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ULTRASTRUCTURE OF THE PERI-INFARCTED ZONE OF THE MYOCARDIUM

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KEY WORDS: ischemia and infarction of the myocardium; peri-infarct zone; ultrastructure of cardiomyocytes; intracellular regeneration.

Particular attention is currently being paid to the peri-infarct zone (PIZ) of the myocardium, bordering on the infarct [3, 4, 6], as the point of application of various therapeutic procedures aimed at limiting the spread of necrosis of cardiomyocytes. The undisputed beneficial clinical effect of these procedures [10] calls for further structural and functional analysis of processes taking place in PIZ in the course of spontaneous experimental occlusive myocardial infarction and under the influence of drugs. However, the structural characteristics of PIZ have not yet been adequately studied and changes in the cardiomyocytes at the periphery of zones of necrosis of heart muscle and on the boundary with normal tissue are variously interpreted [6].

The aim of this investigation was to study the dynamics of ultrastructural changes taking place in PIZ of the myocardium in dogs after occlusion of the coronary artery.

EXPERIMENTAL METHOD

Occlusive myocardial infarction was induced in 26 mongrel dogs weighing 16-20 kg by ligation of the anterior descending branch of the left coronary artery in its middle third [2]. Material for morphologic investigation was taken 2, 4, 6, 8, and 12 h, 1, 2, 3, and 15 days, and 1 month after occlusion of the coronary artery. Samples of myocardium were excised from the center and periphery of the area of ischemic necrosis, from PIZ, and from areas of myocardium remote from the zone of infarction and were fixed in a 12% solution of neutral formalin. Sections cut from blocks of tissue embedded in paraffin wax were stained with hematoxylin–eosin and by the PAS reaction, with counterstaining of the muscular nuclei with hematoxylin, and embedded in polystyrene. The investigation was carried out in direct and polarized light by means of a Docuval universal light microscope (Carl Zeiss, East Germany). Specimens for electron-microscopic study were fixed in cold 4% paraformaldehyde in 0.1 M phosphate

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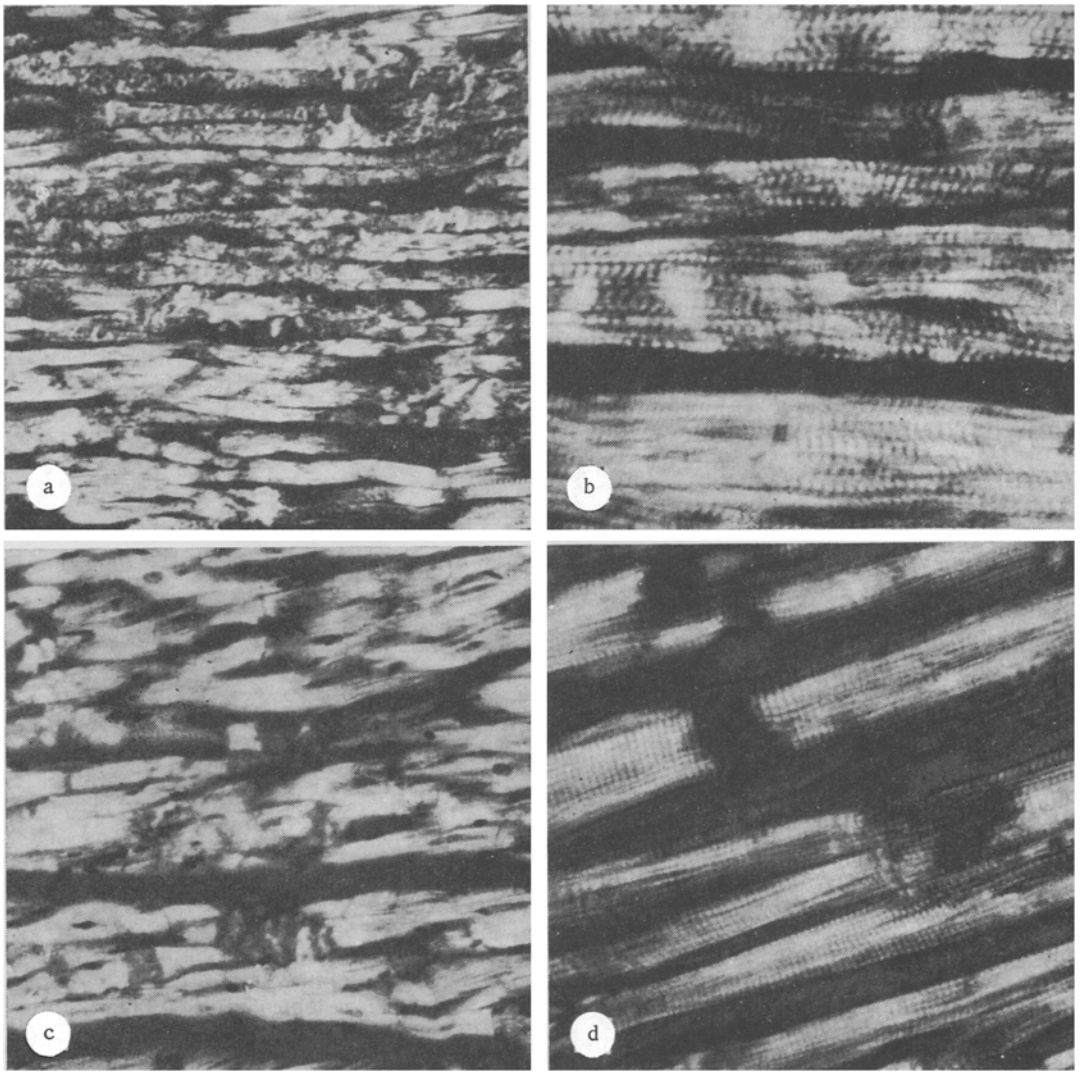

Fig. 1. Polarization microscopy of myocardial infarct following occlusion of anterior descending branch of left coronary artery in its middle third. a) Fragmentation of myofibrils in cardiomyocytes of peripheral zone of myocardial ischemia (320×); b) cytolysis of muscle fibers (uneven widening of isotropic disks of myofibrils) in central zone of myocardial infarct (1000×); c) contractural changes and fragmentation of myofibrils in single cardiomyocytes of PIZ of myocardium (800×); d) foci of intracellular myocytolysis of cardiomyocytes (disaggregation and lysis of myofibrils) in PIZ of myocardium (900×). a) 4 h, b, c, d) 6 h after coronary occlusion.

buffer, pH 8.0 [8] for 24 h. After postfixation with 1% osmium tetroxide solution in the same buffer, pH 7.2-7.4, the material was embedded in Epon-Araldite and, simultaneously, in an equimolar mixture of styrene and methacrylate. Ultrathin sections, cut on an LKB III Ultrotome (Sweden) were stained with uranyl acetate and lead citrate. The sections were examined in the IEM-100B electron microscope (Japan).

EXPERIMENTAL RESULTS

When choosing material for ultrastructural study of PIZ of the myocardium we were guided by the presence of lipid cytoplasmic inclusions, characteristic of this zone, in the cardiomyocytes [12, 15]. The method of polarization microscopy [3, 9], which is more sensitive to changes in the cardiomyocytes, also was used for the morphological study of PIZ.

In the early stages after acute coronary occlusion, multiple small and large foci of injury to the cardiomyocytes, characterized by fragmentation of the myofibrils (Fig. 1a), located at the periphery of the area of distribution of the ligated branch of the coronary

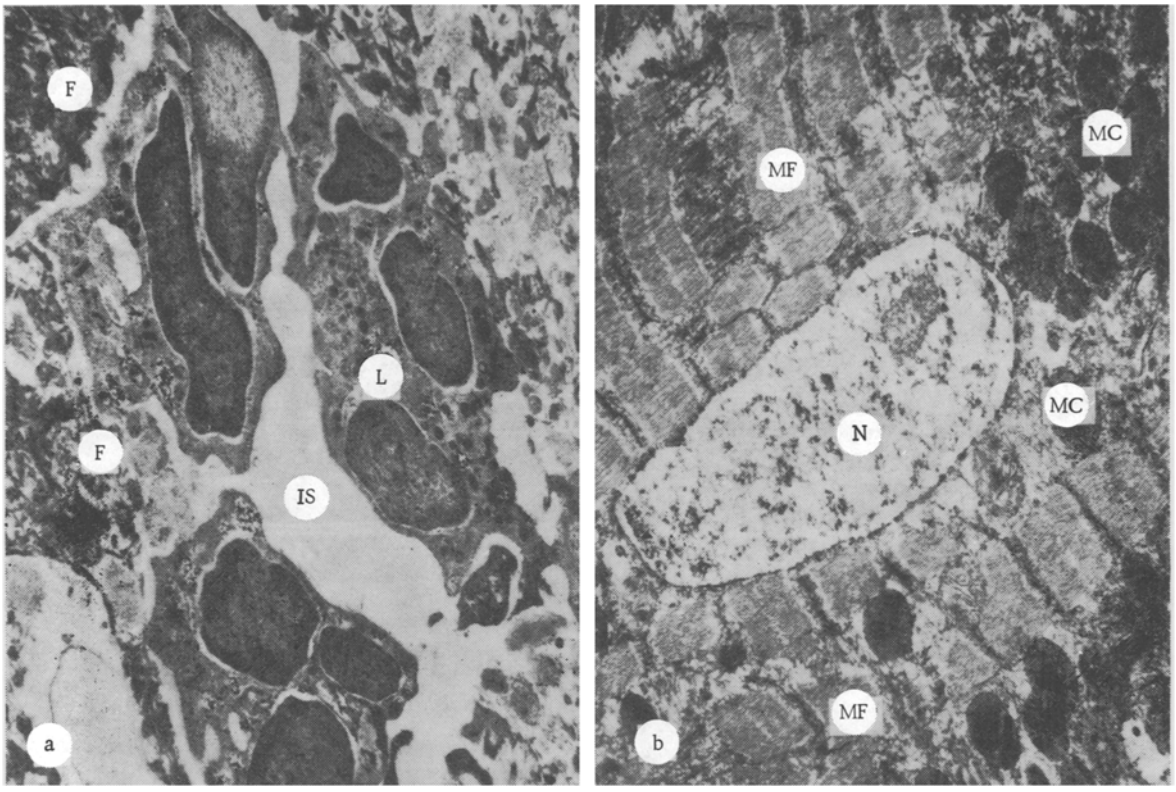

Fig. 2. Ultrastructure of central zone of myocardial infarct 24 h after coronary occlusion. 9200 ×. a) Escape of leukocytes (L) into intercellular space (IS) and deposition of fibrin (F) in necrotic cardiomyocytes; b) cardiomyocyte in a state of cytolysis (intravital autolysis). Autolytic changes in nucleus (N) and cytoplasmic organelles. MF) Myofibrils, MC) mitochondria.

artery, were detected in the myocardium in polarized light. A focal pattern of myocardial damage also was characteristic of the first 2-4 h of interference with the coronary circulation, during which time PIZ could not be identified. Starting with 4 h, foci of fragmentation in the zone of myocardial infarction grew larger, merged with one another, and formed a peripheral zone of ischemia. Some cells with fragmentation of the myofibrils became saturated with plasma proteins and began to give an amylase-resistant positive PAS reaction.

Around the periphery of the zone of ischemia 4-6 h after the beginning of disturbance of the coronary blood flow, muscle fibers undergoing cytolysis were observed in polarized light (Fig. 1b): signs of "overstretching" of the myofibrils were found in them: Dark isotropic disks became wider; the height of the isotropic disks became equal to or exceeded that of the anisotropic disks.

Electron microscopy 6 h after occlusion of the coronary artery revealed marked degenerative changes in the capillary endotheliocytes in the central and peripheral zones of the myocardial infarct. In the central zone of the infarct single erythrocytes were seen in the capillary lumen, the capillary endothelium was flattened, its nuclei were pycnotic and its cytoplasm was dense and homogeneous. In the peripheral zone of necrosis disturbances of the microvessels were more severe. The lumen of some capillaries was blocked by microthrombi consisting of aggregated platelets, in other capillaries intercellular junctions between degenerating endotheliocytes were destroyed, and as a result, blood cells and concentrations of polymerized fibrin were found in the intercellular spaces (Fig. 2a). Cardiomyocytes in the central zone of the myocardial infarct were in a state of cytolysis or intravital autolysis [9] and no lipid inclusions could be found in them (Fig. 2b).

After 6-8 h the zone of myocardial infarction began to be visible under the light microscope (eosinophilia and floccularity of the sarcoplasm, necrobiotic changes in the nuclei of the damaged cardiomyocytes, stasis in the capillaries, and escape of blood cells from the capillaries). Fragmentation of the myofibrils, observed in the cardiomyocytes under the polarization microscope was characteristic of the peripheral zone of the myocardial infarct.

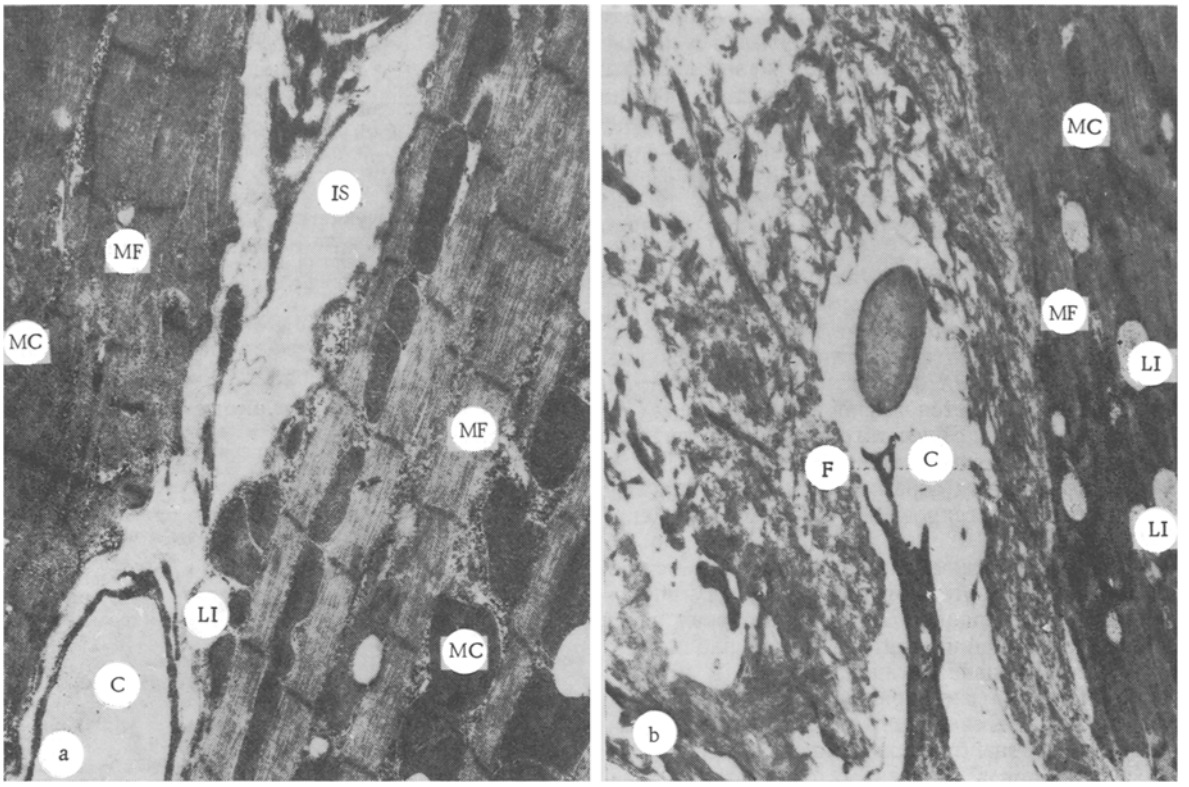

Fig. 3. Ultrastructure of PIZ of ischemic myocardial necrosis. 9200 ×. a) Structure of capillaries (C) and cardiomyocytes unchanged. Large pale vacuoles in sarcoplasm corresponding to lipid inclusions (LI); MF) myofibrils, MC) mitochondria; b) deposits of fibrin (F) in intercellular space.

Irregular alternation of cardiomyocytes, undergoing coagulation necrosis, and of cardiomyocytes with varied degrees of cytolysis was observed in the peripheral zone of ischemic myocardial necrosis on electron microscopy after 8 h. Coagulation necrosis of the cardiomyocytes was manifested as pycnosis of the nuclei, focal destruction and contracture of the fragments of myofibrils, and intracellular fibrin deposition. On the whole the morphologic changes in the peripheral zone of ischemia were evidence of progressive spread of necrosis on account of hypoxia, due to occlusion of the coronary artery, and focal anoxia due to microthrombosis of the capillaries at the periphery of the zone of ischemia. Lipid inclusions in the damaged cardiomyocytes of the peripheral zone of necrosis were not found at this period of observation. The structure of the capillaries and of most cardiomyocytes in PIZ of the myocardium was unchanged. Absence of glycogen and the appearance of lipid droplets in the cytoplasm of the cardiomyocytes was noted (Fig. 3a). In polarized light, contractural changes and foci of intracellular myocytolysis of individual cardiomyocytes were observed in PIZ (Fig. 1c, d). At later times of observation autolytic changes in the muscle cells progressed in the central zone of the myocardial infarct and in the peripheral zone of necrosis, processes of resorption of necrotic tissues by macrophages and leukocytes became clearly apparent. Examination under the ordinary microscope after 12-24 h revealed a zone of cytolysis, identified by the reduced staining intensity of the muscular nuclei or by their total lysis. After 24-48 h of myocardial infarction examination in polarized light revealed muscle fibers in zones of infiltration by macrophages and leukocytes that were in a state of fragmentation with evidence of cytolysis, similar to those found in the early stages of myocardial ischemia. In PIZ and in areas of myocardium remote from the infarct, small foci of myocytolysis, contractures, and fragmentation of myofibrils were observed inconstantly. The distinguishing feature of the peripheral zone of ischemic necrosis after 1-3 days was a higher frequency of discovery of necrotic cardiomyocytes with abundant lipid inclusions in their cytoplasm with the passage of time. Capillaries accompanying these cardiomyocytes were either thrombosed or destroyed; abundant concentrations of fibrin could be seen in the intercellular spaces (Fig. 3b).

The cause of the increase in volume of necrotic myocardial tissue after occlusion of the coronary artery is progressive microthrombosis, leading to disturbance of the blood

supply and necrosis of cardiomyocytes of PIZ, infiltrated with lipids. According to observations by some workers [13, 14] the zone of lipid infiltration is formed toward the end of the first day in the myocardium outside the limits of the zone of ischemia due to ligation of the coronary artery. It is assumed that under optimal conditions ischemic necrosis spreads only within the region of disappearance of glycogen from the sarcoplasm of the cardiomyocytes, and the zone of lipid infiltration remains unchanged in volume during the first 3 days, when it accounts for about 10% of the myocardium of the left ventricle. Only if intervention of pharmacologic, metabolic, and hemodynamic factors is unfavorable do cardiomyocytes infiltrated with lipids undergo necrosis [11].

Our own observations do not confirm this hypothesis. Constancy of extent of the zone of lipid infiltration is evidently due to the character of specific metabolic disturbances in the myocardium, leading to tissue necrosis, and not to the fact that it is not involved in the process of necrosis. Infiltration of viable cardiomyocytes by exogenous lipids has repeatedly been observed in the myocardium in focal metabolic necrosis [1, 5]. As a result, despite the constant width of the zone of lipid infiltration the volume of necrotic myocardium continues to increase for at least 3 days.

It follows from our data that PIZ could more correctly be considered as a zone of lipid infiltration of cardiomyocytes and not the zone of disappearance of glycogen and of a modest reduction of enzyme activity adjacent to it from inside, as a group of American workers has postulated [11]. Moreover, as our own investigations have shown, the zone of lipid infiltration is not a static formation, but moves in the course of progression of ischemic myocardial necrosis.

Repair processes in PIZ of the myocardium are purely of the intracellular regeneration type [7]. In the early period, destruction and lysis of organelles together with active intracellular regeneration are observed in cardiomyocytes of PIZ. A particular feature of intracellular regeneration of the cardiomyocytes of PIZ (compared with their regeneration in areas of myocardium remote from the infarct) is that its course is the same as in plastic cardiac insufficiency [3], often with the formation of a small number of irregularly arranged myofibrils in the sarcoplasm. Muscle cells undergoing atrophy as a result of focal degradation of the sarcoplasm, intracellular necrosis, and lysis of some organelles, are observed in PIZ 15 days after coronary occlusion. Signs of intracellular regeneration (an increased number of ribosomes, signs of formation of new myofilaments, increased activity of the nucleolus) are found in some of these cells. Together with atrophic cardiomyocytes, hypertrophic muscle cells can be seen, with both normal orientation of their myofibrils and also with intracellular foci of transverse orientation of the myofibrils. In the zone surrounding the scar (the former PIZ), 30 days after occlusion, heterogeneity of the cardiomyocytes is found on account of normal, atrophied and hypertrophied cells.

On the basis of the results of this investigation and data in the literature, it can be concluded that there are good prospects for methods of treatment directed toward metabolism and intracellular regeneration of the cardiomyocytes of PIZ, for, being viable, these cells are in a state of increased functional loading, leading to dystrophy, followed by atrophy, pathologic intracellular regeneration, and hypertrophy, with irregular orientation of the myofibrils.

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