CHANGES IN MYOCARDIAL ULTRASTRUCTURE DURING TREATMENT OF EXPERIMENTAL CARDIOGENIC SHOCK BY MEANS OF AN ARTIFICIAL LEFT VENTRICLE

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Acute heart failure developing in patients after complicated operations on the heart and its vessels still accounts for many deaths in the immediate postoperative period. This necessitates a search for new and more effective ways of treating heart failure. One such method is an assisted circulation and, in particular, bypassing of the left heart [2]. By this method the work load on the myocardium is reduced and the arterial blood pressure (BP) is maintained at a level adequate for the needs of the body. There have been many investigations of effects of bypassing the left heart in man and animals, but the morphological aspect of this problem has not been adequately studied. There is virtually no information in the literature on processes taking place in the myocardium outside the zone of ischemia during the treatment of cardiogenic shock by an assisted circulation. Nevertheless, it is well known that compensation of heart failure is largely determined by the state of the structure and metabolism of these areas of heart muscle.

The object of this investigation was to study changes in the ultrastructure of areas of myocardium remote from the zone of ischemia during the development of cardiogenic shock and its treatment by bypassing the left ventricle.

EXPERIMENTAL METHOD

Twelve mongrel dogs weighing from 16 to 34 kg were used. The animals were anesthetized with barbiturates by the standard method. Artificial ventilation of the lungs was carried out with an RO-6 respirator through an endotracheal tube. Acute heart failure was reproduced by successive ligation of the descending branch of the left coronary artery. This led to a gradual decrease in the cardiac output (CO) and BP. After BP had fallen below 70 mm Hg, during continuous recording of parameters of the central hemodynamics, an artificial ventricle (AV) was connected to the arterial system between the left atrium and thoracic aorta. The AV was controlled by an AVK-5M apparatus, with pneumatic drive synchronized with the R wave of the ECG. In all experiments the work of the AV was synchronized with the animal's heart under counterpulsation conditions.

Material obtained by punch biopsy of the myocardium from the posterior wall of the left ventricle obtained at different stages of the experiment was used for the morphological investigations. The first biopsy was carried out at the time of development of cardiogenic shock immediately before the beginning of the bypass procedure, the second after operation of the assisted circulation for 2 h. The material was fixed in glutaraldehyde and in a buffered solution of OsO_4, dehydrated, and embedded in a mixture of Epon with Araldite. Sections were cut on the LKB-8800 Ultratome, stained with uranyl acetate and lead citrate, and examined in the IEM-100B electron microscope. A stereologic method of quantitative analysis was used to study the myocardium [6, 8]; mitochondria of cardiomyocytes were investigated. The surface area of the outer membrane of the mitochondria, the surface area of their cristae, and the relative volume of the mitochondria were determined. These parameters are volume fractions of the various structures in 1 μ^3 of sarcoplasm of the cardiomyocytes. The numeri-

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TABLE 1. Central Hemodynamics after Bypassing of Left Ventricle in Animals with Cardiogenic Shock

Parameter of central hemodynamics	Intact heart	Cardiogenic shock	Bypass	After discontinuation of bypass
Maximal systolic pressure, mm Hg	125 ± 8	61 ± 6	52 ± 5	76 ± 7
Maximal diastolic pressure, mm Hg	80 ± 5	40 ± 7	119 ± 9	50 ± 6
Mean intra-aortic pressure, mm Hg	86 ± 7	43 ± 6	74 ± 9	58 ± 5
CO, ml/min	2227 ± 148	1201 ± 30	761 ± 18	1630 ± 28
Work of the heart, kg·m/min	3.78	0.96	0.6	1.48

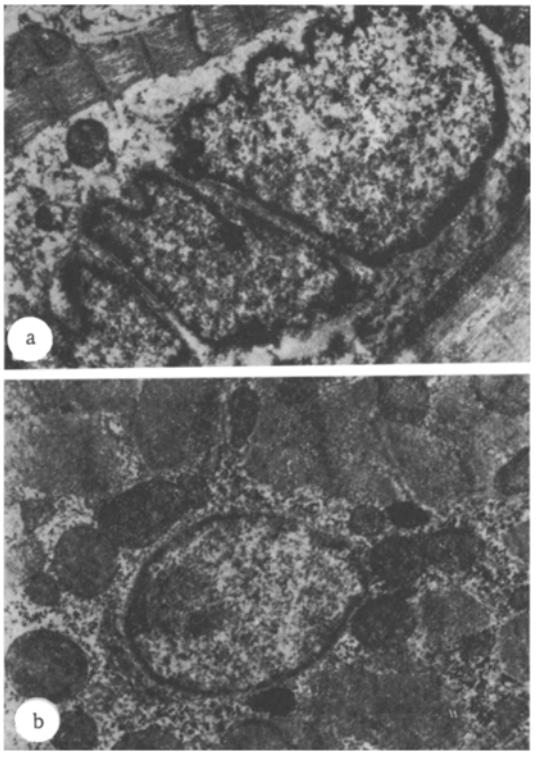
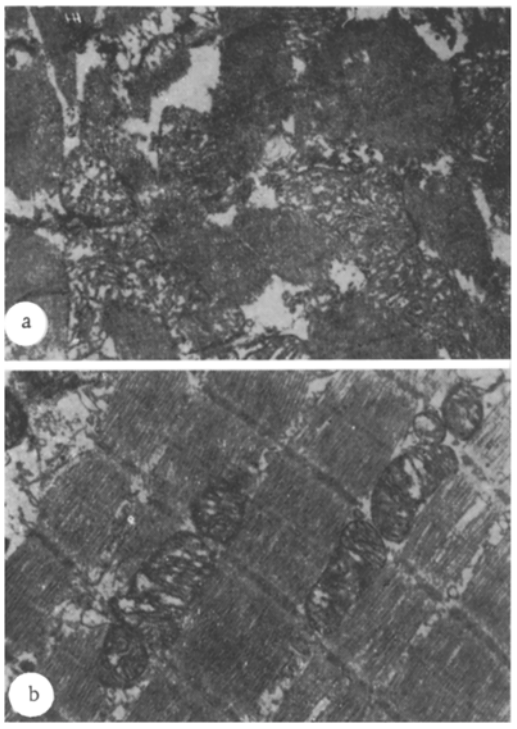


Fig. 1. Electron-micrograph of myocardium at time of development of cardiogenic shock (a) and 2 h after commencement of bypass (b): a) perinuclear edema, numerous evaginations of sarcolemma, disappearance of glycogen granules; b) disappearance of intracellular edema, outlines of nucleus smoothed, abundance of glycogen granules and secondary lysosomes in perinuclear space. 12,000 ×.



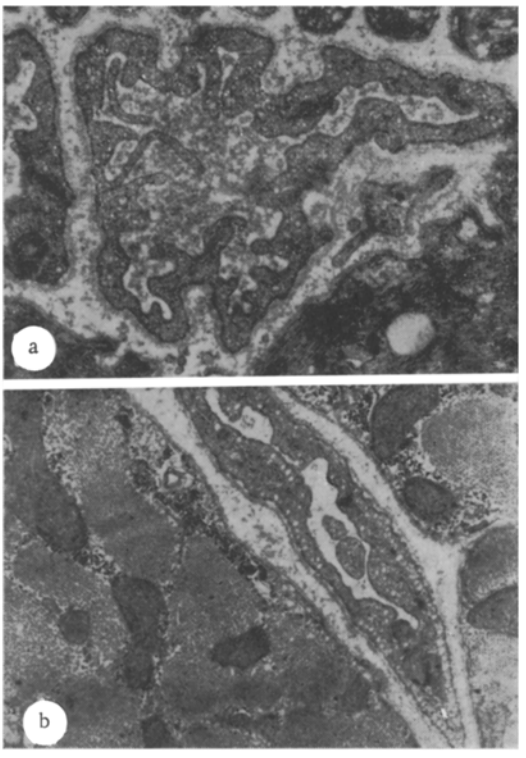


Fig. 2

Fig. 3

Fig. 2. Ultrastructure of mitochondria at moment of development of cardiogenic shock (a) and 2 h after beginning of bypass (b): a) marked contracture of myofibrils with injury to Z bands, lysis of myofilaments, fragmentation and homogenization of cristae of mitochondria; b) usual structure of myofibrils, parallel arrangement of cristae in mitochondria. 12,000 ×.

Fig. 3. State of endothelial cells of capillaries at moment of development of cardiogenic shock (a) and 2 h after beginning of bypass (b): a) festooning of sarcolemma, high pinocytotic activity and cytoplasmic evaginations of capillary endothelium. 12,000 ×; b) outlines of sarcolemma smoothed, accumulation of glycogen granules beneath sarcolemma, decrease in pinocytotic activity and reduction of cytoplasmic evaginations of capillary endothelium. 9000 ×.

cal results were subjected to statistical analysis. The significance of differences between the mean values of the samples was determined at a 95% level of significance ($P < 0.05$).

EXPERIMENTAL RESULTS

Ligation of the coronary arteries led to a gradual fall in BP (Table 1), CO, and the external work of the heart.

Electron-microscopic study of the myocardium at the moment of development of cardiogenic shock revealed intracellular edema of the sarcoplasm of the cardiomyocytes, which was most marked in the perinuclear zone. The surface of the nucleus was enlarged because of numerous evaginations (Fig. 1a).

The outer membrane of the mitochondria was preserved, but the internal structure of these organelles was considerably damaged. The injuries consisted of translucency of the matrix and focal vacuolation. The cristae had lost their parallel arrangement and exhibited focal fragmentation and homogenization (Fig. 2a).

Contractural changes were observed in the myofibrils with curving of the Z bands. Meanwhile sarcomeres with focal unwinding of the fibers and lysis of the myofilaments were seen. Contact between actin filaments and structures of the intercalated disks was disturbed. The lumen of the tubules of the T system was considerably dilated. Lipid inclusions were found in the sarcoplasm of the cardiomyocytes. No glycogen granules were found. The sarcolemma

of the muscle cells of the heart was festooned in appearance. The endothelial cells of the capillaries contained many micropinocytotic vesicles, the luminal surface of their plasmalemma formed numerous so-called digital evaginations (Fig. 3a). The assisted circulation, which was started immediately after cardiogenic shock was recorded, led to an increase in the mean intra-aortic pressure from 43 ± 6 to 74 ± 9 mm Hg. Under these circumstances the external work of the heart decreased. The volume of bypassed blood was 65-70% of the total volume flowing to the heart. After 2 h of bypassing the AV was disconnected. The mean intra-aortic pressure fell from 74 ± 9 to 58 ± 5 mm Hg, and the work of the heart increased.

Electron-microscopic study of the myocardium 2 h after the beginning of the bypass revealed changes in the ultrastructure of the heart muscle confirming the restoration of cardiac activity. The intracellular edema in the cardiomyocytes had subsided considerably. The outlines of the nuclei were smoothed. An abundance of glycogen granules appeared in the perinuclear zone and beneath the sarcolemma (Fig. 1b).

The structure of the mitochondria was extremely heterogeneous, even within the same cardiomyocyte. However, the number of cristae in the mitochondria was increased, and their arrangement became parallel. No vacuolation of the mitochondria was found. Contracture of the myofibrils could not be determined. The sarcomeres had a regular structure with clearly defined H and Z bands (Fig. 2b).

The lumen of the T tubules remained dilated. Lipid inclusions were found extremely rarely, and secondary lysosomes appeared where the organelles were destroyed. The outlines of the sarcolemma were smoothed.

The configuration of the capillaries varied. The pinocytotic activity of their endothelial cells was depressed and the cytoplasmic evaginations were reduced in size (Fig. 3b).

The results of stereometric analysis showed a marked increase in the surface area of the mitochondrial cristae, which increased from 4.32 ± 0.29 to 8.40 ± 0.41 μ^2 in 1 μ^3 of sarcoplasm. The surface area of the outer membrane of the mitochondria increased from 2.91 ± 0.14 to 3.14 ± 0.18 μ^2; however, differences between these values were not statistically significant ($P < 0.05$). The relative volume of the mitochondria remained unchanged after bypassing for 2 h (0.31 ± 0.02).

The results of morphological investigations of areas of myocardium remote from the zone of ischemia at the moment of development of cardiogenic shock thus revealed ultrastructural changes characteristic of hypoxia and functional overloading. Hyperfunction of the "intact" areas of heart muscle in experimental myocardial infarction has been observed by many workers [1, 3, 4, 7, 9].

The early application of cardiosynchronized bypassing of the left ventricle abolished the effects of certain pathological factors on the myocardium. Under bypass conditions the external work of the heart was reduced and the coronary blood flow was maintained at close to its initial level. These circumstances favored myocardial functioning at a lower level of energy consumption, leading to abolition of the reversible injuries to the ultrastructure of the heart muscle cells and to the accumulation of glycogen reserves in them. During left-ventricular bypassing other energy-yielding substrates evidently also accumulated. Quantitative investigations showed a marked increase in the area of mitochondrial cristae in functionally active zones of the myocardium. The work of Chernukh and Kopteva [5] showed that most enzymes of the respiratory chain are in fact synthesized on the inner membranes of mitochondria. Accumulation of energy-yielding substrates points to the possibility of restoring myocardial contractility. This is confirmed by the satisfactory cardiac activity after discontinuation of the AV.

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AN INTRAVITAL METHOD FOR THE EXPERIMENTAL STUDY OF THE PULMONARY CAPILLARIES

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In recent decades considerable attention has been paid to the study of the microcirculation as a possible means of explaining the mechanism of disturbances of the activity of various organs and systems. Progress has been made with the study of such living organs as the liver, kidney, muscle, spleen, nerve tissue, mesentery, retrobuccal pouch, and so on [5]. However, some vitally important organs, such as the lungs, are difficult to investigate. A study of the microcirculation of the lungs could shed light on the pathogenesis and treatment of many pathological processes taking place in the respiration system.

The object of this investigation was to modify existing methods of intravital microscopy of the lung [1, 2, 4, 7] in order to carry out experiments on cats under open chest conditions and during artificial ventilation of the lungs. The aims of the investigation were as follows: to halt the cardiorespiratory movements of the lung but preserve physiological conditions at the site of observation; to choose optimal conditions for observing and recording the object, and to construct devices suitable for carrying out experiments on cats.

EXPERIMENTAL METHOD

A Luman 13 luminescence microscope was used for recording and observing, and the OLK-2 system (Fig. 1) was used for investigations by contact microscopy. RF-3 film was used for microphotography. An important and laborious stage was the construction of a number of devices and adaptations for performing experiments on cats, for the microscope itself is not designed for work with large and medium-sized laboratory animals.

A universal table serving simultaneously for surgical preparation of the animal and for manipulations with it under the microscope, was produced by the Institute's workshops (awarded Efficiency Suggestion No. 1, 1981, State Register of Inventions, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR). A halter with connections for the air pipes and a wound retractor, fixed with bolts, were mounted on the table. Complete and reliable fixation of the animal was secured in this way.

Another design innovation was the creation of a strong removable mechanism, made in the experimental workshops of the Institute (awarded Efficiency Suggestion No. 2, 1981, State Register of Inventions, Institute of General and Pathological Physiology, Academy of Medical Sciences of the USSR). This device is an independent lifting mechanism, fixed to the table by means of a strong pedestal (Fig. 2). By turning a wide wheel the rod of the lifting device is moved, and it can be additionally fixed by means of a set screw. Support for the table is provided by a wide metal platform. It is fixed to the rod by means of a collar with flange and lock. The universal stage with the dissected animal is placed on the platform of the lifting device and moved horizontally. In this way the table with the animal can be moved in the necessary directions.

The problem of halting movements of the lung was solved by the use of an original suction chamber, suggested by the All-Union Research Institute of Pulmonology, Ministry of Health of the USSR (G. M. Kudryashev et al.) and modified by P. S. Aref'ev (awarded Effi-

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