METHODS

Quantitative Morphological Analysis of Acute Focal Damage and Myocardial Infarction

E. L. Lushnikova and L. M. Nepomnyashchikh

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Some approaches based on polarization microscopy and stereology are proposed for quantitative evaluation of acute dystrophic and necrotic lesions in the myocardium. It is shown that in sudden death and myocardial infarction the degree III contractures predominate in cardiomyocytes, while lumpy degradation of myofibrils under these conditions is half as often.

Key Words: *myocardial infarction; acute damage of cardiomyocytes; polarization microscopy; stereology*

Application of polarization microscopy for pathomorphological studies provides ways for diagnosing the prenecrotic alteration in cardiomyocytes (CM), i.e., for detection of early stages of cell damage [3,4]. High sensitivity of this method in detection of CM damage is due to the fact that disturbances in energy metabolism immediately affect the state of their contractile apparatus, myofibrils [6]. Myofibrils respond to various damaging factors (ischemic and metabolic) by a number of nonspecific structural changes [5,6].

Previously, using polarization microscopy for examination of experimental, postmorten, and biopsy myocardial samples, we revealed stepeotypic independent forms of acute damage to CM: contratures, intracellular myocytolysis, primary lumpy degradation, and cytolysis [2,4,8]. For scientific and practical purposes, quantitation of the above types of alteration is often important for determination of the primary type and the total damage to the myocardium [1].

Taking into account the segmentary nature of the above-mentioned types of cell pathology (i.e., the damage involves the whole cell), their quantitation can employ tissue stereological analysis.

Laboratory of Histocytometry and Stereology, Laboratory of Pathological General Pathological Anatomy, Institute of Regional Pathology and Pathological Morphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

MATERIALS AND METHODS

Quantitative morphological analysis of acute focal lesions was performed on myocardial samples obtained from patients suddenly died from acute cardiovascular failure (group 1, 30-55 years old) and myocardial infarction (group 2, 36-67 years old).

Myocardial samples from the left and right ventricles and intraventricular septum were routinely fixed in 10% neutral formalin and embedded in paraffin. Paraffin sections stained with hematoxylin and eosin were examined under usual and polarized light using a Docuval microscope (Carl Zeiss).

For tissue stereological analysis [2], the same random area were fotographed in usual and polarized light ×320. The relative volume (volume density) of CM with different types of damage and of stromal components was measured on photographs at a final magnification of 1300 using a transparent test grid consisting of short lines.

Since blood vessels and connective tissue cells cannot be clearly visualized in polarized light, these structures were analyzed by photographs made in

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usual light, after which the volume density of normal CM and CM with contractures, intracellular cytolysis, lumpy degradation of myofibrils was assessed.

Stereological analysis of focal myocardial lesions can be carried out immediately under a microscope equipped with a polarization device and test grid build into the ocular. This approach allows one to speed up the analysis by omitting photographic processes, but requires great concentration and, therefore, some human errors cannot be excluded.

The data were processed statistically [7] using Statgraphics software.

RESULTS

No macroscopic changes were found in myocardial samples from patients suddenly died from acute cardiovascular failure. Microscopic examination revealed marked venous and capillary plethora, pericapillary and interstitial edema, and in some samples focal fragmentation and segmentary degradation of muscle fibers.

Irregular staining of muscle fibers was noted in all myocardial layers. Many muscle fibers were eosinophilic and had no striation. The observed focal changes

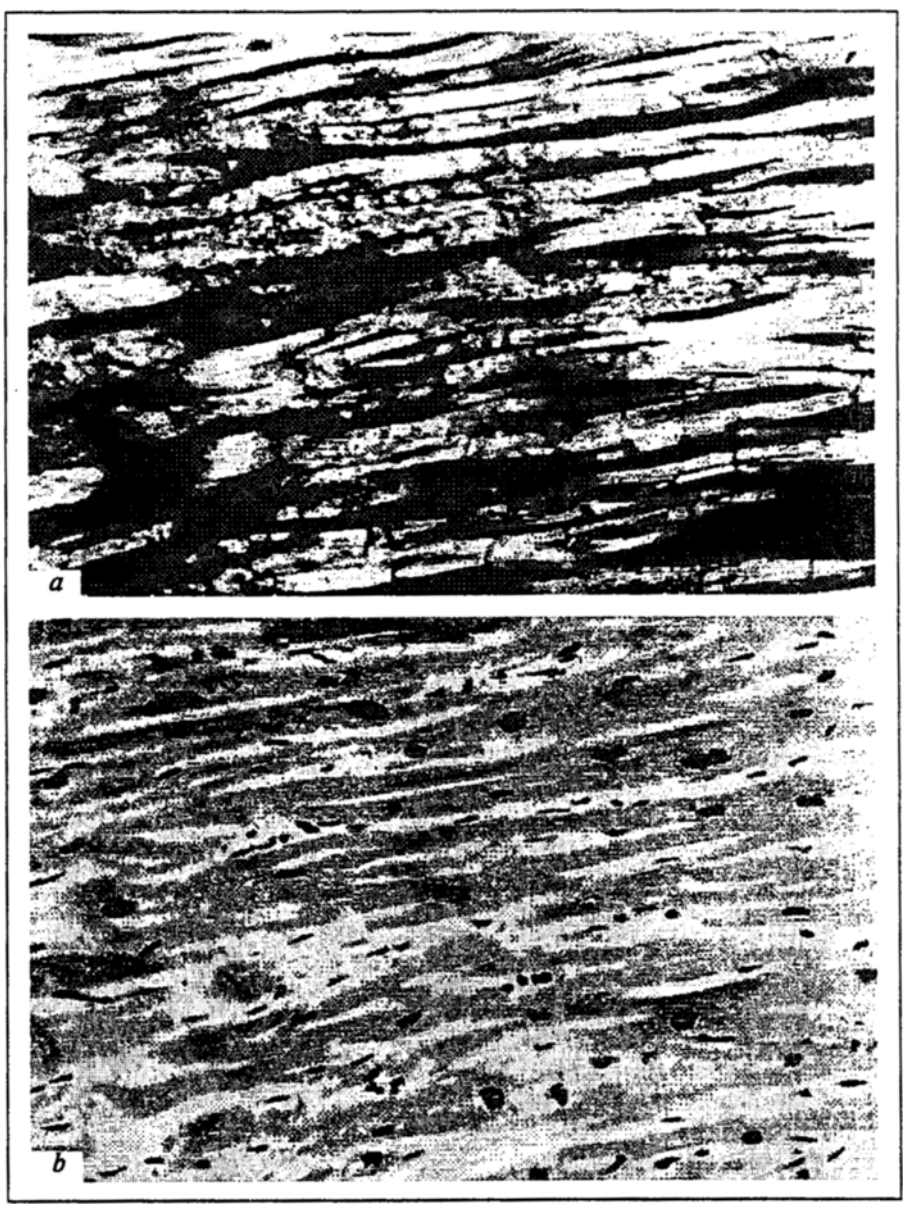

Fig. 1. Prenecrotic stage of subendocardial myocardial infarction. Patient B. 63 years old, sudden death. *a*) degradation of myofibrils in damaged muscle fibers into anisotropic lumps. Extension of intercalated disks in intact myocardium (weak fragmentation). Hematoxylin and eosin staining. Phase-contrast microscopy, ×125. *b*) the same fragment under light microscope, the majority of damaged fibers look like intact.

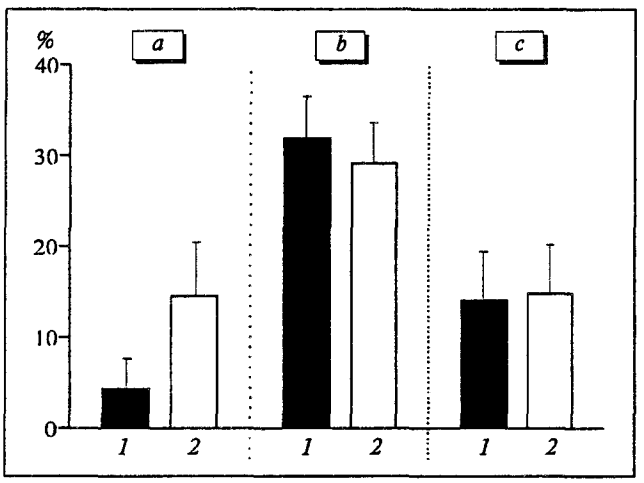

Fig. 2. Volume density (%) of cardiomyocytes with different lesions. *a*) intact cardiomyocytes; *b*) cardiomyocytes with degree III contractures; *c*) cardiomyocytes with lumpy degradation of myofibrils. 1) sudden death; 2) myocardial infartion.

were found in various regions of the myocardium, primarily in the left and right ventricles and interventricular septum.

Phase-contrast microscopy showed that anisotropic bands in damaged CM draw together so that cross-striation completely disappeares (degree III contracture), somewhere myofibrils degrade into anisotropic fragments (Fig. 1). In some CM anisotropic bands completely disappeared. These changes were found in all myocardial layers, being most pronounced in the subendocardial and subepicardial fibers of the left ventricle and interventricular septum.

In patients died from myocardial infarction, the location of necrotic zones was different, but anterior infarctions of the left ventricles were predominant. Microscopy of necrotic zone revealed coagulation necrosis and vascular disorders. Outside the infarction zone we observed dystrophic and necrobiotic alteration in CM. Phase-contrast microscopy of necrotic tissue revealed primarily large foci of CM with lumpy degradation of myofibrils. In prenecrotic CM, multiple large and small foci of lumpy degradation of myofibrils were also seen, moreover, in many CM degree III contractures were noted.

Tissue stereological analysis of focal myocardial lesions shows that degree III contractures were predominant type of acute lesions in CM of both patient groups. The volume density of CM with contractures in groups 1 and 2 was 31.9±7.0 and 29.1±4.4%, respectively (fig. 2). Lumpy degradarion of myofibrils was less abundant: volume density of CM with these changes constituted 14.0±5.6 and 14.8±4.9% respectively. Intracellular cytolysis in acute myocardial lesions was extremely rare (0.2-0.8%).

Normal muscle fibers were found in myocardial samples from both groups: the volume density of normal CM was 4.3% and 14.5% for groups 1 and 2, respectively. The higher number of normal CM in samples from patients with myocardial infarction is probably due to the fact that these samples were taken from outside the necrotic (infarction) zone. In group 1 necrotic changes in the myocardium cannot yet be detected by macro- and microscopic examination, and therefore, myocardial samples were randomly excised and were found to contain irreversibly altered fibers.

When evaluating general quantitative composition of the myocardium in focal acute damage, it should be noted that the total volume density of CM in groups 1 and 2 were relatively low and constituted 51.1 and 58.6%, respectively, the volume density of capillaries was 2.9%, connective tissue cells accounted for 3.5 and 3.9%, respectively, and the extracellular matrix constituted 42.5 and 34.5%, respectively. The high volume density of extracellular matrix is due to interstitial edema, which was more pronounced in the myocardium from patients suddenly died from acute cardiovascular failure. This results in a higher volume stroma/parenchyma ratio in group 1 compared with group 2 (0.957 and 0.705, respectively).

Thus, the combination of quantitative morphological analysis and phase-contrast microscopy allowed us to conclude that acute irreversible damage to CM (degree III contracture and lumpy degradation of myofibrils) is the immediate cause of death in patients of both groups, contractures being predominant type of alterations in acute cardiac pathology.

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