Prenecrotic Contracture Damage in Cardiomyocytes: Photochemical Fluorochrome Staining and Fluorescent Microscopy of the Myocardium

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Prenecrotic contracture changes in cardiomyocytes during ischemic and metabolic alteration of the myocardium are detected at the photooptic level by photochemical fluorochrome staining and examination in fluorescent light. Comparison of the fluorescent and polarization microscopic pictures of damaged cardiomyocytes showed relationships between optically active and isotropic constituents of the cell myofibrillar system. Contracture changes are determined by local mass redistribution in contractile compartments and transformations of the myofibril components, caused by protein structure coagulation in cardiomyocyte sarcomers.

Key Words: *acute myocardial disease; cardiomyocytes; myofibril contractures; photochemical fluorochrome staining; fluorescent, polarization, and interference microscopy*

Myofibrillar system of cardiomyocytes (CMC) is a morphological indicator of early stages of muscle cell damage [1-3,5,9,12,14]. In primary acute ischemic or metabolic damage of CMC the morphological signs of alteration are nonspecific and manifest by stereotypical changes in the CMC contractile structures [1,2, 5,9].

The potentialities of investigation of the CMC contractile structures are largely determined by the microscopic methods. We introduced into morphological analysis of the myocardium methods of CMC examination in polarization light in parallel with examinations in fluorescent light after photochemical fluorochrome staining (PFC) of myocardial specimens [6,8,10].

The former method is based on specific features of birefringence of the cell myofibril actomyosin complex. This method shows changes in anisotropic (caused by the myosin component) and isotropic (depending on the actin constituent of the myofibril) myofibril strips in longitudinally oriented muscle fibers [8]. The latter method consists in imaging of the muscle cell contractile system as a result of the formation of photoproducts fluorescing in visible spectrum band during irradiation of the preparation with short-wave ultraviolet (SUV) spectrum band. The patterns of muscle cell fluorescence depended on the mass distribution in the contractile structures compartment of sarcomere [11].

Classification signs of the main morphological forms of acute muscle cell injury are based on the data of polarization microscopy [4,8,9] preceding the PFC method [10,13,15]. Morphological changes of prenecrotic states of the CMC most often manifest by contracture damage of the myofibrils [1,2,4,5,16].

The aim of this investigation was fluorescent microscopy of the CMC after PFC of the myocardium in acute ischemic and metabolic damage of the contractile structures during pathological sarcomere contractions and comparison of the results with polarization microscopy data.

MATERIALS AND METHODS

Morphological study of early signs of prenecrotic and necrotic states of the CMC was carried out on a model

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of acute myocardial disease. Occlusive myocardial infarction was induced in 39 male C57Bl mice (17-20 g) by ligation (under Nembutal narcosis) of the left coronary artery at the level of the middle third of the left ventricle. The animals were sacrificed 5 min to 24 h after ligation. Catecholamine damage of the myocardium was induced in 34 male Wistar rats (160-270 g). Novodrine (isopropyl norepinephrine) was injected intraperitoneally in a single dose (8-10 mg/100 g) 30 min to 18 h before decapitation. Specimens of human myocardium in heart failure, heart injury, and sudden coronary death were examined (103 cases).

Paraffin sections were stained with hematoxylin and eosin in combination with Pearls reaction, periodic acid-Schiff (PAS) reaction was combined with colloid iron staining by Hale' method) and hematoxylin orange staining; SIA test was carried out after Van Gieson staining with hematoxylin-picrofuchsin and poststaining with resorcine-fuchsin after Weigert.

Fluorescent microscopy was carried out under ML microscopes after exposure of the sections to SUV. Polarization microscopy was carried out under Amplival pol.u. microscope. Interference analysis was carried out under Peraval interhako microscope. The study was described in detail previously [5,7,8,10].

RESULTS

Examination of the myocardium showed that CMC myofibrils responded to alteration by total and mosaic pathological contractions of the sarcomers and by focal destruction and lysis of the muscle cell. These processes determine the dynamics of cell necrobiosis, expressed in changes of the fluorescent and polarization optic characteristics of the myofibrillar system.

Primary morphological signs of cell damage have no features specific of a certain pathogen. The histological picture of pathological changes of different origin depends on the ratio of contribution of different forms of cell alteration and heterogeneous stromal and vascular myocardial reactions to the total pool of damaged parenchyma. Rat and mouse myocardium and section material visually did not differ by the fluorescent characteristics after PFC staining.

Myocardial damage presented as mosaic fluorescence of altered CMC after PFC staining; parallel examination under polarized light showed changes in the myofibrillar system.

Total pathological contraction of CMC sarcomers was denoted as segmented contracture [4] and was associated with increased fluorescence intensity. This helps to detect CMC with contractures among intact muscle cells. If the contractures involved a solitary cell, spatial disproportion of pathological contractions leading to oblique position of insertion disks and longitudinal cleavage of the cell contractile structure, were even better seen (Figs. 1, 2). It is seen under fluorescent light (Fig. 1, *b*) that cleavage of cell contractile structures does not result from postmortem longitudinal ruptures, as the fluorescence intensity of the fissures is higher than the background (extracellular) level.

Comparison of the polarization optic and fluorescent pictures of CMC contractures (Fig. 1) can detect primary foci of uneven density in the monolithic anisotropic conglomeration of a pathologically contracted cell. The configuration of fragments during cell degeneration in the subsequent secondary lumpy degeneration can be determined by the density heterogeneity [5]. This picture can also reflect the initial fusion of the processes of segmented contractures and primary lumpy degeneration [4,5]. Morphological dimorphism of CMC alterations is determined by intracellular differences in the ratio of coagulation/lysis processes and their relationships with local fenestration of the sarcolemma.

The formation of morphological picture of pathological contractions of CMC is determined by heterogeneous manifestation of changes in optically active and passive components of the myofibrillar system in sarcomers. It was shown previously that along with increased birefringence in pathological contraction of CMC, damaged myocardium contains cells in a state of contracture, where anisotropy of massive conglomeration is weakened [5].

Contractions of CMC with approximation of A disks is associated with enhanced anisotropy and intensification of fluorescence (Fig. 3). In other cells more intense contractions lead to fusion of contractile structures into a massive anisotropic conglomeration, which is associated with further increase in fluorescence intensity.

Comparison of the fluorescent picture and birefringence of cells with contractures showed that changes in the insertion disk area are different in different CMC. A narrow isotropic fissure between the cells (Fig. 2, *a*) in fluorescent light is seen as a more extensive area of decreased fluorescence and density (Fig. 2, *b*). Presumably, the molecular structure of this area is heterogeneous, as relatively high anisotropy of sites adjacent to the interface between the cells sharply decreases at the site of cell contact without signs of mechanical disconnection between them.

In some cases the borderline areas of CMC were characterized by morphologically homogeneous fluorescence and anisotropy: isotropic intercellular region corresponds to its decreased fluorescence intensity (Fig. 3).

In addition to these variants, we often observed strips of high anisotropy and synchronous increase of

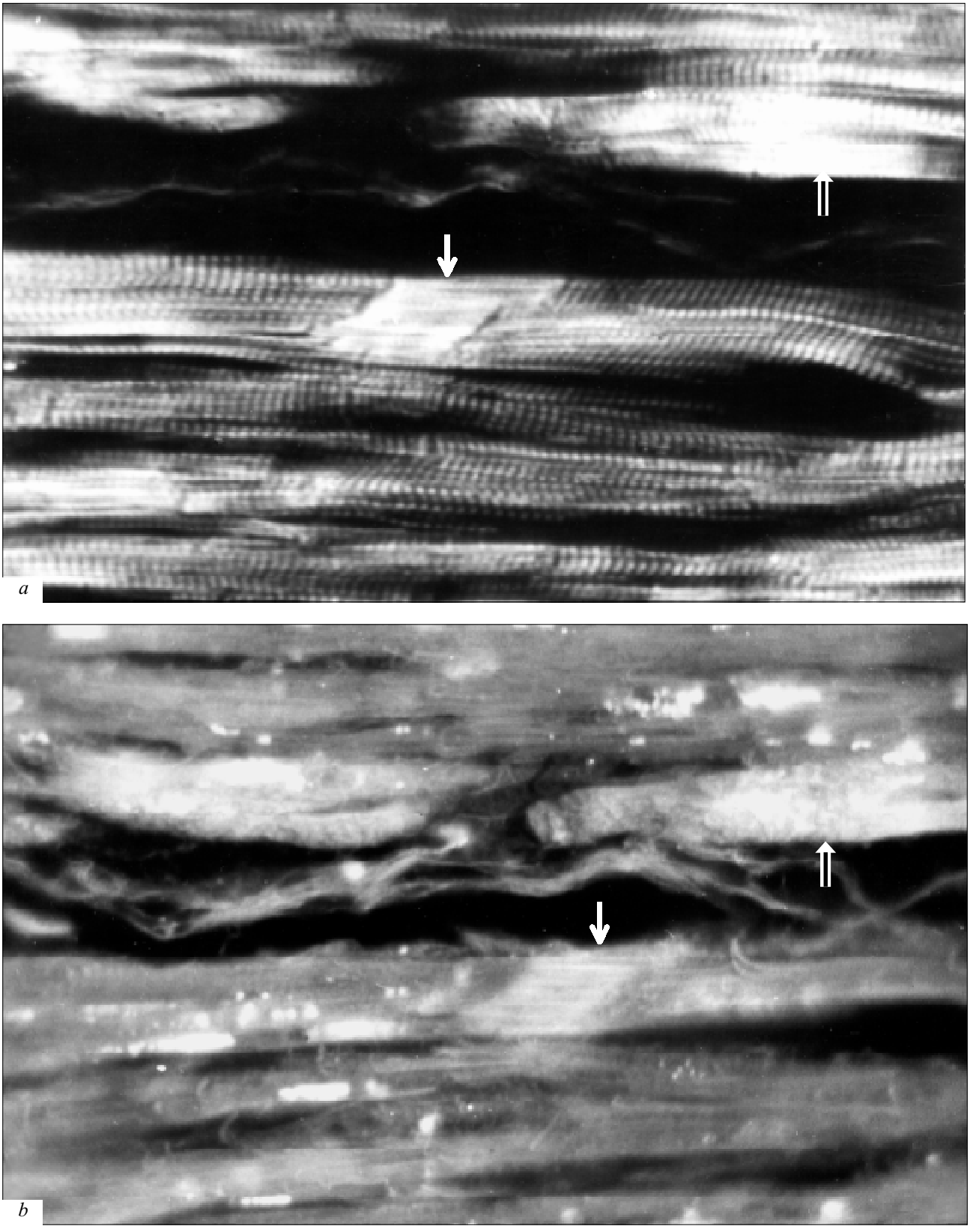

Fig. 1. Segmented contractures of cardiomyocytes in a longitudinal section of human myocardium in acute heart failure in polarized (*a*) and fluorescent (*b*) light. Longitudinal cleavage of the myofibrillar system (arrows), lumpy fluorescence of anisotropic material (double arrows), ×500.

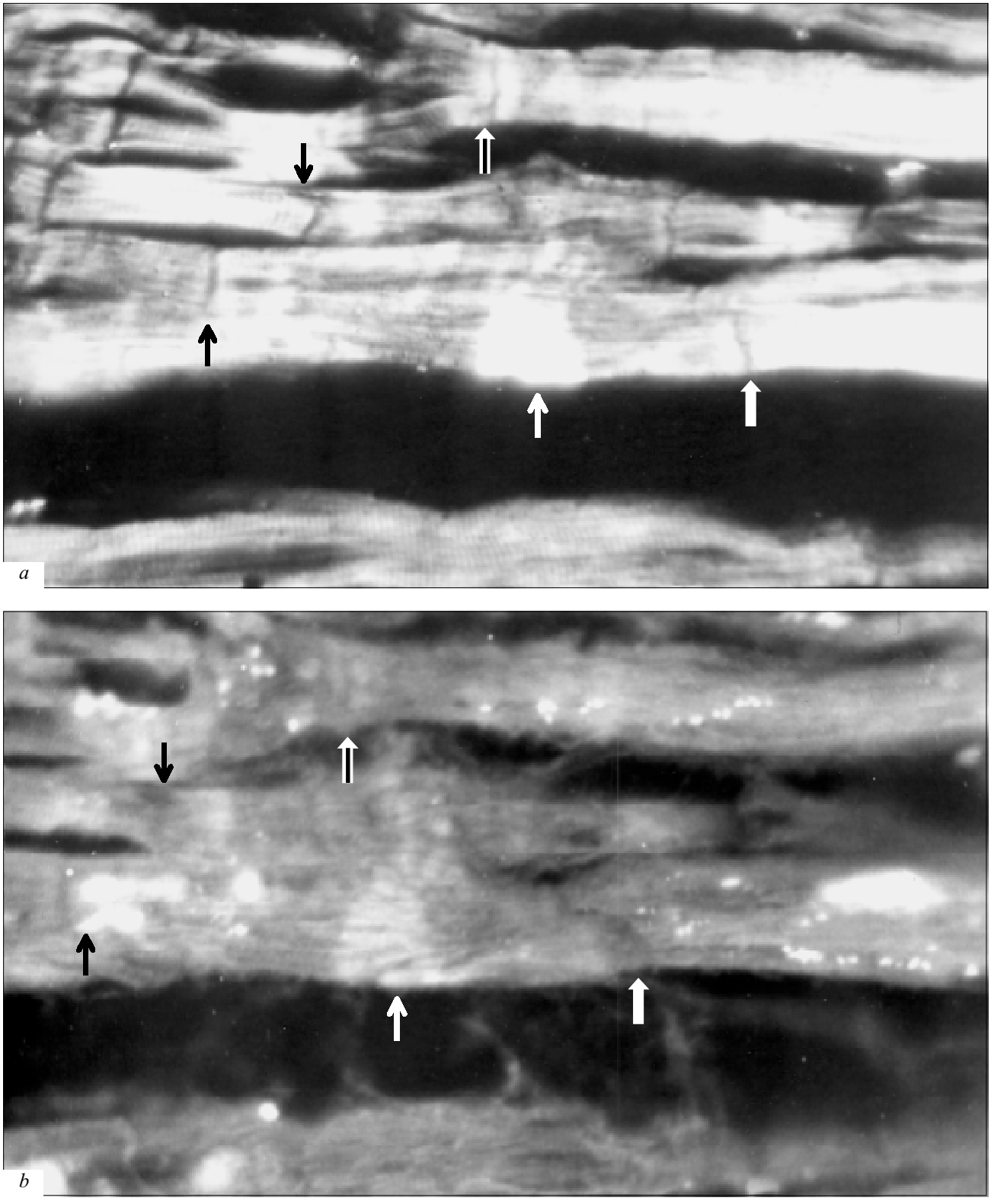

Fig. 2. Contracture injuries of cardiomyocytes in a longitudinal section of human myocardium in sudden coronary death in polarized (*a*) and fluorescent light (*b*). Segmented myofibril contractures (white arrows), decreased density of the isotropic component of contractile structures in the presence of widened insertion disks (bold white arrows), increased density of the substance at the interface of cell disconnection (double arrows), differences in the damaged cell borders by fluorescence intensity in the disconnected areas (black arrows), ×500.

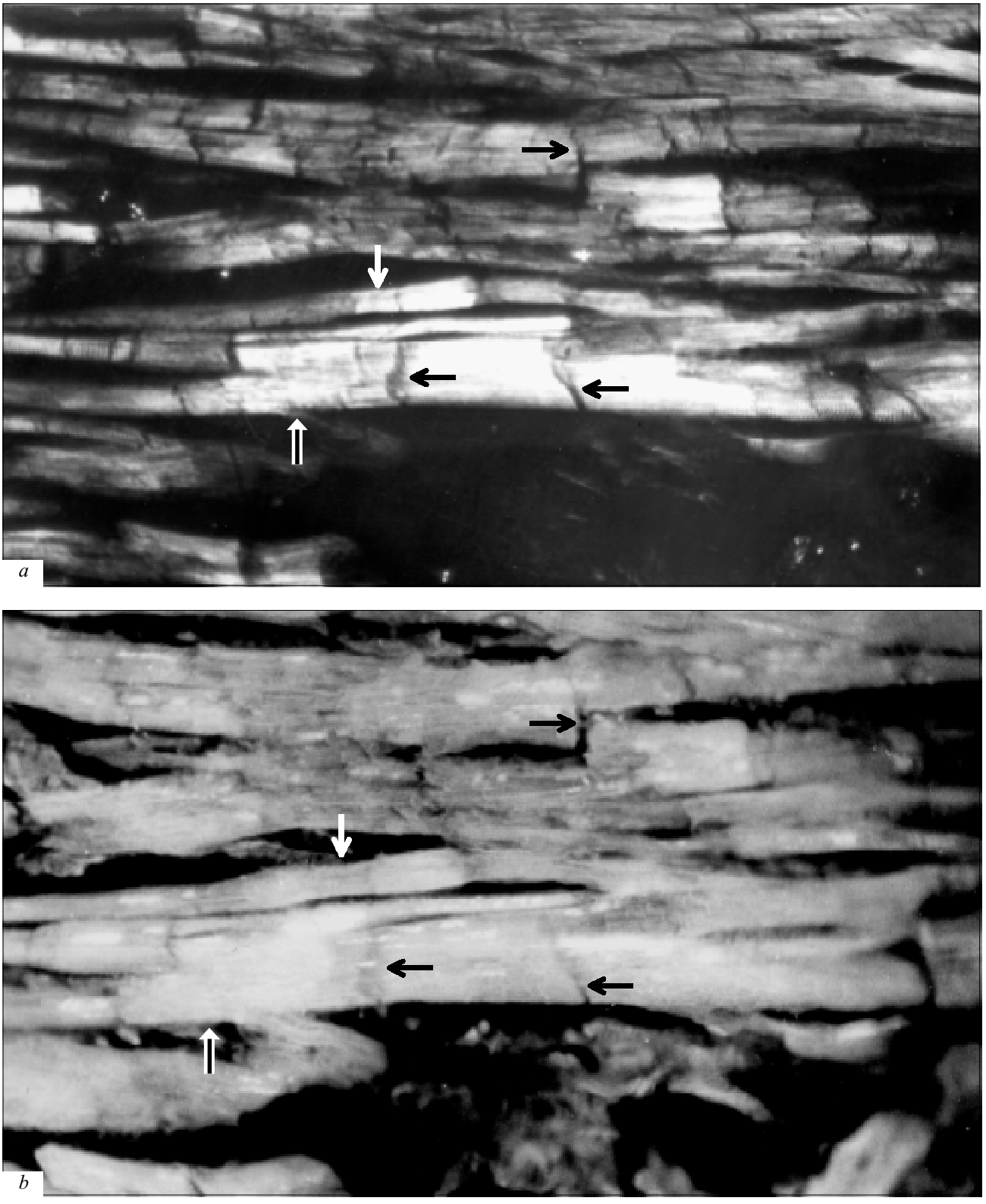

Fig. 3. Contracture injuries of cardiomyocytes in a longitudinal section of human myocardium in small focal infarction in polarization (*a*) and fluorescent light (*b*); ×190 (*a*). ×210 (*b*). Segmented contractures of myofibrils of unidirectional increase of birefringence and fluorescence intensity (white arrows), contractures of different ratios of fluorescence intensity and birefringence of anisotropic conglomeration (double arrows), differences in the sites of contact of altered cells (black arrows).

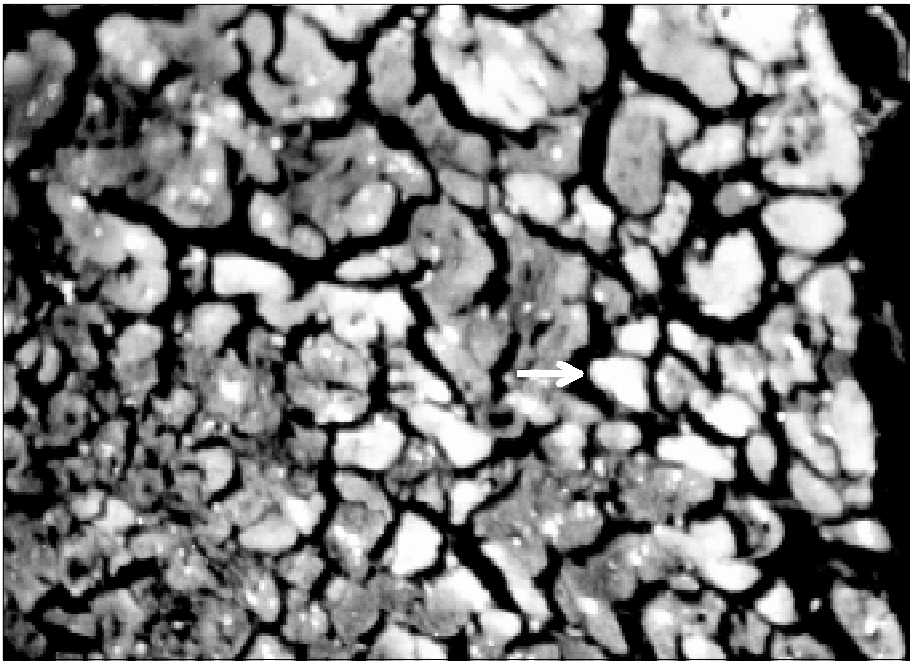

Fig. 4. Cardiomyocyte contracture in a longitudinal section of human myocardium during acute heart failure, ×120. Mosaic pattern of fluorescence in muscle fibers in the focus of lesion under fluorescent light. Arrow shows segmented contractures of myofibrils: high fluorescence intensity over the entire transverse section.

fluorescence intensity (Fig. 2, *b*), adjacent to the isotropic interface between altered cells (Fig. 2, *a*).

Muscle cells in intact myocardial tissue are not separated from each other or from stromal components. The detection of elements of CMC disconnection is one of the initial signs of pathological functioning of muscle tissue. Relationship between various forms of destruction of cell terminal sites eventuating in muscle fiber fragmentation, pathological opening of insertion disks, and local fenestration of the sarcolemma deserves further investigation.

The picture of such changes in fluorescent light helps to differentiate the degree of cell disconnection and isolation by the level of fluorescence, as it reflects the amount of substance in these sites. Such details are difficult to discern by their polarization characteristics, but in fluorescent light they differ from each other by the type of disconnection. These interfaces can be differentiated from obvious disconnection of damaged CMC; differences in cell-to-cell contacts are seen (Fig. 2, *b*), which look more homogeneous in polarized light (Fig. 2, *a*).

Resolution of segmented contractures into coagulation necrosis followed by its degradation into lumps (secondary lumpy degradation of CMC) was seen as a conglomeration of lumps with high fluorescence intensity, separated by nonfluorescing (against the background) spaces. The fluorescence intensity of necrobiotic CMC decreased during macrophagic resorption.

In contrast to polarization microscopy, fluorescent microscopy after PFC staining easily detected CMC contractures on transverse sections of muscle fibers. Segmented contractures of CMC were seen due to higher fluorescence intensity of the entire transverse section of the cell (Fig. 4).

Hence, contracture damage of longitudinally oriented CMC in the fluorescent light after PFC are interpreted within the framework of polarization optic changes. Comparison of fluorescent and polarization microscopic pictures of damaged CMC gave new data on the relationships of optically active and isotropic components of the CMC myofibrillar system, which develop during cell necrobiosis.

Analysis of the fluorescent and polarization images of the entire spectrum of prenecrotic CMC states can be a prerequisite for more complete morphological evaluation of acute failure of myocardial contractility.

REFERENCES

1. M. E. Evseeva, *Byull. Eksp. Biol. Med.*, **130**, No. 10, 378-381 (2000).
2. L. V. Kakturskii, *Sudden Cardiac Death: Clinical Morphology* [in Russian], Moscow (2000).
3. V. K. Lazutin, N. N. Beskrovnaya, V. G. Popov, and A. S. Smetnev, *Kardiologiya,* No. 10, 35-39 (1979).
4. L. M. Nepomnyashchikh, *Byull. Eksp. Biol. Med.*, **121**, No. 1, 4-13 (1996).
5. L. M. Nepomnyashchikh, *Alterative Insufficiency of Cardiomyocytes in Metabolic and Ischemic Injuries* [in Russian], Moscow (1998).
6. L. M. Nepomnyashchikh, G. I. Nepomnyashchikh, E. L. Lushnikova, *et al., Morphogenesis of The Most Significant General Pathological Processes in Human and Animal Organs and Tissues: 5 Scientific Discoveries in Biology and Medicine* [in Russian], Moscow (1998).
7. L. M. Nepomnyashchikh and V. G. Tsimmerman, *Byull. Eksp. Biol. Med.*, **128**, No. 9, 356-360 (1999).

8. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, *Pathoanatomical Diagnosis of Prenecrotic Changes and Myocardial Infarction by Polarization Microscopy* [in Russian], Moscow (1979).
9. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, *Arkh. Patol.*, No. 12, 3-13 (1980).
10. Yu. G. Tsellarius, V. G. Tsimmerman, L. M. Nepomnyashchikh, and L. A. Semenova, *Photochemical Fluorochrome Staining for Pathoanatomical Diagnosis of Prenecrotic Changes and Myocardial Infarction* [in Russian], Moscow (1984).
11. V. G. Tsimmerman, and Yu. G. Tsellarius, *Arkh. Patol.*, No. 7, 72-74 (1976).
12. I. D. Shperling, *Ibid.*, No. 1, 24-29 (1981).
13. B. N. Carle, *Human Pathol.*, **12**, No. 7, 643-647 (1981).
14. H. David, K.-F. Lindenau, J. Bohm, *et al., Exp. Pathol. (Jena)*, **14**, No. 3-4, 141-156 (1977).
15. R. B. Jennings, *Arch. Inst. Cardiol. Mex.*, **34-35**, Suppl. 3, 156-172 (1980).
16. Z. Matejkova, A. Bozner, J. Dostal, and K. Boda, *Cesk. Patol.*, **29**, No. 2, 61-63 (1993).