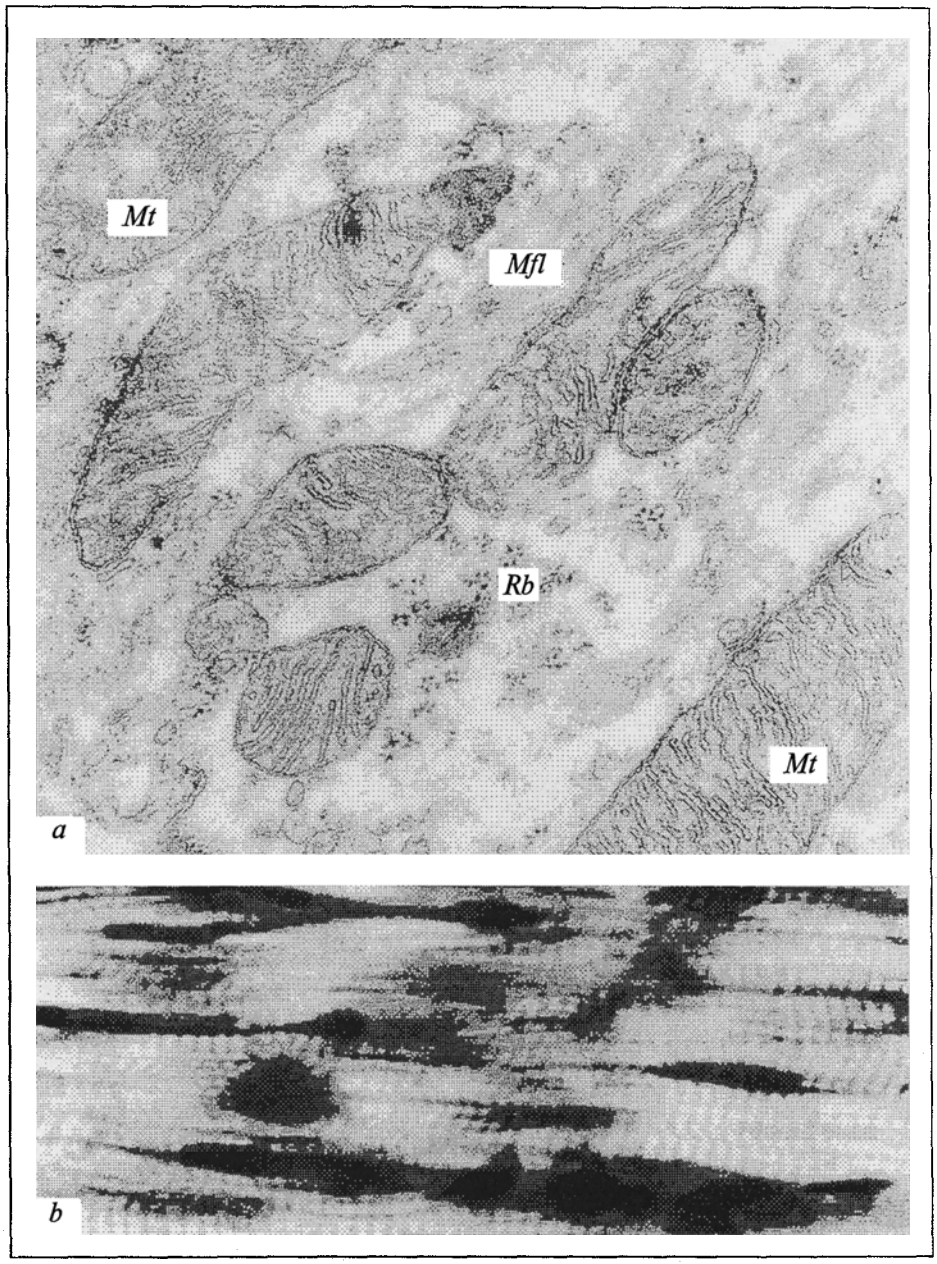

Fig. 3. Intracellular myocytolysis in rat myocardium 18 h after administration of isopropylnorepinephrine. *a*) changes in the center of a focus of intracellular myocytolysis at the stage of regeneration. *Mfl*) myofilaments; *Mt*) mitochondria; *Rb*) ribosomes, ×26,000. *b*) the same center of myocytolysis in polarized light, ×800.

Intracellular myocytolysis is an acute damage characterized by desaggregation and lysis of some myofibrils in a cardiomyocyte. Electron microcopy revealed rapid lysis of myofibrils and sarcoplasmic reticulum in this type of myocardial damage. Myofibrillar destruction occurs at the Z line level: Z lines are fragmented and lysed simultaneously with the myofilaments of isotropic disks. Sarcoplasmic reticulum is not seen. Sarcolemma that limits the myocytolysis focus lacks T tubules. There were no substantial changes in mitochondrial ultrastructure, although the number of mitochondria decreased considerably.

Complete lysis of myofibrils and chaotic distribution of mitochondria in the sarcoplasmic matrix were observed 1-4 h after the onset of acute damage (peak of alterative process). Considerable number of CM with intracellular myocytolysis is under conditions preventing regenerations. In these cells the intensity of nuclear staining after 4-6 h markedly drops (nuclear ghosts) or the nuclei disappear; density of the sarcoplasm decreases (colliquative necrosis).

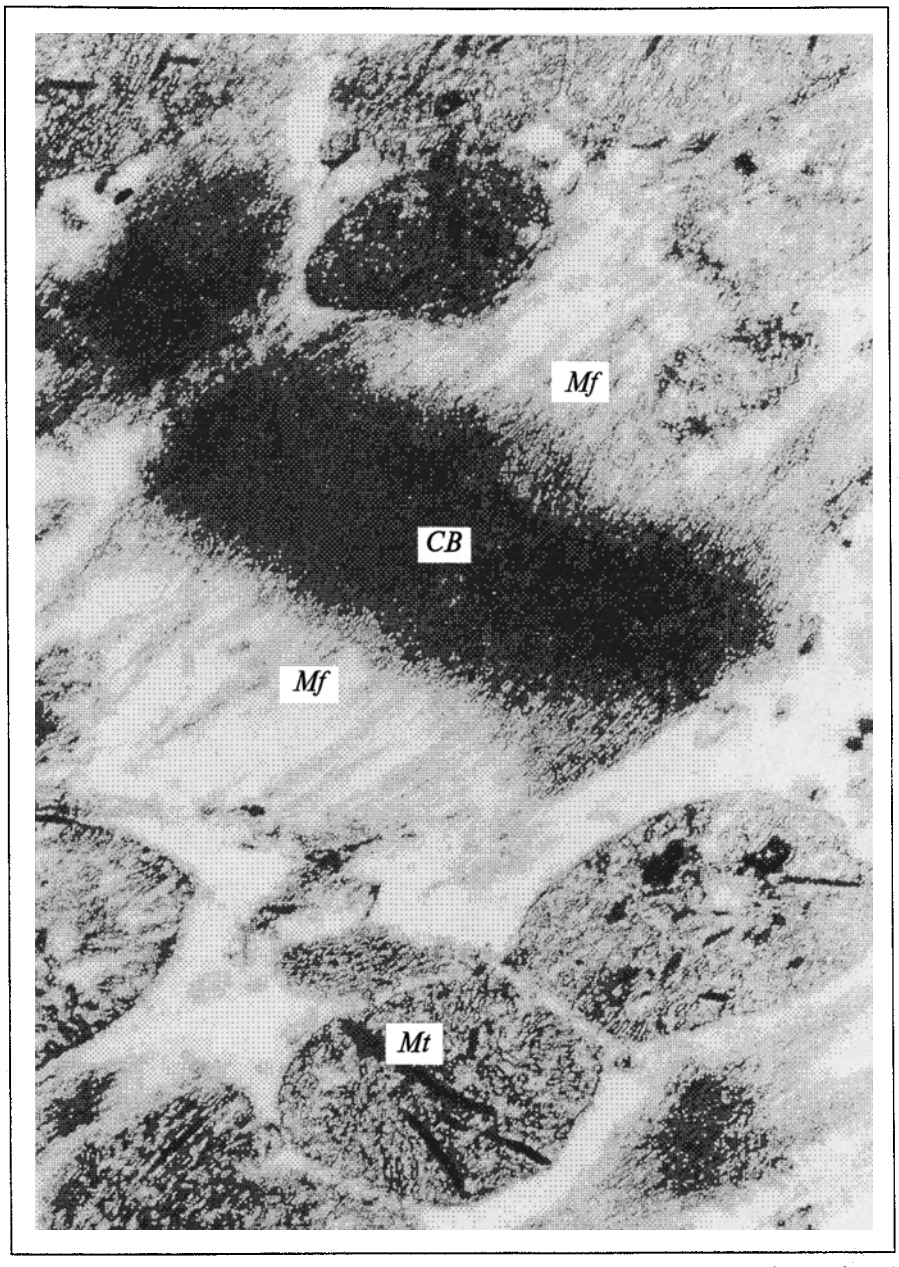

Fig. 4. Clumpy degradation of myofibrils in a cardiomyocyte in the zone of occlusive infarction. A clump of contracted myofibrils (*Mf*) is seen in the center of the microphotograph. *CB*) contraction bands; *Mf*) mitochondria, ×26,000.

Ultrastructural analysis showed that CM with colliquative necrosis contain fine detritus with occasional destroyed mitochondria. After complete lysis, the contents probably diffuses into the extracellular space. The sarcolemma is impregnated by acid glycosaminoglycans with subsequent formation of a fine linear collagen scar.

In small foci of diffuse damage, the structure of almost all cells with intracellular myocytolysis is restored during 1-2 days. Intracellular regeneration starts within the first hours after the onset of myocytolysis: free ribosomes and polyribosomes are accumulated at the periphery of the foci, particularly at myofibrillar slumps. Then ribosomes spread over the entire focus and form polysomes on which myofilaments appear. Numerous tubules and vesicles of smooth and rough endoplasmic reticulum appear in the cytoplasm.

After 12-18 h, the myocytolysis foci become small and round; dark rings consisting of myosin are seen at their periphery in polarized light. Newly formed myofilaments are arranged in bundles and myofibrils not differentiated to sarcomeres. These myofilaments connect myofibrillar slumps (Fig. 3). The mitochondria are seen as chains located between myofibrils. Sarcomeres and Z lines are formed at the

last step of intracellular regeneration, after which the cell acquires normal structure.

Primary clumpy degradation of myofibrils is an acute myocardial pathology characterized by focal mosaic lysis and contracture of individual groups of sarcomeres. Electron microscopy revealed conglomerates of hypercontracted myofibrils (Fig. 4) alternating with zones of lysis in which both myofibrils and elements of sarcoplasmic reticulum are destroyed. The structure of mitochondria is preserved for several hours after damage. Then changes similar to autolysis appear in the mitochondria and deformed nuclei.

Cytolysis (vital autolysis) of undamaged cardiomyocytes is developed upon rapid and complete cessation of anterograde and retrograde blood flow. It is characterized by complete destruction and lysis of intracellular structures, including Z lines and myofilaments of isotropic disks, which increases the distance between anisotropic disks.

In the literature, the contracture type of damage was described as protein dystrophy, eosinophilic degeneration, or fuchsinophilic degeneration. The same terms were applied to coagulation necrosis arising after imbibition with plasma proteins of damaged cells with the third degree segmental contracture or primary clumpy degeneration of myofibrils. The definition "myofibrillar degeneration" may include both subsegmental contractures and clumpy degradation [14]. It is believed that these changes are reversible [14]. In fact, this is true only for subsegmental contractures. The types of CM damage have some similarity, but the fate of cells is different: subsegmental contractures are reversible, while primary clumpy degradation is always followed by coagulative necrosis. Myofibrillar changes occurring in so-called zonal lesions and described for hemorrhagic shock [13] should be classified as subsegmental contractures.

Intracellular myocytolysis has been extensively studied in our laboratory [1,2,6]. It was observed by others and interpreted as edema [9] or lysis [10]. Cytolysis in the central zone of myocardial infarction identified by electron microscopy [12] has been referred to as myofibrillar relaxation at the light microscopy level [16].

Identification of the type of CM damage is fundamental for myocardial pathology [2-5,8]. From theoretical viewpoint, our results contribute to the concept of alterative myocardial insufficiency. This concept is based on a unique distinct characteristic, namely, the state of myofibrils, which are the most important structural and functional unit of cardiomyocyte. Our data provide the basis for morphological diagnosis and prognosis of acute myocardial pathology, particularly of its early stages, as well as for comparison and systematization of pathomorphological findings.

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