

MORPHOMETRIC STUDY OF MYOCARDIAL MITOCHONDRIA IN THE RAT

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Studies of the last few years have shown that the mitochondrial mass of the heart muscle cell undergoes quantitative modifications after different experimental conditions (1-6).

The development of morphometric and stereologic methods for the quantitative analysis of tissue structures permits the determination of the mitochondrial mass of the myocardium with reasonable accuracy.

Since to our knowledge, considerable discrepancy exists with respect to the estimation of the per cent of cytoplasm of the heart muscle cell that is occupied by mitochondria, a description of the results obtained in the hearts of normal rats by means of differential point-counting analysis seemed therefore warranted.

METHODS

Five male albino rats of the Wistar strain, 210 g in body weight, were used. Special care was taken to select animals of the same age and weight. The rats were lightly anesthetized with ether, the chest was opened, and the heart was removed while still beating. 40 small pieces of the right and 40 of the left ventricular outer walls were immediately fixed for 2 hr in cold 1% osmium tetroxide in phosphate buffer (7). The material was embedded in Araldite. From the 40 tissue blocks of each ventricle, five were randomly selected for sectioning. Thick (1μ) sections were mounted on glass slides and stained with alkaline toluidine blue. Thin sections showing gray interference color were mounted on formvar-coated 200-mesh copper grids and stained with uranium acetate and lead citrate. The observations were performed with a Philips 300 electron microscope operated at 60 kv. In order to secure the randomness of the sample from each block of tissue, six electron micrographs were obtained from six consecutive angles of the grid. In this way, 30 micrographs were taken for each ventricular wall of each animal. The

micrographs were recorded on 35 mm film at an original magnification of 3750. At the end of each set of 30 micrographs, a carbon grating replica having 28,700 lines per inch was photographically recorded. The micrographs were enlarged at a final magnification of 10,000. The magnification was calibrated for each set of micrographs by means of the carbon grating replica. From each thick section, two micrographs were obtained with the light microscope and enlarged at a final magnification of 1000.

The morphometric analysis of the mitochondrial mass of the heart muscle cells was done according to the methods described by Weibel (8), and Weibel et al. (9).

The volumetric densities of the mitochondria and the myofibrils were determined by means of a test screen containing a frame of known area ($225\mu^2$) and a quadratic lattice of 225 points. The same frame was used for counting mitochondrial profiles and determining the numerical density. All the mitochondria present within the frame were counted.

When nuclei or extracellular space were present within the frame, appropriate corrections were made for each micrograph. However, since almost 85% of the myocardium appeared to be composed of myocardial cell cytoplasm, the occurrence of nuclei or connective tissue in the electron micrographs was not frequent. The volumetric and numerical densities were calculated according to the equations described by Weibel et al. (9). For the calculation of the numerical density of the mitochondria, a shape coefficient β (10) was assumed to be 2.25. This was based on the estimation of the mean axial ratio of 250 mitochondrial profiles and which was found to be 1.76. The mean volume of the individual mitochondria was estimated by dividing the volumetric density by the numerical density. All the data were referred to 1 ml of heart tissue. In the same study an attempt was made to determine the surface density of the mitochondrial cristae. However, the abundance and close packaging of the cristae made the

measurements extremely difficult and the results unreliable.

RESULTS

Composition of the Myocardium

The light microscopic study showed that 87% of the heart volume was muscle cells. The remaining 13% was composed of connective tissue and blood vessels. The analysis of the heart muscle cells revealed that 2.3% of the cell volume corresponded to nuclei. Accordingly, all the data were corrected by a factor 0.847 in order to establish the true volumetric and numerical densities of mitochondria in 1 ml of heart tissue.

Volumetric Density of Mitochondria

The volumetric density of the heart muscle cell mitochondria in the left ventricular outer wall amounted to 36.6%. The scatter of individual measurements was small according to the standard error, only 0.68% of the mean. In the right ventricle the volumetric density of mitochondria

was smaller than in the left ventricle and represented 29.3%, with a standard error of only 1.18% of the mean. The difference in the volumetric density for the left and right ventricles was significant. The treatment of the data with the *t*-test showed a *P*-value < 0.001.

Numerical Density of Mitochondria

The number of mitochondria existent in the left ventricle was found to be 637×10^9 per ml of heart tissue. In the right ventricle the number of mitochondria amounted to 535×10^9 per ml of heart tissue. The difference in the number of mitochondria for both ventricles was in agreement with the difference existent for the volumetric densities. The mean mitochondrial volume, obtained by the division of the volumetric density by the numerical density, was estimated at $0.57 \mu^3$ for the left ventricle and at $0.53 \mu^3$ for the right ventricle. Although the difference of $0.04 \mu^3$ was significant, it was considered to be too small and could be due to inherent errors of the method. Accordingly, the assumption was made that the

TABLE I
Morphometric Data of Heart Muscle Cell Mitochondria

	Volumetric density	Number of mitochondria per ml heart tissue	Mitochondrial volume (μ^3)
Rat 1			
Left ventricle	36.0	666×10^9	0.54
Right ventricle	30.7	479×10^9	0.64
Rat 2			
Left ventricle	36.6	625×10^9	0.58
Right ventricle	30.4	549×10^9	0.55
Rat 3			
Left ventricle	35.6	625×10^9	0.56
Right ventricle	28.1	572×10^9	0.49
Rat 4			
Left ventricle	38.5	645×10^9	0.59
Right ventricle	29.3	559×10^9	0.52
Rat 5			
Left ventricle	36.7	625×10^9	0.58
Right ventricle	28.2	570×10^9	0.49
Mean			
Left ventricle	36.6 ± 0.6	$637 \times 10^9 \pm 23 \times 10^9$	0.57 ± 0.003
Right ventricle	29.3 ± 1.1	$545 \times 10^9 \pm 45 \times 10^9$	0.53 ± 0.01
	$P < 0.001$	$P < 0.01$	$P < 0.001$

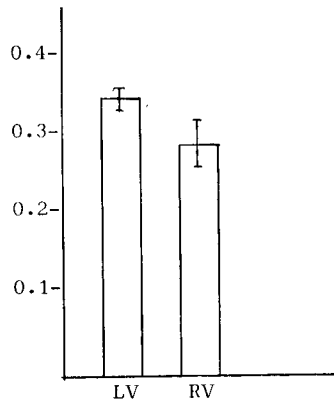


FIGURE 1 Volumetric density of mitochondria in heart muscle cells. *LV*, left ventricle; *RV*, right ventricle. Standard errors are indicated on the ordinate.

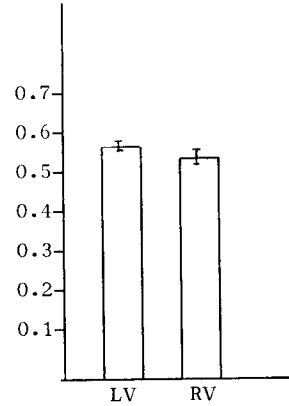


FIGURE 3 Mean mitochondrial volume in μ^3 . *LV*, left ventricle; *RV*, right ventricle. Standard errors are indicated on the ordinate.

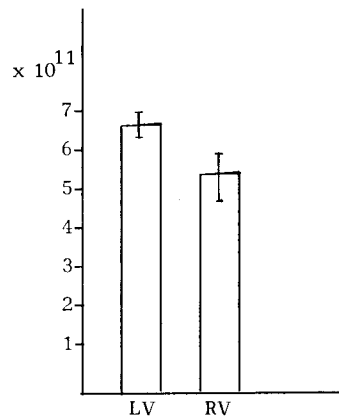


FIGURE 2 Number of mitochondria per cm^3 of heart tissue. *LV*, left ventricle; *RV*, right ventricle. Standard errors are indicated on the ordinate.

mitochondria of both ventricles had approximately the same mean volume. The results of these determinations are summarized in Table I and in Fig. 1, 2, and 3.

Volumetric Density of Myofibrils

The myofibrils were the most abundant component of the myocardial cell cytoplasm. In the left ventricle outer wall the volumetric density of the myofibrils was $46.7 \pm 3.5\%$, and in the right ventricle it represented $43.2 \pm 2.8\%$. On the basis of these determinations, the ratio between the volumetric densities of the mitochondria and the myofibrils was found to be 0.78 for the left ventricle and 0.67 for the right ventricle. The

difference between these ratios was significant ($P < 0.01$).

DISCUSSION

To our knowledge, no studies have yet been reported on the comparative morphometry of the mitochondrial mass in both ventricles. Most of the available reports deal with the quantitative estimation of the mitochondria of the left ventricle muscle cells. The reported data are variable and are summarized in Table II. The discrepancy in the data could be due to the different techniques employed for the morphometric analysis or to the different fixatives used. On the other hand, in most of the reports the randomness of the sampling has not been completely secured, making the results unreliable.

The data presented in this paper indicate that the muscle cells in the left ventricle have a greater mitochondrial mass than the muscle cells in the right ventricle. This is apparently due to an increased mitochondrial number, and perhaps also to a small increase in the mean mitochondrial volume. The ratio between the volumetric densities of the mitochondria and the myofibrils was different for both ventricles. In the left ventricle it was higher than in the right ventricle, suggesting that in the former the myofibrils are supplied by a larger mitochondrial mass.

Although no definitive evidence exists, it appears reasonable to assume that the difference in mitochondrial mass is related to the higher contractile force of the left ventricle and the associated increased need in energy supply.

TABLE II
Per Cent of Cytoplasm Occupied by Mitochondria

Author	Per cent	Method
Arcasoy and Smuckler (6)	Left ventricle: 28.5	Point-counting
Kleitke et al. (3)	Left ventricle: 44	Planimetry
Kment et al. (11)	Left ventricle: 30.5	Planimetry
Laguens and Gómez Dunn (4)	Left ventricle: 40 Right ventricle: 36	Planimetry
Poche et al. (5)	Left ventricle: 32.85	Point-counting
Present paper	Left ventricle: 36.6 Right ventricle: 29.3	Point-counting

In this way the macroscopic difference existent in the development of the walls of both ventricles has also a counterpart at the ultrastructural level.

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