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EFFECT OF THE ASSISTED CIRCULATION ON MYOCARDIAL ULTRASTRUCTURE

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UDC 616.12-008.1-021.6-07:616.127-091.8-07

The effect of a method of assisted circulation (counterpulsation) on the ultrastructure of the myocardium was studied in dogs. Electron microscopy revealed a sharp increase in the glycogen content in the heart muscle cells, mitochondria with a highly osmophilic, finely granular matrix, and high pinocytotic activity of the capillary endothelial cells. The results are evidence of metabolic changes in the myocardium and, in particular, that the myocardial muscle cells are functioning at a lower energy level. The changes discovered in the myocardial ultrastructure evidently account for the beneficial therapeutic effect of the method.

KEY WORDS: assisted circulation; myocardial metabolism; glycogen; mitochondria.

Despite many investigations of the assisted circulation, the true character of the processes taking place under these circumstances in the body is still far from completely understood [3, 7, 10].

The object of this investigation was to study the effect of the assisted circulation, using the counterpulsation method, on the ultrastructure of the myocardium in intact animals.

EXPERIMENTAL METHOD

Seven dogs weighing 15-25 kg were used. Under endotracheal anesthesia (morphine, thiopental sodium, listhenon) the thorax was opened in layers in the third-fourth intercostal spaces on the left side and the subclavian artery was isolated. A cannula, connected to the output tube of a valveless blood pump, with a common inlet and outlet for the blood, was introduced into the lumen of the artery. The pump was connected to a pneumatic drive mechanism and cardiosynchronizer. The ECG and the pressure in the ascending aorta were recorded during the experiments, the acid–base balance and the free hemoglobin concentration in the blood plasma were determined, the "time-tension index" (TTI) and the external work of the left ventricle were calculated.

After the initial parameters of the hemodynamics had been measured and blood samples taken, the pneumatic drive mechanism was switched on and the assisted circulation commenced under counterpulsation conditions as described previously [3, 13].

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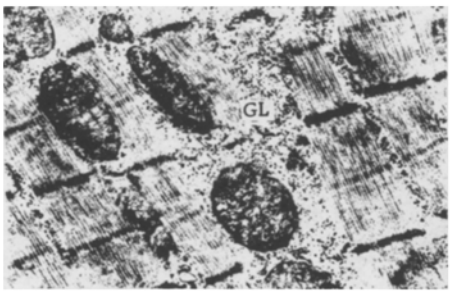

Fig. 1

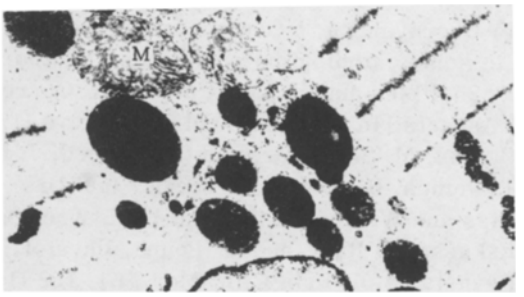

Fig. 2

Fig. 1. Accumulation of glycogen granules (Gl) in muscle cell from left ventricle, 25,000×.

Fig. 2. Muscle cell from left ventricle. Accumulation of mitochondria (M) with strongly osmophilic matrix and numerous cristae.

The duration of assisted perfusion in all the experiments was 1.5-2 h. After the end of counterpulsation pieces of myocardium were taken from the left and right ventricles and the left atrium for electron-microscopic study. The material was fixed in 1% buffered OsO_4 solution by Caulfield's method, dehydrated, and embedded in Araldite. Electron micrographs were obtained on the JEM-100 electron microscope.

EXPERIMENTAL RESULTS

Changes in the hemodynamics recorded in the experiments were the same as usual [3, 5, 6] and were as follows: The maximal systolic pressure in the ascending aorta was reduced during counterpulsation by 35% of its initial level, the diastolic pressure was increased by 76%, and TTI and the external work of the left ventricle were reduced by 38%. The acid–base balance was virtually unchanged in the course of the experiments and hemolysis by its end did not exceed 20-25 mg%.

Electron-microscopic investigation of the myocardium after 1.5-2 h of counterpulsation revealed changes in the ultrastructure of the muscle fibers and capillary endothelium.

Considerable accumulation of glycogen granules was observed in the muscle cells of the myocardium from the left ventricle. The granules were round or polygonal in shape, 20-40 nm in diameter, and were distributed between the myofibrils, myofilaments, and mitochondria, filling nearly the whole of the sarcoplasm (Fig. 1). Marked accumulation of glycogen granules was observed under the sarcolemma, which formed arcadelike projections.

Mitochondria located between the myofibrils in most cases had the characteristic structure for dog muscle cells. The mitochondria were oval or elongated in shape, 2.0-2.5 μ in diameter, and contained 10 to 12 cristae each. The cristae were sharply curved and wavelike in configuration and anastomosed frequently with each other. The matrix of the mitochondria was finely granular and of average electron density. In the perinuclear zone of some muscle cells, which was packed with glycogen granules, oval mitochondria measuring 1.5-1.8 μ with an extremely dense, finely granular, osmophilic matrix (Fig. 2) were found. The cristae were arranged strictly parallel to each other, and their number per unit area was 1.5-2 times greater than in ordinary mitochondria.

The myofibrils had the characteristic structure for the myocardium, with clearly defined A and I disks, H and Z bands, and regularly arranged myofilaments. Between the myofibrils, at the level of the Z bands, dilated transverse tubules of the sarcoplasmic reticulum were visible. They attained a diameter of 0.5-0.8 μ. Floccular filamentous structures of average electron density were observed in the lumen of the tubules.

The state of the basement membranes of the myocardial capillaries and of the osmophilic layer of the sarcolemma was indistinguishable from normal. However, the outlines of the capillaries were frequently highly convoluted and considerable variation was observed in the thickness of the cytoplasm of the endothelial cells. The cytoplasm formed numerous short and wide, or narrow and long projections into the capillary lumen. A sharp increase in the pinocytotic activity of the capillary endothelium also was noted. Meanwhile, the sarcoplasm in areas adjacent to the capillaries did not exhibit such marked pinocytotic activity.

Numerous pinocytotic vesicles 30-50 nm in diameter were detected in the cytoplasm of the endothelial cells, where they lay near both the inner and the outer membrane (Fig. 3). The electron density of their con-

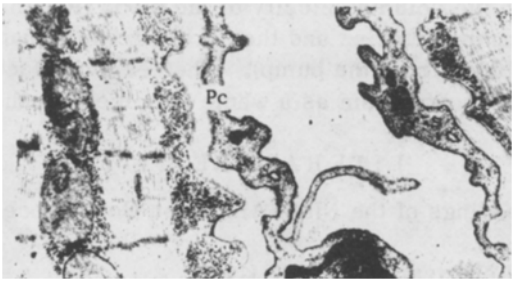

Fig. 3. High pinocytotic activity (Pc) of endothelial cells of myocardial capillaries, 22,000×.

tents was low. The endothelial cells contained few organelles and only the number of ribosomes and polysomes in them was increased.

Numerous floccular filamentous structures and also a finely-granular substance, more osmophilic than normal, were observed in the capillary lumen.

The sharp increase in the glycogen content in the myocardial muscle cells and dilatation of the T system of the sarcoplasmic reticulum observed in these experiments indicate that under the influence of the assisted circulation the heart muscle cells were functioning at a low energy level. This could have been due to a reduction in the working load on the heart, which in the case of the left ventricle amounted to almost 40% of its initial level. A decrease in the working load on the myocardium in the presence of an assisted circulation has been proven conclusively in other investigations [5-7].

The mechanisms of glycogen accumulation by the myocardial muscle cells during the operation of the assisted circulation is not sufficiently clear. Comparison of the results now obtained with those of the experiments of Safonova et al. [2], who observed increased extraction of glucose by the myocardium from the coronary blood during the operation of an assisted circulation, suggests that the utilization of glucose under these circumstances remained at its previous level or was even reduced, and the excess of glucose was deposited in the myocardium as glycogen.

A possible explanation is that the accumulation of glycogen in the myocardium was connected with a decrease in the concentration of catecholamines and, in particular, of adrenalin in the heart muscle in the presence of counterpulsation [4]. Adrenalin, by its action on cyclic 3,5-AMP [11, 12], is known to lead through a series of intermediate reactions to the activation of phosphorylase, an enzyme hydrolyzing glycogen [8, 9]. A decrease in the adrenalin concentration could therefore inhibit the hydrolysis of glycogen and so promote its accumulation by the myocardial tissues.

The increase in the number of small mitochondria with an extremely dense osmophilic matrix in the perinuclear zone is evidence of the accumulation of "juvenile," younger forms of mitochondria, not yet involved actively in metabolism, in the myocardium. It has been shown [1] that mitochondria with a dense, osmophilic matrix, and with large numbers of densely packed, parallel cristae, are in the state of energy accumulation. An increase in the number of such mitochondria in the muscle cells during the operation of the assisted circulation was evidently connected with a reduction in the energy requirements of the myocardium associated with the reduced working load on it. Other evidence in support of the depressed metabolism of the heart muscle during the operation of an assisted circulation was obtained by Chazov et al. [5], Shumakov et al. [3], Clauss et al., [6], and other workers who found a decrease in the oxygen consumption of the myocardium and in the arteriovenous oxygen difference of the coronary blood.

Considering that in myocardial infarction during the first few minutes after coronary occlusion and the development of ischemia glycogen disappears from the muscle cells, the mitochondria swell, and their matrix becomes translucent, it seems likely that the accumulation of glycogen and the appearance of "juvenile" forms of mitochondria are evidence of the creation of a favorable level of energy metabolism for myocardial function under counterpulsation conditions, so facilitating the beneficial therapeutic effect of the assisted circulation in clinical practice.

The increase in pinocytotic activity of the endothelial cells of the myocardial capillaries, the increase in the number of ribosomes and polysomes, and also the presence of numerous projections and evaginations of the cytoplasm and endothelium can be interpreted as a compensatory and regulatory response of the myocardial

vascular system to the increase in the volume velocity of the blood flow in it and to the change in the character of the pulse wave in the main coronary arteries and the aorta (the appearance of an additional peak of pressure in diastole due to the injection of blood from the pump). Whether these factors have a positive or negative influence on the myocardium and on the organism as a whole is something that only future research will decide.

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IDENTIFICATION OF SMOOTH MUSCLE MYOSIN IN MYOID CELLS OF THE TESTES

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UDC 612.734.14:612.616

Smooth muscle myosin was found by the Coons' immunomorphological test in the outer wall of the seminiferous tubules of man, rats, and mice. The results of the investigation confirm the smooth-muscle nature of the myoid cells.

KEY WORDS: testis; myoid cells; smooth-muscle myosin.

The so-called myoid cells (myofibroblasts, contractile fibroblasts) which, together with fibrous structures, form the outer wall of the seminiferous tubules, have many common features with smooth muscles. The cytoplasm of these cells is rich in actinlike microfibrils 50-70 Å thick, which by themselves and with the plasmalemma form dense bodies. To correspond to the pinocytotic vesicles, on the outer surface of the cell membrane there are craterlike depressions. At the site of contact of the cells with each other desmosome-like formations and nexuses have been identified [3, 10]. Contraction of myoid cells in isolated seminiferous tubules [8] and in tissue culture [5] has also been observed.

This morphological and functional similarity between the myoid and smooth-muscle cells suggested that the composition of their contractile proteins would be identical.

In this investigation an attempt was made to identify smooth-muscle myosin in the myoid cells.

EXPERIMENTAL METHOD

Myosin was identified by the indirect Coons' method using pure donkey antibodies against rabbit immunoglobulin G, labeled with fluorescein isothiocyanate. Antiserum against human uterine myosin was obtained in rabbits in several stages by the method described previously [2]. Sections through the testes, and

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1499-1501, December, 1976. Original article submitted May 18, 1976.