surface of type 2 alveolocytes. It can accordingly be supposed that activation of the basal type of secretion of alveolar surfactant in the lung of the hypothermic animal is based on a similar mechanism, for the necessary conditions are present for its realization: the presence of active synthesis of phospholipids in type 2 alveolocytes and partial limitation of the secretion of these phospholipids from the apical surface of the cells. Liberation of phospholipids from the interstices evidently took place through the lymphatic system of the lung and the general lymphatic circulation.

Changes in the lung in hypothermia are thus characterized by a local disturbance of integrity of the air–blood barrier, disintegration, aggregation, and lysis of membranes of the alveolar surfactant, activation of synthesis and secretion of surfactant by type 2 alveolocytes, and the accumulation of surface-active material in the interstices of the alveolar septa, which in the writers' opinion, promotes destruction of their collagen and elastic fibers and also an increase in functional activity of the alveolar macrophages, accompanied by destruction of some cells.

Activation of synthesis and secretion of alveolar surfactant, and intensive utilization of disintegrated surfactant by alveolar macrophages and by type 1 alveolocytes are an expression of compensatory and adaptive reactions against the background of disturbance of ventilation, the hemodynamics, and also of lipid metabolism in the lungs, due to acute general hypothermia.

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ULTRASTRUCTURAL MANIFESTATIONS OF RUBOMYCIN-INDUCED ABNORMAL SYNTHESIS OF CONTRACTILE PROTEINS BY RAT CARDIOMYOCYTES

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Toxic injury to the myocardium by antibiotics of the anthracycline series disturbs the synthesis of specific contractile proteins in cardiomyocytes and leads to the development of heart failure [11, 12]. Administration of these antibiotics, especially rubomycin, to animals can be used as an experimental model of chronic cardiac pathology [7, 11, 13].

The present investigation was carried out with the aim of studying the time course of ultrastructural changes arising in the cardiomyocytes of albino rats in response to administration of rubomycin.

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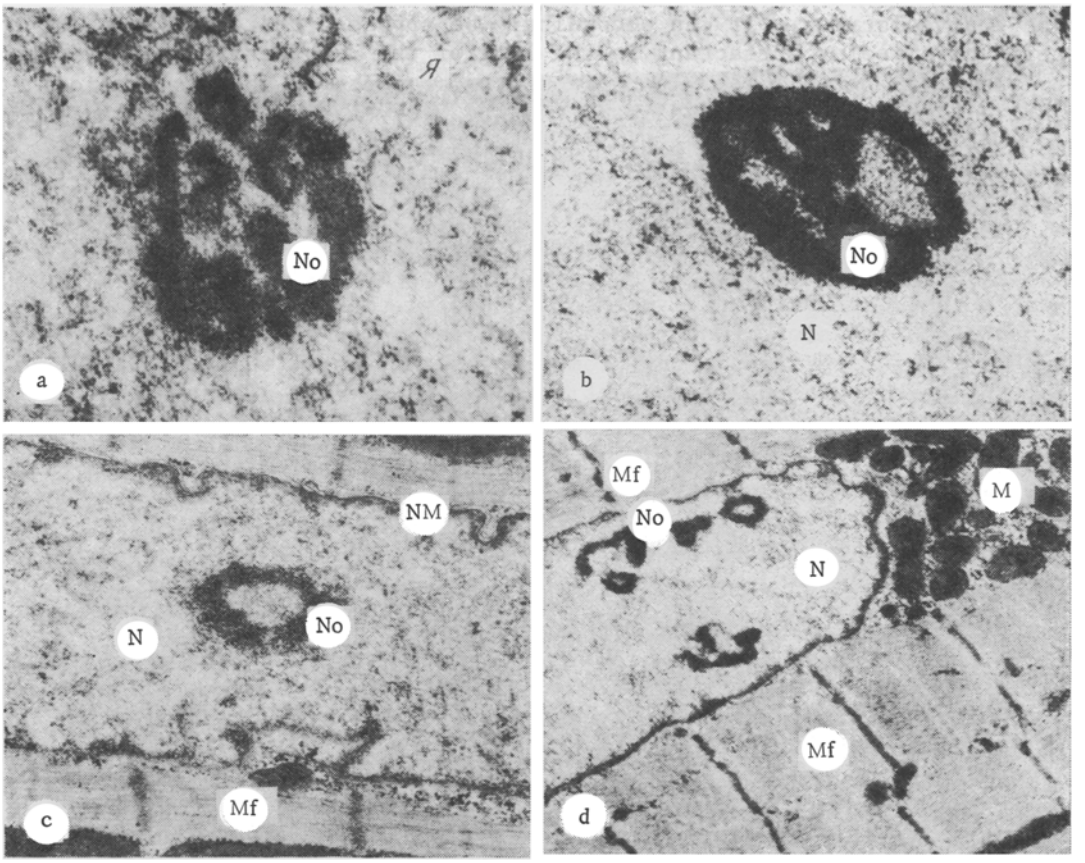

Fig. 1. Changes in nucleoli and nuclei of cardiomyocytes of albino rats receiving rubomycin. a) Nucleolus (No) in nucleus (N) of intact cardiomyocyte. Looped nucleonema is mainly granular in structure. 36,000 ×; b) segregation of nucleolus 3 h after injection of rubomycin. 36,000 ×; c) annular form of segregation of nucleolus. Nuclear membrane (NM) forms folds 3 days after repeated injection of rubomycin. 18,000 ×; d) fragmentation of cardiomyocyte nucleolus 2 days after single injection of rubomycin. 12,000 ×. b, d) Series I; c) series II of experiments. Mf) Myofibrils, M) mitochondria.

EXPERIMENTAL METHOD

Experiments were carried out on 67 male Wistar albino rats weighing 180-220 g. A solution of rubomycin hydrochloride was injected into 33 rats in the experiments of series I intraperitoneally as a single dose of 30 mg/kg. The animals were killed 1-3, 6, 12, 18, and 24 h and 2-4 and 5 days after injection of the antibiotic. In the experiments of series II rubomycin was injected intraperitoneally into 12 rats in a dose of 5 mg/kg once a week. The animals were killed on the 3rd day after having received a total dose of 20, 25, and 30 mg/kg (after 24, 31, and 38 days respectively). Control animals (22 rats) received physiological saline in a similar volume to the experimental animals and were killed 1, 3, 12, and 24 h and 2-5, 23, 31, and 38 days after the injection. The rats were decapitated under chloroform anesthesia. The heart was removed from the thorax, its contractions were stopped by cold, after which the material was fixed with cold 4% solution of paraformaldehyde in 0.1 M phosphate buffer, pH 8.0, and postfixed with 1% osmic acid in the same buffer at pH 7.2-7.4 [5]. Samples of tissue fron the papillary muscle of the left ventricle were embedded in a mixture of styrene and butyl methacrylate [15]. Ultrathin sections were stained with uranyl and lead. The sections were examined in the Tesla BS-500 electron microscope with an accelerating voltage of 60 kV.

EXPERIMENTAL RESULTS

Injury to the myocardium and pathomorphological changes associated with stasis in the systemic circulation developed in the experimental rats of series I on the 3rd-5th day and in the rats of series II by the 3rd day after receiving a total dose of rubomycin of

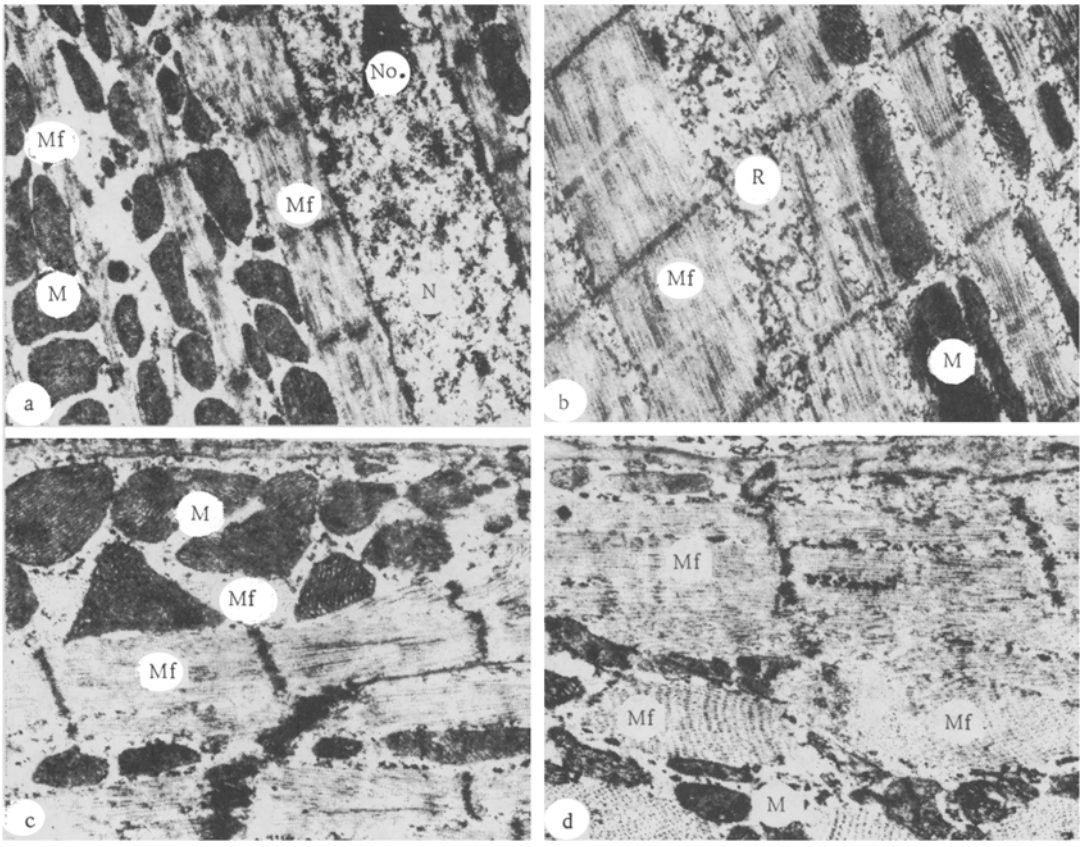

Fig. 2. Changes in myofibrils in cardiomyocytes of albino rats under the influence of rubomycin. a) Thinning and lysis of myofibrils, collapse of nucleolus in injured cardiomyocyte. No glycogen or ribosomes present in denuded cytoplasmic matrix. 12,000 ×; b) thinning and lysis of myofibrils, numerous free ribosomes (R) in cytoplasm 5 days after injection of rubomycin. 24,000 ×; c) distribution of myofibrils in subsarcolemmal zone of cardiomyocyte at right angles to long axis of cell. 24,000 ×; d) disturbance of orientation of myofibrils in cardiomyocyte. 30,000 ×; b) Series I; a, c, d) series II of experiments.

25-30 mg/kg. At the time of decapitation the animals were in an extremely severe terminal state. The character and time course of the structural changes in the cardiomyocytes of the rats of the two series of experiments were similar, since the cardiotoxic action of rubomycin is dose-dependent [7, 11].

The first signs of injury appeared in the nucleoli of the cardiomyocyte nuclei. The time course of this process was studied for 24 h after a single injection of the maximal dose of rubomycin. Starting from the 2nd hour, segregation of the granular and fibrillary components of the nucleonema appeared in single cardiomyocytes or in groups of them (Fig. 1b), whereas the nucleoli in the adjacent cardiomyocytes preserved their normal appearance (Fig. 1a). After 6 h some of the nucleoli became fragmented, others condensed and collapsed. The dense nucleoli consisted mainly of the fibrillary component of the nucleonema. The changes described in the nucleoli were constantly found in the cardiomyocytes until the end of the experiment (Fig. 1c, d).

Segregation, fragmentation, and collapse of the nucleoli are the morphological reflection of reduction or cessation of ribosome production in the cell [2, 6, 14]. These changes have not previously been described in the model used in these experiments.

Changes in the cytoplasmic components became visible 3 h after a single injection of the maximal dose of rubomycin. They consisted of sequestration of collections of α-glycogen, the formation of myelin-like structures around areas of the cytoplasm with cytoplasmic granules and elements of the smooth endoplasmic reticulum, and the appearance of secondary lysosomes. The changes increased progressively, so that by the end of the 1st day glycogen could no longer be found in the α-form in the cardiomyocytes, the number of secondary lyso-

somes was increased, and isolation of sequestered areas of cytoplasm in the intercellular space could be observed until the end of the experiment.

The disappearance of glycogen and reduction of the cytoplasmic elements combined with changes in the nucleoli can be interpreted as a manifestation of reduced synthetic activity of the cell [2, 6, 9], which evidently occurs also in the cardiomyocytes of rats under the influence of rubomycin.

Thinning of the myofibrils and their focal lysis developed between 3 and 18 h after administration of rubomycin and progressed during the first 48 h. Lytic changes in the myofibrils, as shown in other investigations [2, 4, 6, 10], are the most demonstrative features of a disturbance of contractile protein synthesis in cardiomyocytes. By the end of the 1st day, granules corresponding to ribosomes were almost completely absent in the cytoplasm between the thin myofibrils (Fig. 2a), but on subsequent days ribosomes reappeared in the cells (Fig. 2b). Nucleoli in these cardiomyocytes as a rule had a near-normal structure.

The number of ribosomes was visually increased in the cardiomyocytes of the rats of series I, killed after 24 h-5 days, and in rats of series II on the 31st-38th day. At these times irregularly oriented myofibrils could be found in the subsarcolemmal zones, in the perinuclear space, and between the residual myofibrils (Fig. 2c, d). The appearance of abnormally oriented newly formed myofibrils, it can be concluded, depended on the previous resumption of contractile protein synthesis and on disturbance of the functions of the nucleus and nucleolus as a result of incorporation of rubomycin molecules in the DNA structure [8]. Disturbance of certain regulatory mechanisms evidently continues to persist even after the nucleoli have regained their ability to synthesize RNA. The irregular orientation of the myofibrils in cardiomyocytes has been described in idiopathic cardiomyopathies and myocardial hypertrophy in rats due to high-altitude hypoxia [1, 12], but it is not mentioned in descriptions of ultrastructural studies of the myocardium under the influence of anthracycline antibiotics.

The structure of the mitochondria during the first 2 days of the experiment was indistinguishable from normal. On the 3rd day a mosaic pattern of distribution of the cardiomyocytes began to be found, all the mitochondria in the cells were enlarged, the matrix translucent, and the cristae fragmented, although all other intracellular organelles were completely preserved. Such cardiomyocytes also were constantly found during later observations. The static nature of the picture of injury to the mitochondria and the absence of any time course of the progress suggest that swelling and destruction of the cristae took place during histological treatment of the tissues and were due to intravital loss of stability of the mitochondrial membranes.

It can thus be concluded that the ultrastructural changes in the cardiomyocytes as a whole, resulting from injury to the myocardium by the action of rubomycin, an antibiotic of the anthracycline series, reflects the morphological basis of plastic cardiac insufficiency [3, 6], developing as a result of disturbance of synthesis of specific proteins and inhibition of reproduction of the contractile structures of the heart.

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