

METHODS

A NEW MORPHOMETRIC PARAMETER FOR ANALYSIS OF CARDIOMYOCYTE ULTRASTRUCTURAL CHANGES: THE RELATIVE VOLUME FRACTION

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The volume fraction [1, 2, 12] is a parameter widely used for morphometric evaluation of the state of cardiomyocyte ultrastructure. It denotes what fraction of volume of the cardiomyocyte (sarcooplasm) is occupied by the test structure. This parameter is often used to compare not only different ultrastructures in the same cell, but also a particular ultrastructure in cells of different experimental groups. However, when changes in the parameter are analyzed for a test ultrastructure in different experimental groups, the true change in volume of that particular ultrastructure can be determined only if it is not accompanied by a change in the total volume of the cardiomyocytes. Actually, when cardiomyocyte ultrastructure is studied in cases of myocardial hypertrophy, many workers analyze the change in the volume fraction of the ultrastructures while disregarding any increase in size of the cardiomyocytes [6, 11]. Some workers, however, have noted that reduction of the volume fraction of structures may be connected with general growth of the cell [3], and they used absolute parameters for structures calculated on the basis of their volume fractions and the dimensions of the cardiomyocytes [4].

During analysis of cardiomyocyte ultrastructure in the course of ischemic myocardial damage, some workers, while demonstrating changes in the volume fraction of the ultrastructures, do not discuss the possibility of a change in cell volume, even though they describe edema of the sarcooplasm of some myocytes [5, 10]. Some workers have suggested that the decrease in the values of the volume fractions of subcellular structures, recorded by them in ischemic damage, can be explained by swelling of the cells as a result of intracellular edema [8, 9], and the absence of significant changes in their value can be attributed to a proportional increase in the volume of the structure analyzed and the whole cell [7].

It is impossible at present to assess changes in the intrinsic volume of an ultrastructure chosen for analysis with the aid purely of the generally accepted morphometric parameters, and without resorting to measuring the general dimensions of the cardiomyocytes.

To estimate changes in the intrinsic volume of a test ultrastructure during the analysis of acute processes taking place in cardiomyocytes, accompanied by a change in total cell volume without formation of new or loss of existing myofibrils, the writer suggests the use of a new morphometric parameter: the relative volume fraction of an ultrastructure. The relative volume fraction defines the volume occupied by a given ultrastructure as a ratio of the original volume of the cardiomyocytes (before any change in their volume).

When developing this parameter, we set out from the assumption that the absolute volume of myofibrils (V_{mf}) is directly proportional to the mean length of the sarcomeres (l_{sarc}), since $V_{mf} = l_{sarc} \times n_{sarc} \cdot S_{mf}$, and the number of sarcomeres (n_{sarc}) and the area of cross-section of myofibrils (S_{mf}) in cells are constant if no new ones are formed or existing ones lost.

A change in the mean length of the sarcomeres, which characterizes a change in the absolute volume of myofibrils, and data on changes in the relative volume of the myofibrils (volume fraction) can be used to estimate quantitatively the change in volume of the cardiomyocytes by the equation:

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

where $V_{\text{cell.1}}$, $l_{\text{sarc.1}}$, and $V_{v_{\text{mf1}}}$ denote the volume of the sarcoplasm, the mean length of the sarcomeres, and the volume fraction of the myofibrils of the original cells respectively; $V_{\text{cell.2}}$, $l_{\text{sarc.2}}$, and $V_{v_{\text{mf2}}}$ are the corresponding parameters of the cells after a change in their volume.

The calculated parameter of the change in cell volume of the cardiomyocyte takes into account the swelling of the cell and the change in its size during contraction and relaxation. By means of this parameter it is possible to determine the relative volume fraction of any cardiomyocyte ultrastructure after a change in its volume by the equation:

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

where $V_{v_{i2}}$ is the volume fraction of the given cardiomyocytes structure after a change in its volume.

The use of the relative volume fraction as a parameter in investigations to determine quantitative ultrastructural criteria of acute ischemic damage to the myocardium confirmed its high informativeness.

During analysis of processes accompanied by a change in volume of the cardiomyocytes without the formation of new or loss of existing myofibrils, there are sound reasons for using the parameter of relative volume fraction of the ultrastructure, which defines the volume occupied by the given structure as a ratio of the initial volume of the cell, and enables a change in the intrinsic volume of the ultrastructure to be estimated.

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