

QUANTITATIVE ULTRASTRUCTURAL CRITERIA OF MYOCARDIAL DAMAGE DURING POSTISCHEMIC REPERFUSION

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Ultrastructural signs of myocardial damage in the course of ischemia and postischemic reperfusion have been described by several investigators [7-11, 13, 14]. Some of them attempted to study ultrastructural changes by morphometry [7, 8, 10, 11, 13]. However, quantitative ultrastructural criteria of the presence and depth of ischemic myocardial damage, essential for an objective evaluation of the effectiveness of cardioplegia, have not been finalized.

The aim of this investigation was to quantify ultrastructural criteria of myocardial damage in isolated rat hearts subjected to ischemia and reperfusion.

EXPERIMENTAL METHOD

Experiments were carried out on eight male Wistar rats weighing 250-300 g. The hearts were removed under thiopental anesthesia (0.2 mg/g), cannulas were introduced into the aorta and left atrium, and the left heart was perfused with Krebs-Henseleit buffer by the method in [12]. In the control group four rats were perfused for 90 min. After control perfusion for 10 min the hearts of four experimental rats were subjected to total normothermic ischemia for 30 min, followed by reperfusion for 40 min. At the end of perfusion a piece of myocardium from the region of the apex of the heart was removed for ultrastructural investigation, fixed in a 2.5% solution of glutaraldehyde, postfixed with OsO_4 , dehydrated, and embedded in Epon and Araldite. Longitudinal sections through the myocardium were stained with lead citrate and uranyl acetate and examined in the EMV-100L electron microscope under a magnification of 25,000. The volume fraction of the myofibrils and mitochondria relative to the volume of sarcoplasm, and the relative surface density of the mitochondria were determined by means of a morphometric grid (a multipurpose test system with 270 control points [15]) on photographic plates, the length of the sarcomeres was measured, and the state of the mitochondria was assessed visually on a 5-point scale: 5 points — densely packed cristae, 4 and 3 points — weak and moderate dispersion of the cristae, translucency of the matrix, 2 and 1 points — marked and intensive cristolysis, with translucency of the matrix. From 28 to 39 electron micrographs were analyzed for each rat. The results were subjected to statistical analysis (95% level of probability).

EXPERIMENTAL RESULTS

Acute damage was noticed in hearts which underwent normothermic ischemia for 30 minutes followed by reperfusion for 40 minutes, the characteristic features of which were the presence of cardiomyocytes in the tissue in a state of primary cloudy-swelling degeneration and gross swelling of some of the cells of the capillary endothelium [2]. In the present investigation attention was concentrated on the ultrastructural characteristics of cardiomyocytes with no evidence of primary cloudy-swelling degeneration.

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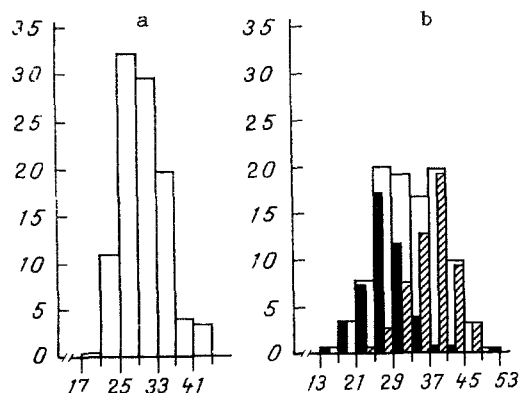


Fig. 1. Frequency of occurrence of sarcomeres of different lengths in cardiomyocytes of control myocardium (a) and myocardium during postischemic reperfusion (b). Abscissa, length of sarcomeres (in conventional units); ordinate, frequency of occurrence of sarcomeres (in %). Unshaded columns — in all cells, obliquely shaded columns — in cells of first group, black columns — in cells of second group.

TABLE 1. Morphometric Parameters of Cardiomyocyte Ultrastructure of Control Myocardium and Myocardium during Postischemic Reperfusion

Parameter	Control	Postischemic reperfusion		
		mean value	group of cells	
			first	second
Mean length of sarcomeres, conventional units	31,04±1,96	33,16±2,80	37,00±1,12*	28,41±1,84
Volume fraction of myofibrils, %	52,25±0,66	42,24±2,06*	43,63±1,68*	39,46±3,34*
Volume fraction of mitochondria, %	28,95±0,61	39,54±2,13*	36,64±0,39*	42,90±3,26*
Relative surface density of mitochondria, μ^2/μ^3	7,29±0,54	5,46±0,13*	5,47±0,22*	5,27±0,20*
Visual index of state of mitochondria, conventional units	4,58±0,17	2,80±0,06*	2,77±0,13*	2,75±0,18*
Relative volume fraction of sarcoplasm, %	100	132,25±10,39*	142,50±6,18*	123,00±17,70
Relative volume fraction of myofibrils, %	52,25±0,66	55,82±4,74	62,05±1,96*	47,63±3,09
Relative volume fraction of mitochondria, %	28,95±0,61	52,30±4,58*	52,18±1,96*	53,56±11,09

Legend. Asterisk indicates significant difference from control.

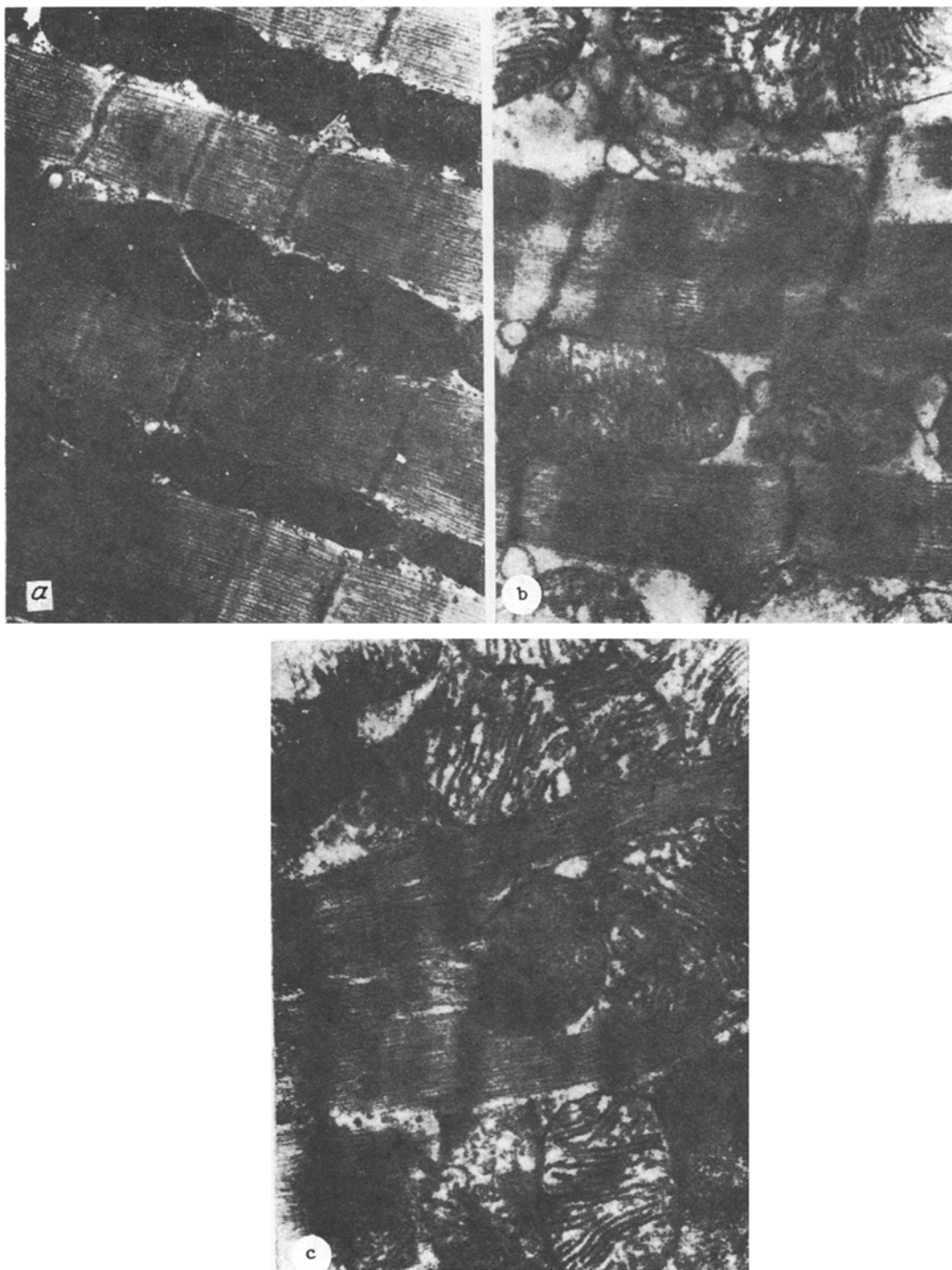


Fig. 2. Changes in ultrastructure of mitochondria and contractile system of cardiomyocytes during postischemic reperfusion. a) Control; b, c) cardiomyocytes of 1st and 2nd groups respectively during postischemic reperfusion. 25,000 \times .

High variability was observed in the myocardium of the heart in the course of postischemic reperfusion despite no change in the average length of the sarcomeres (Table 1; Fig. 1), and it was therefore decided to make a separate analysis of two groups of cells differing in prominence of the I-disks.

Cells of the first group, whose myofibrils were distinguished by clearly defined I-disks, had sarcomeres with an average length significantly greater than that of the sarcomeres in the control and in the second groups of cells, in which the I-disks were

poorly defined and the mean length of the sarcomeres did not differ from the control (Table 1; Figs. 1 and 2).

During separate determination of the parameters for analysis in the two chosen groups of cells a decrease in the volume fraction of the myofibrils, an increase in volume fraction of the mitochondria and a decrease in their relative surface density, and also a decrease in the visual index of the state of the mitochondria were observed (Table 1). The increase in the volume fraction of the mitochondria and the decrease in their relative surface density can be explained by swelling of the mitochondria, whereas the decrease in the visual index of the state of the mitochondria reflects changes described in the literature as translucency of the mitochondrial matrix. Translucency of the matrix and swelling of the mitochondria in the cardiomyocytes, which we assessed quantitatively, were described by other workers in ischemia and reperfusion [7, 9, 14]. Meanwhile the decrease in the volume fraction of the myofibrils cannot be explained by a change in their own state, but suggests an increase in the total volume of the cells and, consequently, that the volume fraction cannot be compared between the groups. Some investigators, who determined the volume fraction of the cardiomyocyte ultrastructures, also emphasize that changes in the characteristic volume of a structure cannot be detected by means of this parameter if accompanied by a change in total cell volume [10, 11].

To obtain results capable of comparison between the groups, we transformed the data into volume fraction of the structures to be analyzed, expressed as a ratio of the volume of the sarcoplasm of the control cells. This parameter, or relative volume fraction, was used to analyze changes in the characteristic volume of structures during processes accompanied by a change in the total volume of the cardiomyocytes without transformation and loss of myofibrils. The value of the parameter for a structure to be analyzed can be determined by the equation:

$$\text{conv. } V_{v_{i2}} = V_{v_{i2}} \cdot \frac{l_{\text{sarc.2}}}{l_{\text{sarc.1}}} \cdot \frac{V_{v_{mf1}}}{V_{v_{mf2}}},$$

where $l_{\text{sarc.1}}$ and $V_{v_{mf1}}$ denotes the average length of the sarcomeres and the volume fraction of myofibrils of the control cells respectively, $l_{\text{sarc.2}}$, $V_{v_{mf2}}$, and $V_{v_{i2}}$ the mean length of the sarcomeres, volume fraction of the myofibrils, and volume fraction of the cardiomyocyte structure to be analyzed respectively after the change in the cell volume.

Comparison of the calculated values of the relative volume fractions showed that the group of cells of reperfused myocardium with distinct I-disks had a significantly increased relative volume fraction of its myofibrils. The relative volume fraction of the mitochondria was high, and gives much clearer evidence of swelling of the mitochondria than the volume fraction of the mitochondria, whose value cancels out the significant increase in volume of the sarcoplasm of the cardiomyocytes (Table 1). Cells of this group evidently correspond to the early stages of cytolysis [4], for they are characterized by distinct I-disks whose length, however, has not yet reached the length of the A-disks, a feature regarded as indicating cytolysis [3]. Cells in a state of overstretching in the myocardium after immersion fixation are found in the presence of profound reversible and also irreversible ischemic damage to the cardiomyocytes [5, 6, 14]. They are recorded in the zone of developing myocardial infarction [3, 5, 6] and in the myocardium of persons after sudden cardiac death [1].

The relative volume fractions of the sarcoplasm and myofibrils in cells of the second group from the reperfused myocardium did not differ significantly from the control. The relative volume fraction of the mitochondria was considerably greater than in the control, although not up to the level of significance (evidently on account of the great scatter of the values), and it was very close to corresponding values in the first group of cardiomyocytes (Table 1).

The essential conclusion is that the parameters of the mitochondria analyzed in cardiomyocytes of the two groups were closely similar. These results suggest that a state of relaxation of the myofibrils observed in some cells in the course of postischemic reperfusion of the myocardium was not predetermined by a special morphological state of the mitochondria of these cells. This observation, which undoubtedly requires further study, is of great practical importance in the analysis of biopsy material. It means that averaged

parameters of the state of the mitochondria of cardiomyocytes can be used to assess the presence and depth of ischemic myocardial damage, regardless of the state of their contractile apparatus.

Averaged parameters of the state of the cardiomyocyte mitochondria can thus be used as criteria of the presence and depth of myocardial damage during reperfusion when the effectiveness of cardioplegia is determined. These parameters include: the relative surface density, the visual index of the state of the mitochondria, and the relative volume fraction of the mitochondria, namely volume fraction relative to volume of control cells.

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PROTECTIVE ACTION OF PANTHETIN ON THE DENERVATED STOMACH

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Vagotomy has achieved widespread popularity in recent years in the surgical treatment of duodenal ulcer [1, 4, 8]. Whereas reduced production of hydrochloric acid and pepsin, observed after this operation, have served as the pathophysiological basis for its use in the treatment of duodenal ulcer, the neurodystrophic changes in the mucous membrane and disturbances of the motor function of the stomach, accompanied by a disturbance of the efflux of its contents from the organ, are regarded as among the leading factors in the pathogenesis of postvagotomy complications [2, 3, 5]. Accordingly the search for pathogenetic approaches to the pharmacotherapy of the complications developing after operations connected

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