POLARIZATION AND ELECTRON-MICROSCOPIC INVESTIGATION OF INTRACELLULAR REGENERATION OF THE MYOCARDIUM AFTER MYOCYTOLYSIS

L. A. Semenova, N. K. Eriskovskaya, and Yu. G. Tsellarius

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Intracellular myocytolysis in the rat heart was induced by injection of adrenalin or novodrin (isoprenaline). During the first hour, foci of destruction of the sarcoplasmic reticulum and myofibrils appeared in the damaged muscle cells. In subsequent hours, intracellular regeneration was observed: polysomes appeared in the foci, new tubules of the sarcoplasmic reticulum and new myofilaments were formed. The process of myocytolysis is unconnected with destruction or formation of lysosomes. The sarcolemma, mitochondria, and nucleus are essentially unchanged in reversible forms of myocytolysis.

Intracellular myocytolysis is a type of acute metabolic injury to the muscle cells of the heart characterized by focal lysis of myofibrils, and readily discovered by examination in polarized light. When the action of the injuring agent is relatively short in duration, the normal structure of cells undergoing myocytolysis can be restored after 1-2 days [5-7].

Electron-microscopic changes in muscle cells of the heart during the first minutes of development of myocytolysis were described in a previous paper [4]. A description of the further changes in the muscle cells during this type of injury is given below.

EXPERIMENTAL METHOD

Parallel polarization- and electron-microscopic studies were made of the papillary muscles of the left ventricle of 12 albino rats weighing 120-180 g between 20 min and 24 h after subcutaneous injection of adrenalin (8 mg/kg) or of novodrin (isoprenaline) (80 mg/kg). The material was fixed in the cold with osmium fixative [9] and embedded in a mixture of methacrylates. Sections, 1 μ in thickness, were examined in polarized light, while ultrathin sections in the parallel series were counterstained with lead [10] and examined in the Tesla BS-513 electron microscope. Full details of the method were given previously [4].

EXPERIMENTAL RESULTS

As the previous investigation showed [4], during the first minutes destruction of the sarcoplasmic reticulum and myofibrils took place in the zone of myocytolysis, starting at the level of the Z-bands, but no significant changes were observed in the nucleus, sarcolemma, or mitochondria.

After 1-3 days, the intracellular myocytolysis reached its maximum development through progression of the destructive changes. At this stage the foci of myocytolysis, when examined in polarized light, appeared optically empty and, as the previous study showed, fixation of the dyes was reduced in the foci and the myosin in the foci of injury could no longer be detected by an immunoluminescence method [6].

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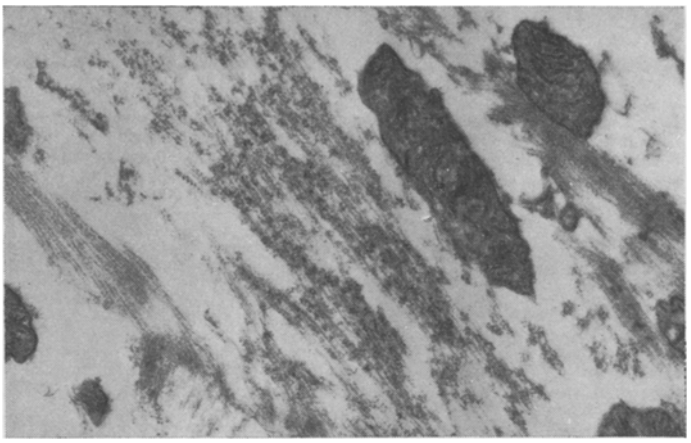

Fig. 1. Area of papillary muscle of a rat (24 h after injection of adrenalin): central zone of focus of myocytolysis; thin bundles of newly formed myofilaments in center between chains of ribosomes (26,000×).

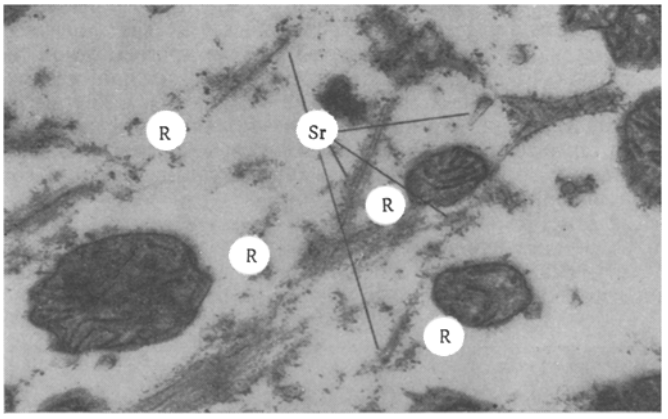

Fig. 2. Another part of the same specimen: central zone of focus of myocytolysis; newly formed tubules of sarcoplasmic reticulum (Sr), surrounded by ribosomes (R) visible in the translucent cytoplasm; mitochondria are circular in shape, and matrix of mitochondria is translucent where cristae have been destroyed (45,000×).

Examination in the electron-microscope showed that at the height of development of myocytolysis the myofibrils in the most severely damaged areas of the muscle fibers were completely destroyed, and circular mitochondria of different sizes were scattered among the translucent and structureless sarcoplasm. The matrix of some mitochondria was condensed, and foci of translucency could be seen in others where some of the cristae had disappeared. At the periphery of a focus of myocytolysis fragments of sarcoplasmic reticulum were visible, with ribosomes lying between the free ends of the remaining myofibrils.

Even after one short exposure to the injurious agent, the foci of myocytolysis did not develop in a strictly synchronized manner, and between 1 and 24 h later foci at different stages of the process could be seen simultaneously in the myocardium.

In the optical microscope, restoration of the damaged muscle cells appeared more than 12 h after injury. In polarized light, the foci grew shorter and birefringent filaments appeared, intersecting the focus longitudinally. In the ordinary histological investigation the foci of intracellular myocytolysis were practically invisible at this stage, because the injured parts fixed the dyes just as strongly as normal fibers.

Electron-microscopic investigation revealed signs of commencing intracellular regeneration during the first few hours after injury. In the central part of the foci of myocytolysis, groups of ribosomes were

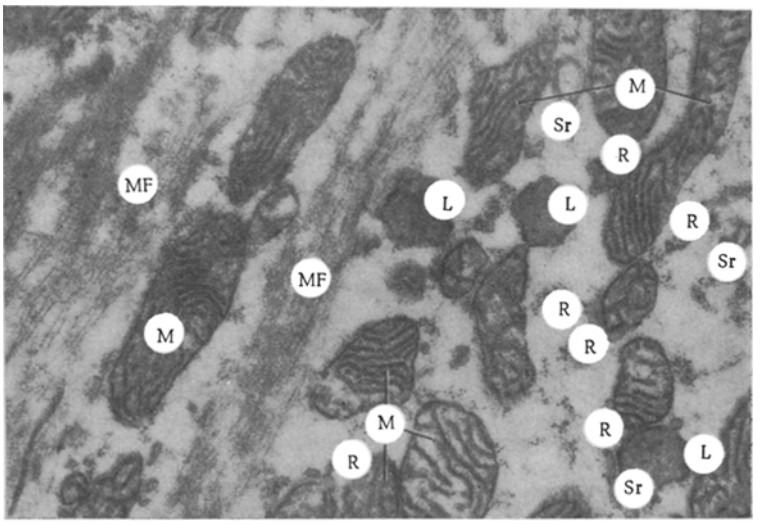

Fig. 3. Periphery of a focus of myocytolysis: newly formed myofibrils, not separated into sarcomeres (MF), and with oval mitochondria between them, can be seen. Cells in center and on right side have no myofibrils; numerous groups of ribosomes (R) and tubules of sarcoplasmic reticulum (Sr) lie in the sarcoplasm between mitochondria (M) and lipid droplets (L) (45,000×).

seen in the spaces between the mitochondria, and they increased in number toward the periphery of the injured focus. Sometimes the ribosomes were arranged in chains (polysomes), with thin bundles of fibrils, evidently myofilaments, alongside them (Fig. 1). This assumption is supported by the fact that at this stage, unoriented myosin began to be detectable in the foci of myocytolysis by the immunoluminescence method [6]. Besides the formation of myofilaments, tubules of sarcoplasmic reticulum also began to appear (Fig. 2). The newly formed bundles of myofilaments at first lay haphazardly, but later, starting at the periphery of the focus, they became longitudinal in direction, and when examined in polarized light they appeared as anisotropic fibrils without cross striation. The mitochondria between the bundles of these myofilaments became elongated in shape. Later, long myofibrils in which Z-bands were only just beginning to appear, but in which alternation of the sarcomeres could be deduced from the packing density of the myofilaments, began to be formed (Fig. 3).

In its general features, the process of intracellular regeneration of heart muscle fibers after myocytolysis resembles the formation of myofibrils of striated muscle during embryogenesis [8]. Similar pictures are given in Glagoleva and Chechulin's atlas [2], where they are identified as myoblasts formed several days afer experimental myocardial infarction. The parallel investigation in light (polarized) and electron microscopes demonstrated clearly that this process can take place in mature muscle fibers of the heart during the first few days after intracellular myocytolysis.

It is important to note that intracellular myocytolysis, which judging from all its features is a process of intravital partial autolysis of intracellular structures, usually begins at the periphery of the cell, where as a rule there are no lysosomes, and in the course of the pathological process no lysosomes are formed in foci of myocytolysis. Since in other pathological situations not accompanied by such widespread processes of autolysis the number of lysosomes in the muscle cells of the heart is known to increase [1], it may be postulated that the formation of new lysosomes is more likely to protect the cell against autolysis than to promote it.

At all stages of intracellular myocytolysis the sarcolemma was completely intact and no signs of permeation of plasma were found. A feature distinguishing reversible intracellular myocytolysis from other types of damage to the myocardium is the comparatively mild injury to the mitochondria, the changes in which did not go beyond those observed in the normally functioning myocardium [3]. The essential condition for reversibility of intracellular myocytolysis is injury to only parts of the cell and integrity of the nucleus. If the nucleus is destroyed the process becomes irreversible and is transformed into primary colliquative necrosis [5].

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