REVIEWS

Principal Forms of Acute Damage to Cardiomyocytes According to Polarization Microscopy Data

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The principal forms of acute cardiomyocyte damage of metabolic and ischemic genesis are accompanied by changes in the contractile apparatus, which is reflected in double refraction of myofibrils. Thus, polarization microscopy is the most sensitive method allowing one to detect the early stages of cardiomyocyte damage. Individual types of cardiomyocyte lesions are identified on the basis of parallel histological studies and polarization and electron microscopy of prenecrotic alterations and myocardial infarction using experimental and autopsy material. They are: I, II, and III degree myofibrillar contracture, intracellular myocytolysis, primary lumpy degradation of myofibrils, and cytolysis. These types of damage form the morphological basis of acute myocardial pathology with a possible outcome of coagulative or colliquative necrosis.

Key Words: *metabolic and ischemic myocardial damage; ventricular fibrillation; myocardial infarction; cardiomyocyte myofibrils; polarization and electron microscopy*

The pathogenesis, diagnosis, and prognosis of various types of cardiomyocyte lesions have preoccupied researchers for a long time and stimulated an ongoing search for new methods of indentifying the pathological processes. Morphological analysis, which permits evaluation of the spectrum of alterations in structural and tissue interactions as well as their intensity and scope, is of great importance. The search for methods of identifying individual types of injury with specific characteristics and outcomes is an important aspect of morphological analysis [7, 11,25,32].

For a long time, dystrophy and necrobiosis were considered to be the major and clinically most important changes taking place in cardiomyocytes (CM). Dystrophic and necrobiotic changes in CM underlie focal metabolic cardiac damage and myocardial infarction. Dystrophic alterations in CM, which often precede necrosis, make up a large group of lesions differing in their mechanisms of development and morphological manifestations. Polarization microscopy has made it possible to identify early stages of CM damage at the light microscopy level [20,33,45-47,49].

Basic research into the nature and dynamics of morphological changes in CM caused by various agents or conditions modeling acute human myocardial pathology has been performed for the first time by Russian scientists with the use of polarization microscopy [1,2,20,25,32,33]. Up to 1985, these studies were carried out under the supervision of Dr. Yu. G. Tsellarius. It was found that different changes in myofibril structure revealed by polarization microscopy provide a basis for the classification of acute CM lesions [34].

Myofibrils respond to alterative stimuli by a complex of structural disorders that are not specific for any

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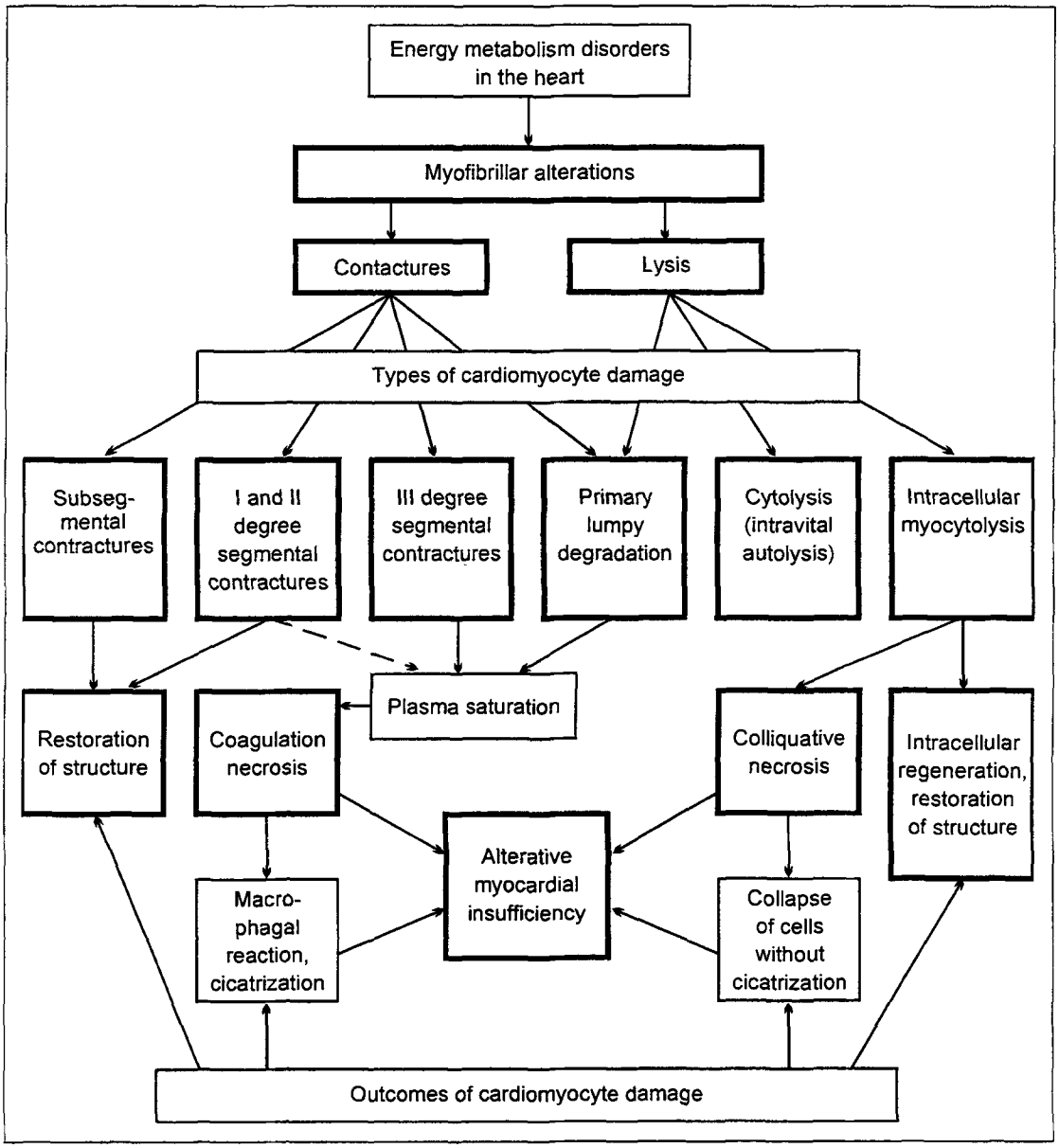

Fig. 1. Principal types of CM lesions, myofibrillar alterations revealed by polarization microscopy, and their outcomes in alterative myocardial insufficiency.

damaging factor or agent. The identified changes in myofibrillar structure are primary, independent types of damage (they do not switch from one form to another) and determine the outcome of cell injury [7,35].

The formulation of these key concepts became possible after many years of systematic experimental and clinical research into acute myocardial pathologies of different genesis. Myocardial damage was reproduced by administering cardiotoxic doses of epinephrine and isopropylnorepinephrine [7,32] and cobalt chloride [7,48], by functional overload [25,32], and by ventricular fibrillation induced by electrical current [23]. Experimental myocardial infarction was induced by coronary occlusion [8]. The myocardium was studied at intervals between 5 min and 48 h after the application of the damaging factor. Hearts of patients who had died of causes unrelated to cardiac disease, acute cardiac insufficiency, acute coronary insufficiency, myocardial infarction [4,7,35], asystolia, or fibrillation (electrocardiogram records) during or following heart surgery [5] were studied.

Cryostat sections of unfixed myocardium and paraffin sections of formaldehyde-fixed myocardium were used for the light microscopy investigations. The sections were stained with hematoxylin and eosin, toluidine blue, and PAS-hematoxylin orange [32] and by methods revealing fuchsinophilia [41, 43]. Cryostat sections were stained by the Coons method using fluorescent antibodies to myosin [37] and serum proteins [30]. For electron microscopy the material was pro-

cessed as described elsewhere [35]. Stained and unstained preparations were studied by polarization, light, phase-contrast, and interference microscopy.

The following types of acute damage to CM have been identified: 1) contracture (segmental and subsegmental); 2) intracellular myocytolysis; 3) primary lumpy degradation of myofibrils; and 4) cytolysis. These types of CM alterations ensue from energy metabolism disorders attendant upon ischemia, toxic influences, and metabolic disturbances and provide a morphological basis of alterative myocardial insufficiency [11]. Following is a detailed description of these processes (Fig. 1).

Contracture types of damage are identified in polarized light by increased anisotropy of myofibrillar A-disks and by a shortening of isotropic disks (Fig. 2). The total contracture of cardiomyocyte myofibrils (Weismann's segment) is denoted as "segmental contracture." Another type of damage, characterized by contraction of separate groups of sarcomeres with preserved striation of unconctracted myofibrillar segments, is termed "subsegmental contractures."

Segmental contractures arise in the myocardium the moment the damaging factor is applied. Normal CM with regular striation and CM with contracted myofibrils are clearly seen under a polarization microscope. Three degrees of segmental contracture have been defined. In I degree contracture, the height of isotropic disks remains unchanged, while anisotropy of A-disks is increased (isometric contraction). In II degree contracture, anisotropic disks are brought closer to each other due to a shortening of isotropic disks, but cross-striation is preserved. In III degree contracture, anisotropic disks are close packed and form a continuous anisotropic conglomerate. Interference microcopy reveals increased optical density of the CM sarcoplasm with I-III degree contracture. The pattern of antimyosin antiserum fluorescence on sections treated after Coons corresponds in all details to the polarization microscopy pattern of myofibrillar structure.

The nuclei in CM with segmental contracture of myofibrils are pycnotic and shrunken. These CM react with cationic and anionic stains more intensely than normal CM. When special methods to identify fuchsinophilia are applied, damaged cells are selectively stained by fuchsin.

Segmental contractures of I, II, and III degree are detected in the myocardium within the first few hours after a single damaging influence. After 12 h, the number of cells with contracture decreases due to the disappearance of I degree contractures and some II degree contractures, which indicates that these alterations are reversible.

After 4-6 h, a PAS-positive diffuse reaction is seen in cells with III degree contracture. This reaction is not abolished by amylase. Plasma proteins are detected in these cells by the method of Coons. After this, CM with III degree contracture display a typical picture of coagulative necrosis; these cells are subsequently destroyed by macrophages, the destruction often being preceded by diminished double refraction of the anisotropic substance and its degradation to lumps (secondary lumpy degradation).

The occurrence of segmental contractures of some CM or CM groups is high. They are seen in metabolic disorders caused by intoxication or functional overload and are always localized at the edge of the infarction zone.

Subsegmental contractures in polarized light these look like bright cross stripes or multiple anisotropic conglomerates between which striated myofibrillar segments are seen. Under a light microscope, subsegmental contractures look more dense and eosinophilic. Fuchsinophilia of these alterations is transient.

In experiments where ventricular fibrillation was induced, we always detected subsegmental contractures occupying 3-4 rows of myofibrils adjacent to the endo- and epicardium. Detection of subsegmental contractures of this localization was facilitated by the use of a polarizing microscope. These structures disappeared 6-12 h after defibrillation, which indicates that these alterations are reversible. Comparison of morphological data obtained on experimental and autopsy material with electrocardiography records showed that the presence of subsegmental contractures in the subendothelium and subepicardium is a diagnostic marker of ventricular fibrillation. Cells with locally contracted myofibrils were found in the deeper layers of the myocardium in cases of metabolic or ischemic damage. In addition, subsegmental contractures caused by mechanical damage were often found in myocardial bioptates.

Intracellular myocytolysis is an acute injury to CM characterized by disaggregation and lysis of myofibrils in a certain part of the cell (Fig. 3). Within the first few hours of the process, myofibrils with intracellular myocytolysis stain less intensely than normal myofibrils. At the regeneration stage staining is the same. Therefore, it is impossible to identify this damage under a light microscope, nor can it be detected by staining for fuchsinophilia.

The fluorescence of antimyosin antibodies disappears simultaneously with the disappearance of myofibrils observed in polarized light. However, diffuse fluorescence appears in the areas of myocytolysis several hours later. Myofibrils are restored on day 2 and can be identified by the Coons method and under a polarizing microscope.

In microfocal diffuse myocardial lesions, almost all cells with intracellular myocytolysis restore their structure within 1-2 days. Intracellular regeneration

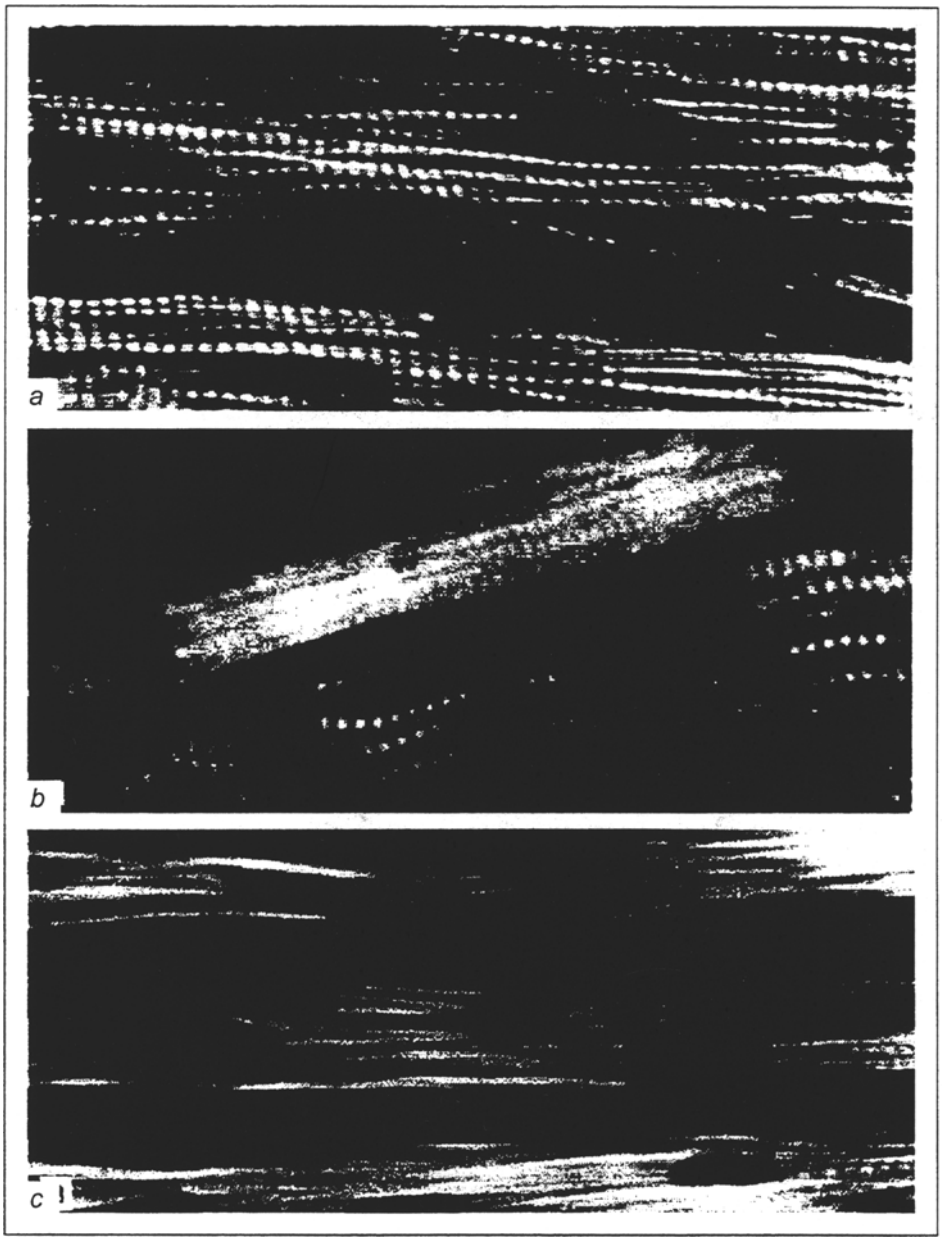

Fig. 2. Epinephrine-induced damage to rat myocardium. Polarization microscopy of CM myofibrils. *a*) intact myocardium in polarized light; *b*) II degree segmental contracture: increased myofibrillar anisotropy; *c*) III degree segmental contracture: continuous myofibrillar anisotropy. Here and in Fig. 3: staining with hematoxylin and eosin. ×1000.

starts within hours of myocytolysis: free ribosomes and polyribosomes accumulate at the periphery of the foci, being particularly numerous at myofibrillar stumps. The ribosomes then spread over the entire area of the focus and form polyribosomes, on which individual myofilaments start to be produced. Numerous tubules and vesicles of smooth and granular endoplasmic reticulum appear in the cytoplasm.

After 12-18 h, the foci of myocytolysis become small and round, polarizing haloes composed of myosin appearing at their periphery. New myofilaments form bundles and myofibrils undifferentiated to sarcomeres. These myofibrils join the myofibrillar stumps. Sarcomeres and Z-disks form at the last stage of intracellular regeneration, after which the cells regain their normal structure.

In cases of extensive myocardial damage, large numbers of cells with intracellular myocytolysis find themselves under conditions that hamper regeneration. In these cells the nuclei stain weakly (ghost nuclei) or disappear, and the sarcoplasm becomes liquefied (colliquative necrosis). After complete lysis of structures, the cell contents probably diffuse into the intercellular space. The shrunken sarcolemma is impregnated by acid glucosaminoglycans and turns into a linear collagenous scar. Colliquative necrosis of CM developing due to intracellular myocytolysis does not lead to the formation of inflammatory infiltrates.

At all stages of the process, the cytoplasm of injured cells remains PAS-negative. Application of fluorescent antisera against plasma proteins confirms the fact that, in contrast to contracture, intracellular myo-

cytolysis is not accompanied by diffusion of plasma proteins into the cells even when colliquative necrosis is developing.

Most often multiple foci of intracellular myocytolysis appear in the myocardium after the administration of high doses of catecholamines, which in some cases causes the death of animals within one hour. Such foci were also observed in orthostatic collapse and physical overload. The occurrence of intracellular myocytolysis in autopsy material was relatively low. Numerous foci of intracellular myocytolysis were found in hearts of patients with hyperaldosteronism and pheochromocytoma who had died of acute heart failure.

Primary lumpy degradation of myofibrils is an acute pathology of CM characterized by degradation of myofibrils as a result of focal mosaic lysis and contracture of individual groups of sarcomeres (Fig. 4). In polarized light this type of damage is identified by the disappearance of cross-striation in CM and the appearance of numerous lumps of an anisotropic substance randomly alternating with areas free of anisotropic structures. The presence of isotropic regions between anisotropic lumps allows one to distinguish between lumpy degradation and subsegmental contractures that are characterized by the presence of distended myofibrils between the foci of sarcomere contracture. Under a light microscope, CM

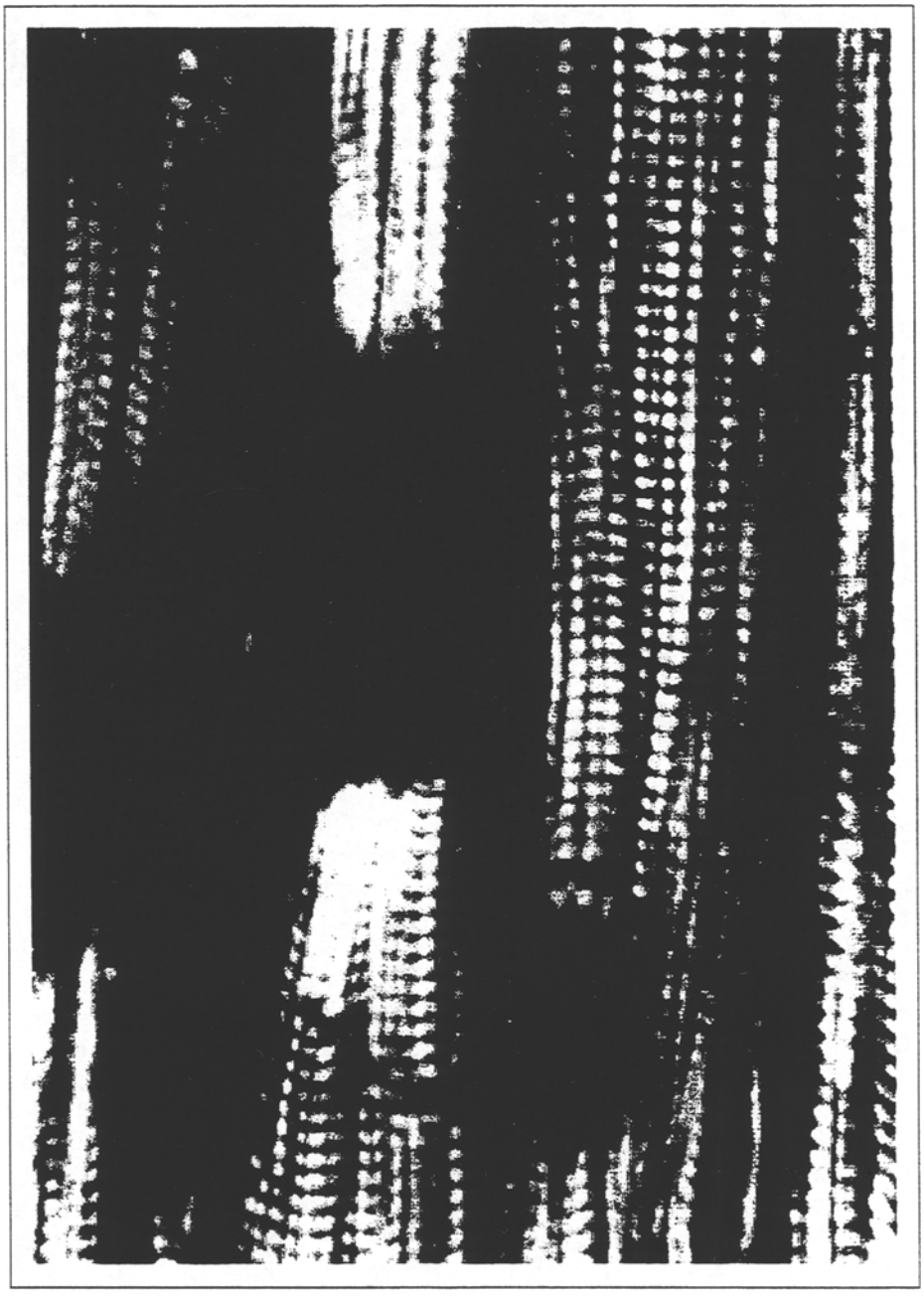

Fig. 3. Epinephrine-induced damage to rat myocardium. Intracellular myocytolysis. Polarization microscopy.

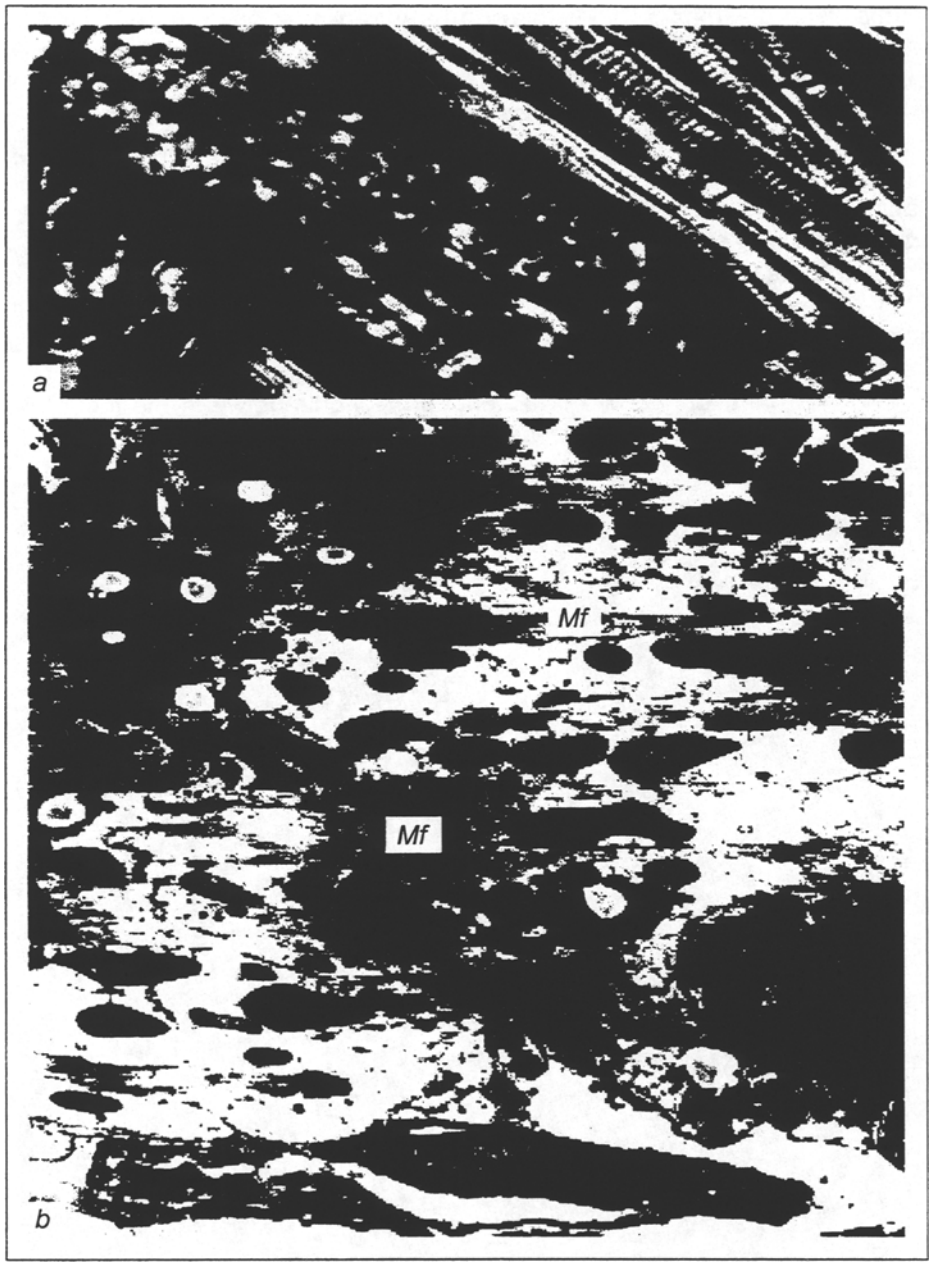

Fig. 4. Isopropylnorepinephrine-induced injury to rat myocardium. Primary lumpy degradation of myofibrils in CM. *a*) staining with hematoxylin and eosin. Polarization microscopy. ×800; *b*) destroyed myofibrils (*Mf*) between foci of hypercontracture. ×4500.

with lumpy degradation stain intensely, their sarcoplasm is lumpy, and the nuclei are hyperchromatic and deformed. Anisotropic lumps in cells with lumpy degradation of myofibrils are occasionally fuchsinophilic, since their staining depends on the fixation period and other uncontrollable conditions. This applies especially to autopsy material. The method of Coons and the PAS-reaction shown that plasma proteins diffuse into cells with lumpy degradation 1-2 h after the onset of the process. Cells that have undergone coagulative necrosis are destroyed by macrophages.

Primary lumpy degradation of myofibrils reflects severe irreversible damage to CM. The discovery of lumpy degradation foci within the first few minutes or hours after the damaging influence indicates CM necrosis.

Primary lumpy degradation is the most frequent alteration of CM in severe myocardial damage. It is found in autopsy material obtained in acute cardiac insufficiency, acute coronary insufficiency, and myocardial infarction. Small foci of damage located at the periphery of the ischemic zone and consisting of CM with primary degradation and segmental contracture of myofibrils [8] are the earliest indication of myocardial infarction [8].

Cytolysis or intravital autolysis of intact CM develops as a result of rapid and complete cessation of linear and retrograde blood flow. Cytolysis develops in occlusive myocardial infarction (central zone

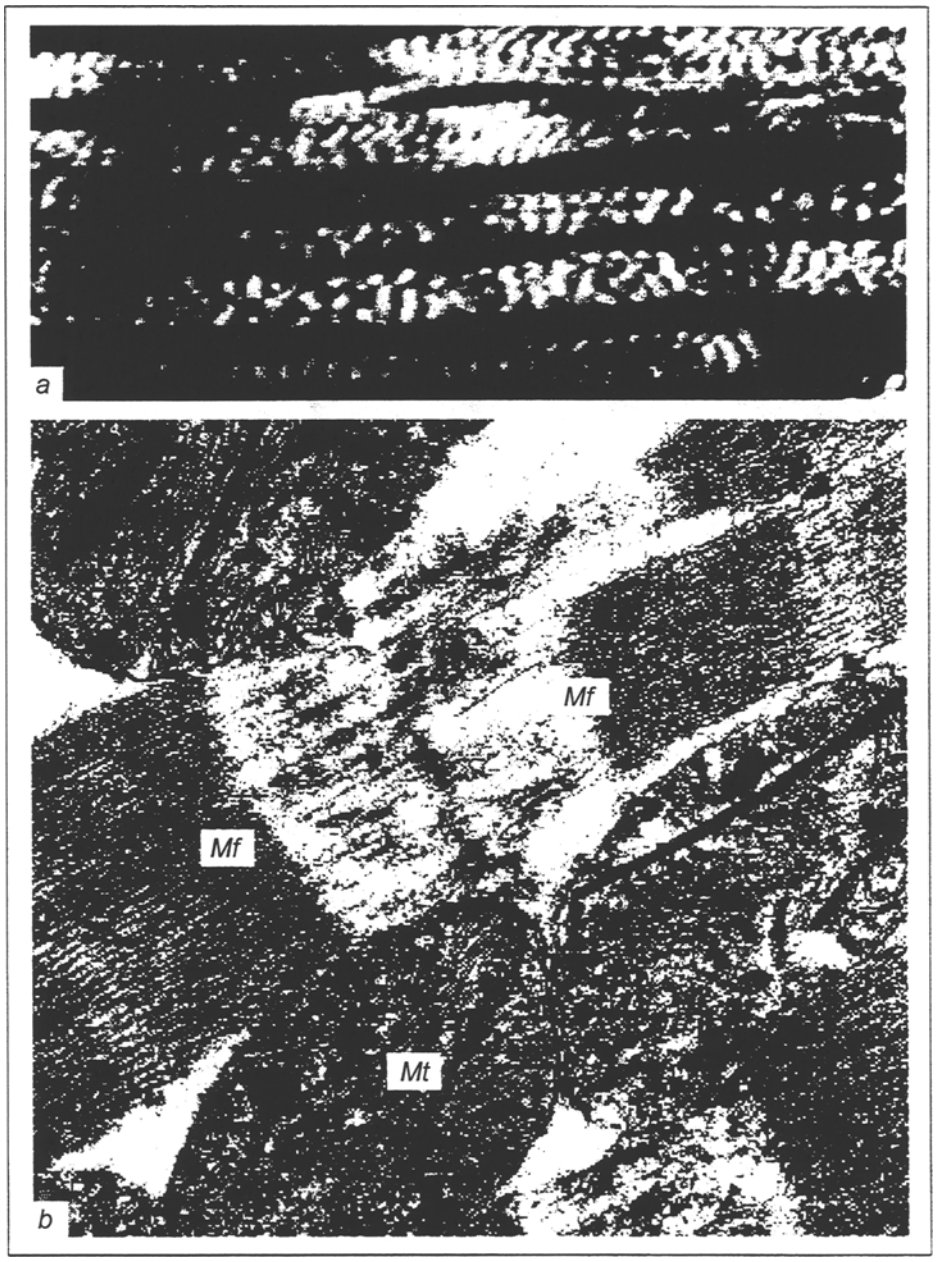

Fig. 5. Experimental myocardial infarction. Cytolysis of CM in the central zone of necrosis. *a*) polarization microscopy. ×1000; *b*) ultrastructural alterations: autolysis of anisotropic disks of myofibrils (*Mf*), diastasis of anisoptropic disks. *Mt* = mitochondria. ×36 000.

of ischemia) and in extensive infarction-like metabolic injuries accompanied by capillary thrombosis. Under a light microscope, CM with cytolysis phenomena can be identified after 12-24 h by weakly stained nuclei or by their absence. Cross-striation of CM with cytolysis is clearly seen after staining with eosin. The PAS-reaction is negative, and fuchsinophilia is absent. Under a polarizing microscope, muscle fibers in which cytolysis has occurred can be identified after 4-6 h by "overdistended myofibrils": their isotropic disks are several-fold higher than the same disks in viable cells (Fig. 5).

The mechanisms underlying the development of the types of CM damage described here require further investigation. The contracture alterations are probably associated with increased permeability of the plasma membrane. The diffusion of plasma proteins into the CM cytoplasm occurring in irreversible injury (III degree segmental contracture and primary lumpy degradation of myofibrils) indirectly confirms this assumption. An increase in plasma membrane permeability has been confirmed by experiments with tracers [38,40].

Some workers have hypothesized that increased anisotropy in injured cells is not associated with contracture, since contraction of the muscle fibers should lead to a decrease in myofibrillar anisotropy [46]. However, this is true only in the case of isotonic contraction, while in reality myocardial contractions are close to isometric, since distending forces from

other cells are applied to the ends of each CM, and anisotropy in this case should increase (photoelastic effect). The partial protein denaturation accompanying with contracture may account for the increase in the concentration of dry matter and the corresponding increase in the ability to bind stains. The concentration of dry matter increases still further due to the diffusion of plasma proteins into the cell. These proteins produce a fixative effect on the cell, as a result of which it is not autolyzed and can be destroyed only by macrophages.

The occurrence of subsegmental contractures, which are particularly frequent in ventricular fibrillation, is probably associated with the desynchronization of contraction not only between individual cells but also in one cell, which is not accompanied by profound damaging processes and may be reversible. Our observations are consistent with those of others [39], demonstrating a possibility of artificial appearance of subsegmental contractures during cardiac biopsy.

Primary lumpy degradation of myofibrils, which has been defined as an independnet type of damage, can be considered to be the most severe irreversible form of contracture, in which myofibrillar destruction occurs between foci of overcontraction, and the cell becomes saturated with plasma proteins and necrotizes more rapidly than in segmental contractures.

The sudden degradation of myofibrils in intracellular myocytolysis is topographically not associated with lysosomes and probably does not result from their destruction. If myofibrillar degradation is enzymatic and is independent of other factors, it must be assumed that the enzymes responsible for the degradation are not localized in the lysosomes. Hydrolases are present in the endoplasmic reticulum [42] and in the myofibrils themselves [44]. The factor activating these enzymes - a pH drop due to respiratory disorders or any other perturbation - remains unclear.

In intracellular myocytolysis the structure of the sarcolemma is not markedly affected, as is evidenced by the absence of plasma protein diffusion in the cell. This may account for the preservation of the cell's ability to regenerate.

Alterations in CM occurring in plastic insufficiency, when the formation of myofibrils is affected due to blockade of protein synthesis, are to a certain degree similar to intracellular myocytolysis [6,13,14, 16,17,19,25,26,28,29]. Preservation of the sarcotubular system and weak intracellular regeneration are specific features of plastic insufficiency [15,18, 21,27].

It is unclear why disorders of energy metabolism lead to myofibrillar contracture in some cells and to lysis in others. This may be due to individual metabolic variations. It has been hypothesized that the organelles in CM are renewed in asynchronous cycles, this being necessary for continuous functioning of the heart [22]. This leads to metabolic heterogeneity manifesting itself in a mosaic pattern of damage to the myocardium [2,32]. Autoradiographic studies have shown that contractures occur primarily in cells with minimal RNA synthesis [3,12]. It can be assumed that intracellular myocytolysis develops in cells which are in the opposite phase of the cycle, namely, at the stage of most intensive renewal of myofibrils.

Thus, acute CM injury caused by intoxication, functional overload, and ischemia induces stable myofibrillar alterations revealed in polarized light. Hence, polarization microscopy is a sensitive method pinpointing the early stages of cardiomyocyte damage [9-11,24,31,36].

Different patterns of myofibrillar alterations under a polarizing microscope make it possible to identify individual types (forms) of CM injury providing the morphological basis of acute myocardial pathology leading to coagulative or colliquative necrosis.

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