MORPHOLOGY AND PATHOMORPHOLOGY

Lytic and Contracture-Lytic Prenecrotic Damage to Cardiomyocytes: Photochemical Fluorochrome Staining and Fluorescent Microscopy of the Myocardium

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Acute prenecrotic damage to cardiomyocytes of lytic (myocytolysis, cytolysis) and contracture-lytic (primary lumpy degradation of myofibrils) types during ischemic and metabolic alteration of the myocardium are detected at the photooptic level by means of photochemical fluorochrome staining and examination under fluorescent light. Comparison of the fluorescent and polarization microscopic pictures showed that changes in cardiomyocytes are determined by local mass redistribution in the sarcomere compartments and transformations of birefraction of myofibrillar system components during necrobiosis. These changes are determined by lysis and coagulation processes in protein structures of sarcomeres.

Key Words: *acute myocardial disease; cardiomyocytes; myofibril lysis and contractures; fluorescent and polarization microscopy*

Morphological studies of the type and dynamics of changes in cardiomyocytes (CMC) in acute myocardial pathology showed that different patterns of primary damage to myofibrils [11] can form the basis for classification of acute CMC injuries and determine the outcome of cell injuries [4,5,9]. Polarization microscopy of contractile structures in CMC showed the dynamics of contractures, lysis, or combination of mosaic lysis and myofibril contracture foci in a cell [10,11].

The following stereotypical injuries of CMC are now distinguished: contractures (segmented and subsegmented), intracellular myocytolysis, cytolysis, and primary lumpy degradation [6]. These types of CMC lesions were studied at a photooptical level by polarization microscopy and by fluorescent microscopy [15] after photochemical fluorochrome staining (PFS) [7,8,12,13]. The contractile system of CMC can be evaluated due to the formation of photoproducts fluorescing in visible area after exposure of the preparation to short-wave UV.

We previously investigated morphological changes in the myocardium during predominance of contracture lesions of the myofibrillar system in altered CMC [8].

The aim of this study was fluorescent microscopic (after PFS) examination of CMC contractile structures in which myofibril lysis or combination of lysis and contractures develop in the same cell during acute ischemic and metabolic myocardial injuries and to compare the findings with the data of polarization microscopy.

MATERIALS AND METHODS

The myocardium of 206 experimental animals of different species (mice, rats, dogs) was examined after modeling of ischemic and metabolic injuries to the heart; specimens of human myocardium (103 cases)

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were obtained at autopsy after myocardial infarction, acute heart failure, and sudden coronary death.

Paraffin sections were stained with hematoxylin and eosin in combination of Pearls reaction, periodic acid-Schiff reaction with colloid iron staining after Hail and with hematoxylin orange.

Fluorescent microscopy under ML microscopes was carried out after exposure of the sections to short-wave UV. We developed this method previously [7, 12,13] and used it along with previously developed microscopic examination of the myocardium in polarized light [10]. Microscopy in polarized light was carried out under an Amplival Pol.u microscope. Interference analysis was carried out using Peraval Interphako microscope. The method was described in detail previously [7,8,12].

RESULTS

The development of lytic processes in contractile structures of damaged CMC (intracellular myocytolysis) presented as loci with weak fluorescence in normally fluorescing sarcoplasma (Fig. 1, *a*); the mosaicity of fluorescence corresponded to the image observed in polarized light (Fig. 1, *b*).

The morphological pictures of myocytolysis in fluorescent and polarized light are similar when foci of myofibrillar system lysis are relatively small. The substance density is low in these foci, and the lysate can be resorbed, while the cell is potentially viable.

In other cases comparison of the myocytolysis images in fluorescent and polarized light showed that the pictures of lytic destruction of myofibrils were different. There were CMC with optically empty foci (Fig. 1, *b*) filled with isotropic material in fluorescent light (Fig. 1, *a*). There were elongated CMC with pathologically compressed side (Fig. 2, *b*) passing into optically empty site bordering with polarization-intact site of the cell. The products of focal lysis partially filled this site as was seen in fluorescent light (Fig. 2, *a*); this material demonstrated no anisotropy.

Extension of CMC myocytolysis foci was paralleled by finely dispersed destruction of the myofibrillar system during transfer into the colliquative necrosis phase. The changes looked different in fluorescent and polarization light (Fig. 3). CMC with intense longitudinally cleaved myofibrils, low density between them and loss of substance from the one side of the cell were distinguished, optically active component of structures in the focus being completely destroyed.

One more variant was extensive myocytolysis of myofibrils with pronounced destruction of the sarcomere myofibrillar system, paralleled by dissemination of the contents in the entire volume and a slight decrease of the fluorescence intensity. Extensive areas of myocytolysis with incompletely lyzed anisotropic component of the sarcomere myofibrillar system were seen. The fluorescence intensity in this site was uneven. Small foci of more intense fluorescence corresponded to optically active material of developing colliquation necrosis of the CMC.

In case of generalization of myocytolysis, colliquation necrosis of the CMC was easier detected in fluorescent than in polarized light, because it possessed residual fluorescence.

Cytolysis can also be referred to stereotypical forms of CMC alterations by lytic destruction of contractile system [5,9]. It is interpreted as an autolytic destruction of intact myocyte under conditions of complete anoxia. CMC subjected to cytolysis are usually situated in the depth of the infarction focus and are surrounded by cells with developing primary lumpy degradation. Early stages of cytolysis formation are little studied. Cytolysis is sometimes regarded as a "relaxation" type of CMC death [14] resulting from early loss of capacity to contractions by ischemic cells.

The fluorescent picture of cytolysis is similar to its picture in polarized light. Strips of higher intensity, corresponding to A strips, were seen against the background of somewhat less intense fluorescence; the distances between these strips were greater than in an intact cell. Fluorescing cross strips of the myofibrillar system were seen in stretched fibers in fluorescent light (Fig. 2, *a*).

Primary lumpy degradation of CMC represents lytic destruction and contracture condensation of contractile structures and sarcoplasma in one cell. The morphological picture of primary lumpy degradation was virtually the same in fluorescent and polarized light, though the level of fluorescence and anisotropy was different (Fig. 4). Altered CMC were clearly distinguished by the fluorescence mosaicity. The lumps of condensed sites of contractile structures with high intensity of fluorescence were situated among damaged cells with fluorescence of different intensity (from low to "intact").

It was possible to differentiate the interface between the cells from spaces between the lumps due to low intensity of the interface on a fluorescent image of primary lumpy degradation; these signs were less demonstrative in polarized light (Fig. 4).

Primary lumpy degradation of a cell is a form of CMC alteration, significant for evaluating acute contractile insufficiency of the myocardium because of early disorders in the sarcolemma, irreversibly eventuating in coagulation necrosis.

Connective-tissue elements of the heart (myocardial stroma, vessels of all diameters, arterial adventition, fibrous rings, plasma, blood cells) could be differentiated in fluorescent light due to higher fluor-

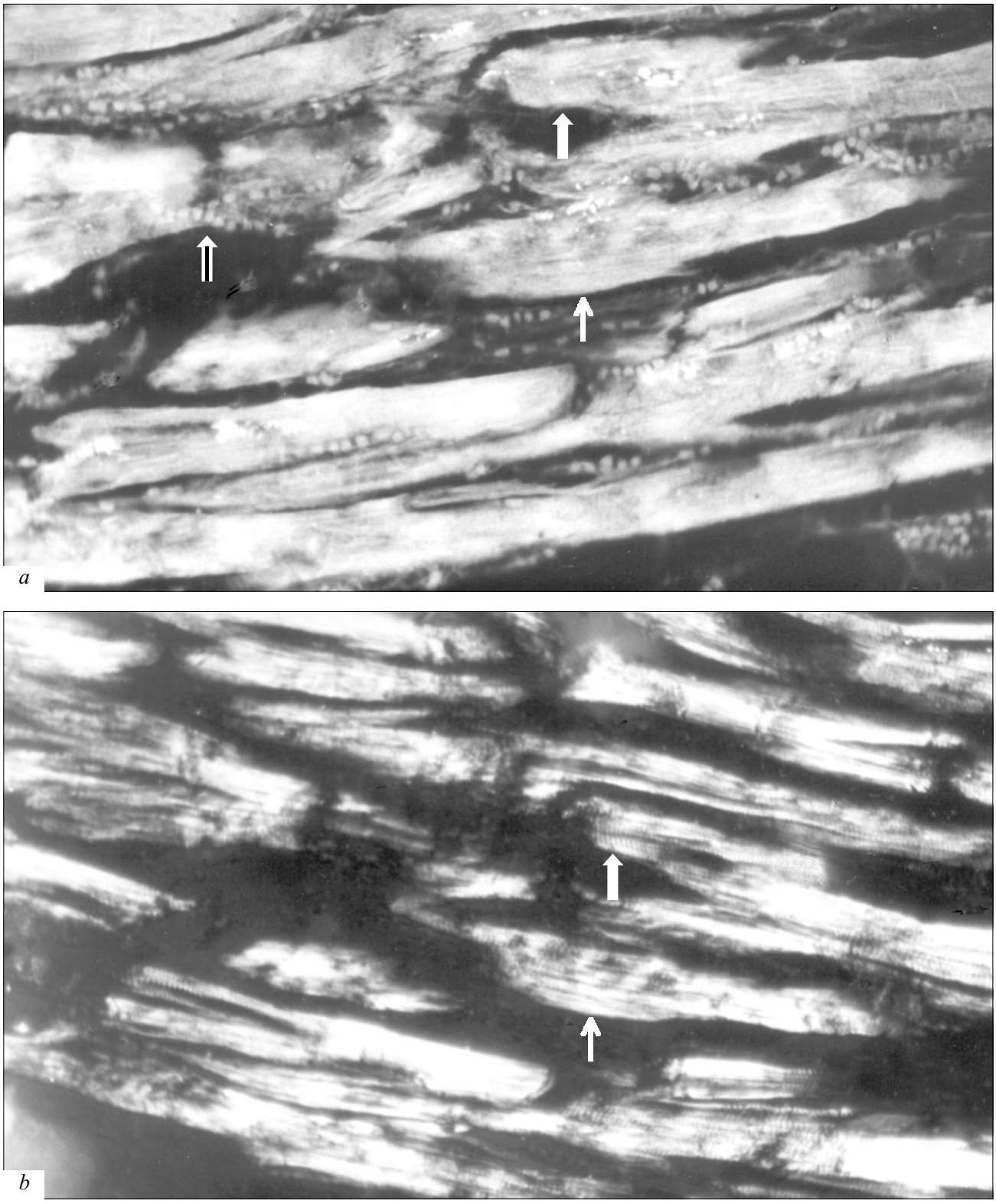

Fig. 1. Longitudinal section of human myocardium in fluorescent (*a*, ×450) and polarized (*b*, ×400) light. Lysis in cardiomyocyte in acute heart failure. Myocytolysis: loci with weak and mosaic fluorescence (thin arrows). Cell with optically empty focus in polarized light in fluorescent microscopy is filled with isotropic material (thick arrows). Blood plasma and cell elements outside vessels (double arrow).

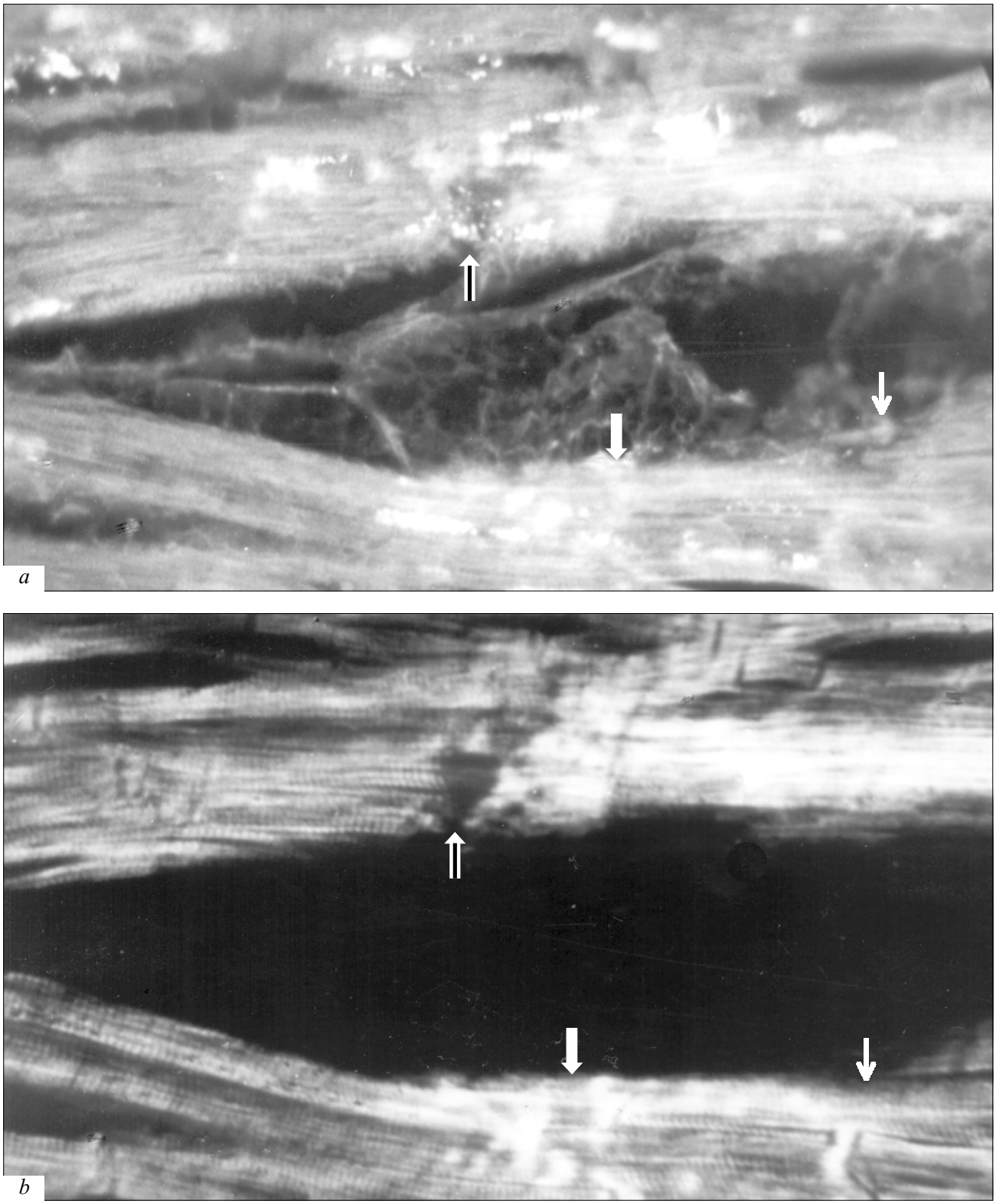

Fig. 2. Longitudinal section of human myocardium in fluorescent (*a*) and polarized (*b*) light, ×900. Contracture-lytic lesions of cardiomyocytes in small-focal myocardial infarction. Focal pathological contraction of myofibrillar system (thick arrows). High density of anisotropic substance at the interface between damaged and intact cell (thin arrows). Myocytolysis (site without anisotropy): high density of isotropic substance (double arrows).

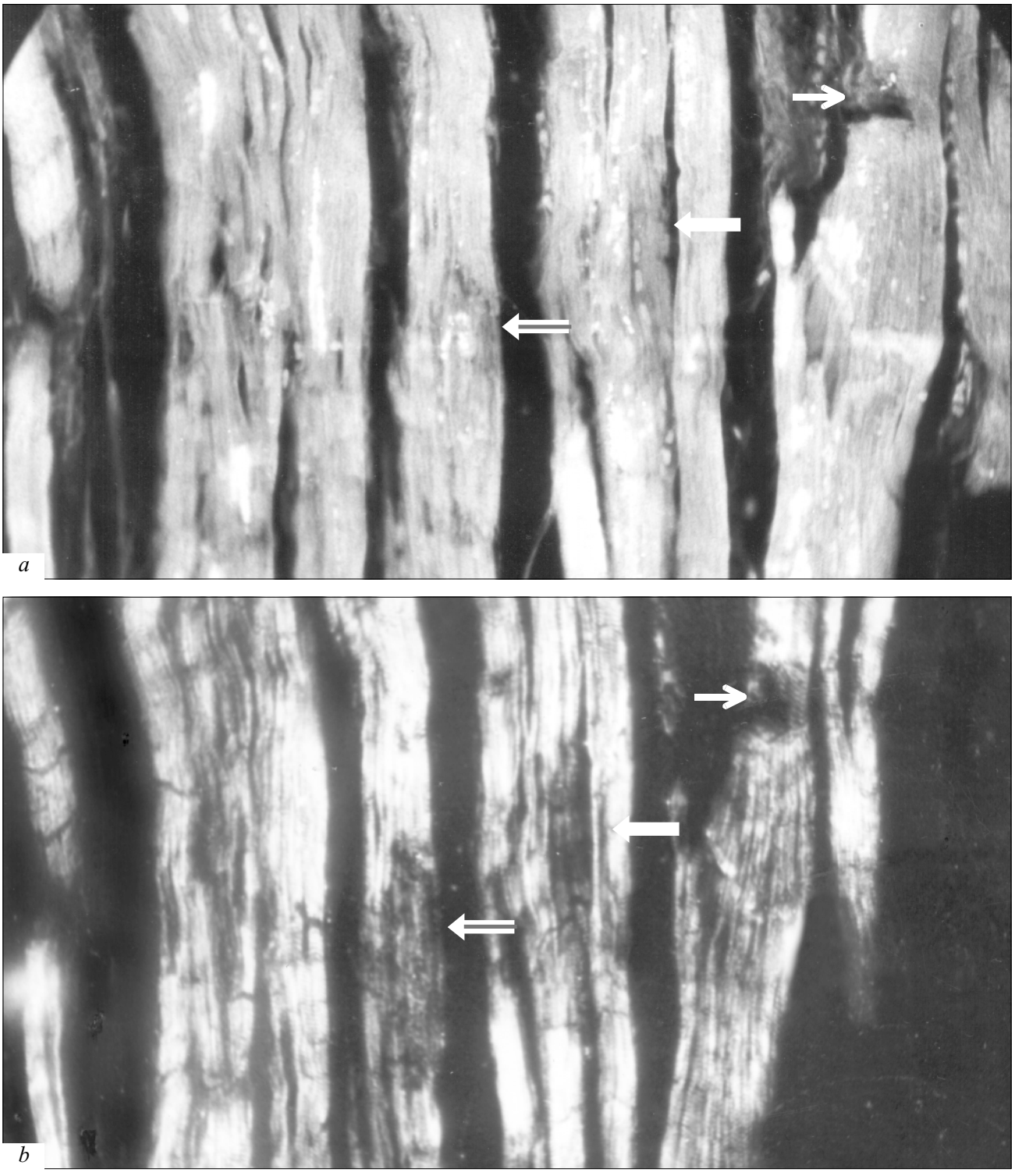

Fig. 3. Longitudinal section of human myocardium in fluorescent (*a*, ×450) and polarized (*b*, ×400) light. Lytic damage to cardiomyocytes in acute heart failure. Lysis and local decrease of the substance density in myofibrillar system with longitudinal cleavage of contractile structures (thin arrows). Myocytolysis focus: lysis and dispersed decrease of the myofibril substance density (thick arrows). Myocytolysis with incomplete myofibril lysis: uneven lysis of anisotropic component of myofibrillar system (double arrow).

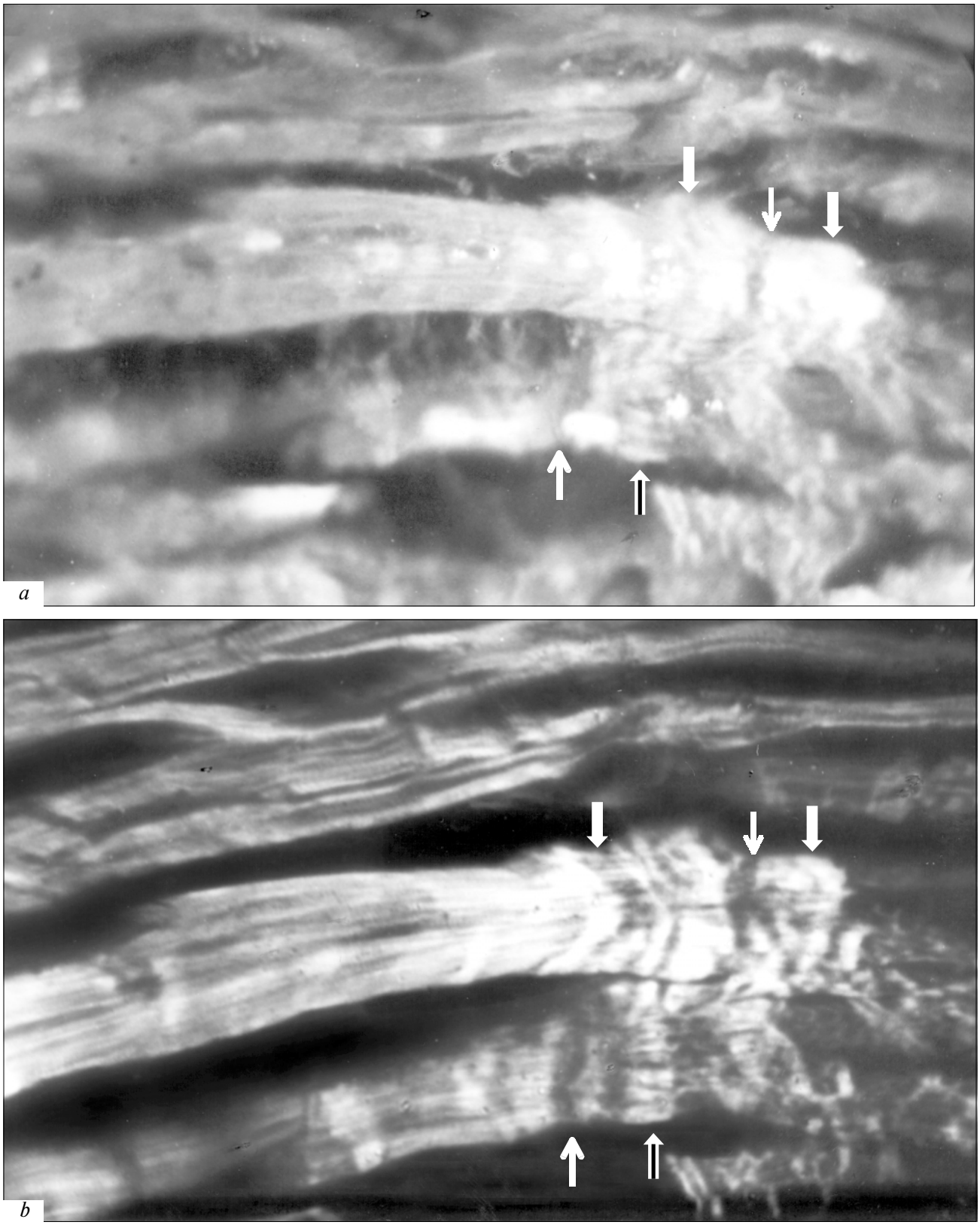

Fig. 4. Longitudinal section of human myocardium in fluorescent (*a*) and polarized (*b*) light, ×900. Contracture-lytic damage of cardiomyocytes in myocardial infarction. Primary lumpy degradation of myofibrils (thick and double arrows): different intensity of fluorescence of spaces between lumps in damaged cells, decreased intensity of fluorescence of cell-cell interface (thin arrows).

escence intensity after PFS (Fig. 1, *a*). Examination of myocardial stroma under polarized light required additional staining.

Hence, acute prenecrotic lesions of CMC of lytic (myocytolysis, cytolysis) and contracture-lytic (primary lumpy degradation of myofibrils) types during myocardial alteration of ischemic and metabolic origin can be detected at the photooptic level by PFS and examination in fluorescent light. Comparison of fluorescent and polarization microscopy findings showed that CMC changes were determined by local redistribution of the substance in the sarcomere compartments of CMC myofibrillar system.

The mechanism of lytic and contracture lesions of CMC deserves further investigation [2]. A relationship between their formation and development of electric instability was detected. On the other hand, the presence of autochtonic sarcomere irregular contractions in a myocyte, spontaneous contractions of sarcomeres outside the framework of electromechanical conjunction, and its potential reversibility into mechanoelectrical conjunction [1,3] essentially extend our notions on the formation of CMC injuries and their role in the development of acute contractile insufficiency of the myocardium.

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