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The surface to volume ratio of mitochondria, a suitable parameter for evaluating mitochondrial swelling

Correlations during the course of myocardial global ischaemia *

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Summary. Cellular changes occuring in the left ventricular myocardium during ischaemia after different methods of cardiac arrest have been evaluated by morphological and morphometric parameters: volume densities of mitochondria (V_{VMi}), sarcoplasm (V_{VSp}), myofibrils (V_{VMf}), surface densities of mitochondria (S_{VMi}). The surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) has been used as an independent parameter of mitochondrial swelling.

Since ischaemic swelling of myocardial cells increases the volume of the reference space and ischaemic swelling of mitochondria decreases the free sarcoplasm, V_{VMi} and V_{VSp} cannot be considered as reliable indicators of the degree of oedema. S_{VMi}/V_{VMf} remains nearly constant after different forms of cardiac arrest, demonstrating the integrity of mitochondrial outer membranes. The inverse linear ratio between $S_V ratio_{Mi}$ and the mean mitochondrial volume indicates that the increase in mitochondrial volume is achieved by surface smoothing.

Loss of matrix structure and fragmentation of cristae occur at an $S_V ratio_{Mi}$ of about 5.8, cristolysis at 5.5 to 5.6 and amorphous matrix densities at an $S_V ratio_{Mi}$ of less than 5.5 $\mu m^2/\mu m^3$.

The $S_V ratio_{Mi}$ is a suitable parameter for evaluating mitochondrial swelling both at the onset and during global myocardial ischaemia, independent of the method of cardiac arrest used. It serves as an indicator of the state of structural preservation of mitochondria during ischaemia.

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Introduction

Global myocardial ischaemia induced by artificial cardiac arrest leads to an immediate loss of function, increasing energy deficit, alterations of metabolism and finally to changes in ultrastructure (Bretschneider 1964, Paulussen et al. 1968; Schaper et al. 1979; Jennings et al. 1983; Gebhard et al. 1989). These changes have been described qualitatively by several authors (Ferrans and Roberts 1971/72; Jennings and Hawkins 1980; Steen et al. 1983).

Ultrastructural investigation of left ventricular myocardium immediately after the onset and during the course of global ischaemia have shown that not only the decay of energy rich phosphates, but also the degree of cellular and particulary mitochondrial alterations is surprisingly variant, depending on the method of cardiac arrest and temperature (Schaper et al. 1979, 1986; Schnabel et al. 1983, 1987a; Gebhard et al. 1989).

Up to now volume densities usually have been used for the morphometric description of ischaemic changes (Trump et al. 1976; McCallister et al. 1978; Goldstein and Murphy 1983; Schaper et al. 1985). The surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) as a morphometric parameter independent of the reference space has been used by a few authors to characterize volume and size of mitochondria under normal and pathological

conditions (Wassilew and David 1977; Anversa et al. 1978; Mall et al. 1986), but has been applied only recently to the processes acting in ischaemia (Greve et al. 1988; Schmiedl 1988).

The aims of this study were: to quantify the course of defined ultrastructural alterations during pure global ischaemia and during ischaemia following perfusion with different cardioplegic solutions, to evaluate $S_V ratio_{Mi}$ as a parameter for mitochondrial swelling, to relate changes of $S_V ratio_{Mi}$ to other mitochondrial changes, and to evaluate how far $S_V ratio_{Mi}$ could serve as a general indicator for ultrastructural preservation of mitochondria during global ischaemia, independent of the method of cardiac arrest used.

Materials and methods

Canine hearts were subjected to global ischaemia at 25° C following cross-clamping of the aorta (pure ischaemia) or cardioplegic perfusion with St. Thomas-solution (composition in mmol/l: 91.6 NaCl, 14.8 KCl, 1.2 KH_2PO_4, 1.2 $CaCl_2$, 25 $NaHCO_3$, 1.2 Procaine·HCl, 1.2 $MgSO_4$, 15 $MgCl_2$) or HTK-solution (in mmol/l: 15 NaCl, 9 KCl, 4 $MgCl_2$, 180 Histidine, 18 Histidine·HCl, 30 Mannitol, 2 Tryptophan, 1 K-Ketoglutarate).

Samples of left ventricular myocardium were taken immediately after cross-clamping of the aorta or after the end of cardioplegic perfusion with St. Thomas- or HTK-solution. The left ventricle was then incubated at 25° C in Tutofusin® (composition in mmol/l: Na^+ 140, K^+ 5, Ca^{++} 2.5, Mg^{++} 1.5, Cl^- 153; Pfrimmer, Erlangen, FRG) during pure ischaemia or in the corresponding cardioplegic solution (Dr. F. Köhler, Alsbach, FRG). Anesthaesia and experimental procedures have been described earlier (Preusse et al. 1981; Gebhard et al. 1984).

At defined time intervals (Schnabel et al. 1987a) subendocardial tissue blocks of about 1 mm^3 were fixed by immersion in a modified fixation solution according to Karnovsky (1965). The fixative contained 1.5% glutaraldehyde, 1.5% paraformaldehyde plus 0.2 M sodium cacodylate buffer at pH 7.35. After frequent rinsing in 0.1 M sodium cacodylate buffer plus 4% sucrose, postfixation followed for 2 h with 1% OsO_4 in 0.1 M sodium cacodylate buffer plus 3.5% sucrose. Subsequent rinsing in sodium cacodylate buffer was followed by dehydration in graded series of ethanol. After treatment with propylenoxide specimens were embedded in araldite. All these steps were standardized, automated and carried out continuously (Histomat, bio-med, Theres, FRG). Semithin sections were stained with Toluidine-Blue for orientation to exclude myocardium at a distance of less than 400 ± 100 µm from the endocardium. Ultrathin sections were stained with uranyl acetate and lead citrate. Qualitative and quantitative investigations of the ultrathin sections were carried out with an EM 10 electron microscope (Zeiss, Oberkochen, FRG).

In each group 6 canine hearts were investigated. In each heart and at each time point 15 specimens were excised and 6 of them were chosen by chance for embedding. Three random blocks were cut according to the random sectioning system of Weibel (1979). 50 test fields per ultrasection were evaluated according to the systematic random sampling system (Weibel 1979).

All morphometric studies were performed on-line (TV-construction, Bosch special with monitor), using a 72 point test system with test lines. Provided that criteria of random selection are met, the volume density (V_{Va}) of a structure "a" is equivalent to the ratio of the lattice points falling on the structure (P_a) to the lattice points falling on the reference space (P_{ref}):

$V_{Va} = V_a/V_{ref} = P_a/P_{ref}$ (Weibel 1979).

For the determination of V_V the three points defined by the ends of the lines and the vertex of the lines of the test system were counted. The volume densities of mitochondria (V_{VMi}), of free sarcoplasm (V_{VSp}) and of myofibrils (V_{VMf}) were determined.

The surface density (S_V) of a structure, e.g. mitochondria, is the ratio of the total surface area to the volume of the reference space, in this case the myocardial cell. Provided that there is random orientation of the sectioning planes the general stereological equation for the determination of S_V is:

$S_{VMi} = 2I/L$ (Weibel 1979).

Where I is the total number of intersections of the test lines with the circumferences of the object and L the true length of the test lines within the reference space. Using our specific lattice test system which was developed for counting on sections of anisotropic tissue the equation then becomes:

$S_{VMi} = 2I/(P_{cell} \cdot Z/3)$

Where Z is the true length of the test lines used here and is the sum of the length of the two legs of the angle. This length is divided by three as this angle defines three test points as described above. The reference space for V_V and S_V is in all cases the myocardial cell volume.

The surface to volume ratio of particular structures, e.g. the mitochondria ($S_V ratio_{Mi}$), is determined by size and shape of the structures (Mall et al. 1986). The general stereological equation for the estimation of $S_V ratio$ on isotropic uniform random sections reads:

$S_V ratio = 2I/P \cdot Z$ (Weibel 1979).

Using our specific lattice test system the equation for the determination of the $S_V ratio_{Mi}$ becomes:

$S_V ratio_{Mi} = 2I/(P_{Mi} \cdot Z/3)$

P_{Mi} is the total number of test points overlying the mitochondria.

All results are given as mean values ± SEM unless indicated otherwise. Significant differences have been noted for P values of 0.05 or less using the Wilcoxon-Mann-Whitney (U)-test, for unpaired samples or the Wilcoxon matched pairs signed rank test.

Results

There are striking differences in myocardial fine structure at the onset and during the course of ischaemia depending on the method of cardiac arrest (Figs. 1a–3d). The morphological condition of the myocardial fine structure observed at various times after cardiac arrest by the indicated method are shown in Table 1 and in Figs. 1a–3d.

Immediately after the initiation of ischaemia there are remarkable morphometric differences in left ventricular ultrastructure depending on the different forms of cardiac arrest (Figs. 1a–3a, 4, 5, 6).

Table 1. Qualitative changes of working myocardium during global ischaemia at 25° C as shown in Figs. 1a–3d

	Pure Ischaemia				Cardioplegia with							
					St. Thomas solution				HTK solution			
Time of ischaemia	0	60	120	180	0	120	180	240	0	120	300	360
Cellular alterations:												
Cellular oedema	1–2	4	4	6	2–3	3–4	4	4	0	1	4	4
Mitochondrial alterations:												
Mitochondrial swelling	2	4	4–5	6–7	2	3	4	5–6	0	2	4	6–7
Loss of matrix granules	1	7	7	7	0–1	6–7	7	7	0	6	7	7
Clearings of matrix structure	1–2	4	6	6–7	1	4	5–6	6	0–1	2–3	5–6	6
Loss of matrix material	0	3	5	5	0	2	4–5	5	0	0	4	5
Fragmentations of cristae	0–1	4	5	7	0	3–4	5	5–6	0	0	5	6
Cristolysis	0	3–4	4	5–6	0	0	3–4	5	0	0	3	5–6
Amorphous matrix densities	0	0	1	3	0	0	0	1	0	0	0	0

grade of alterations: 0 = unchanged, 1 = slight, 2 = moderate, 3 = remarkable, 4 = striking, 5 = considerable, 6 = extreme, 7 = maximal

V_{VMi} (Fig. 4) immediately after aortic cross clamping ($22.2 \pm 0.9\%$) or after St. Thomas-perfusion ($20.8 \pm 0.8\%$) is considerably higher than after HTK-perfusion ($18.5 \pm 0.3\%$).

V_{VSp} (Fig. 4) after aortic cross clamping amounts to $8.8 \pm 0.5\%$; the cells are slightly swollen (Fig. 1a); after St. Thomas-perfusion it is $10.5 \pm 1.1\%$; the cells are remarkably swollen (Fig. 2a); and after HTK-perfusion it is $5.1 \pm 0.6\%$; the cells are unswollen (Fig. 3a).

V_{VMf} (Fig. 4) after the onset of pure ischaemia ($68.6 \pm 0.8\%$) and after St. Thomas-perfusion ($68.9 \pm 1.3\%$) are nearly identical and considerably lower than with HTK-perfusion ($76.3 \pm 0.6\%$).

S_{VMi} (Fig. 5) amounts to 1.39 ± 0.02 after the onset of pure ischaemia, to 1.44 ± 0.04 after coronary perfusion with St. Thomas-solution and to 1.47 ± 0.02 $\mu m^2/\mu m^3$ after coronary perfusion with HTK-solution.

S_{VMi} divided by V_{VMf} (Fig. 5) at the onset of pure ischaemia (2.02 ± 0.05 $\mu m^2/\mu m^3$) is slightly lower than after St. Thomas-perfusion (2.11 ± 0.09). After perfusion with HTK-solution it is 1.93 ± 0.05 $\mu m^2/\mu m^3$.

The $S_V ratio_{Mi}$ immediately after cross clamping at the onset of pure ischaemia amounts to 6.3 ± 0.2 $\mu m^2/\mu m^3$ (Figs. 6, 1a) – mitochondria are moderately swollen. Perfusion with St. Thomas-solution leads to values of 6.9 ± 0.02 $\mu m^2/\mu m^3$ (Fig. 2a) – mitochondria are moderately swollen. After HTK-perfusion the $S_V ratio_{Mi}$ of 7.8 ± 0.1 $\mu m^2/\mu m^3$ (Fig. 3a) is considerably higher when compared with the onset of pure ischaemia and immediately after the end of St. Thomas-perfusion – mitochondria are not at all swollen.

V_{VMi} (Fig. 4) after 180 min of pure ischaemia ($23.7 \pm 0.6\%$) does not show any significant increase when compared with the onset of ischaemia, although mitochondria are obviously swollen (Figs. 1a, d). 120 min after St. Thomas-perfusion V_{VMi} is significantly higher than immediately after the end of perfusion ($23.2 \pm 0.7\%$, $p < 0.05$, Fig. 2b). 300 min after perfusion with HTK-solution V_{VMi} has increased significantly to $23.0 \pm 0.8\%$ ($p < 0.05$).

Maximal values of V_{VMi} during ischaemia are reached after 180 min of pure ischaemia ($23.7 \pm 0.6\%$, Fig. 1a), 180 min after St. Thomas-perfusion ($23.6 \pm 0.5\%$, Fig. 2c) and 360 min after HTK-perfusion ($25.2 \pm 0.5\%$, Fig. 3d).

V_{VSp} (Fig. 4) during pure ischaemia already increases significantly to $11.8 \pm 1.0\%$ ($p < 0.05$) within the first 10 min. In contrast, after perfusion with both cardioplegic solutions V_{VSp} remains nearly constant during the first 10 min of ischaemia. There is a significant difference compared with the onset of ischaemia 180 min after St. Thomas-perfusion ($14.5 \pm 0.6\%$, $p < 0.05$) and 60 min after HTK-perfusion ($7.8 \pm 0.6\%$, $p < 0.05$). Maximal values of V_{VSp} are reached after 180 min of pure

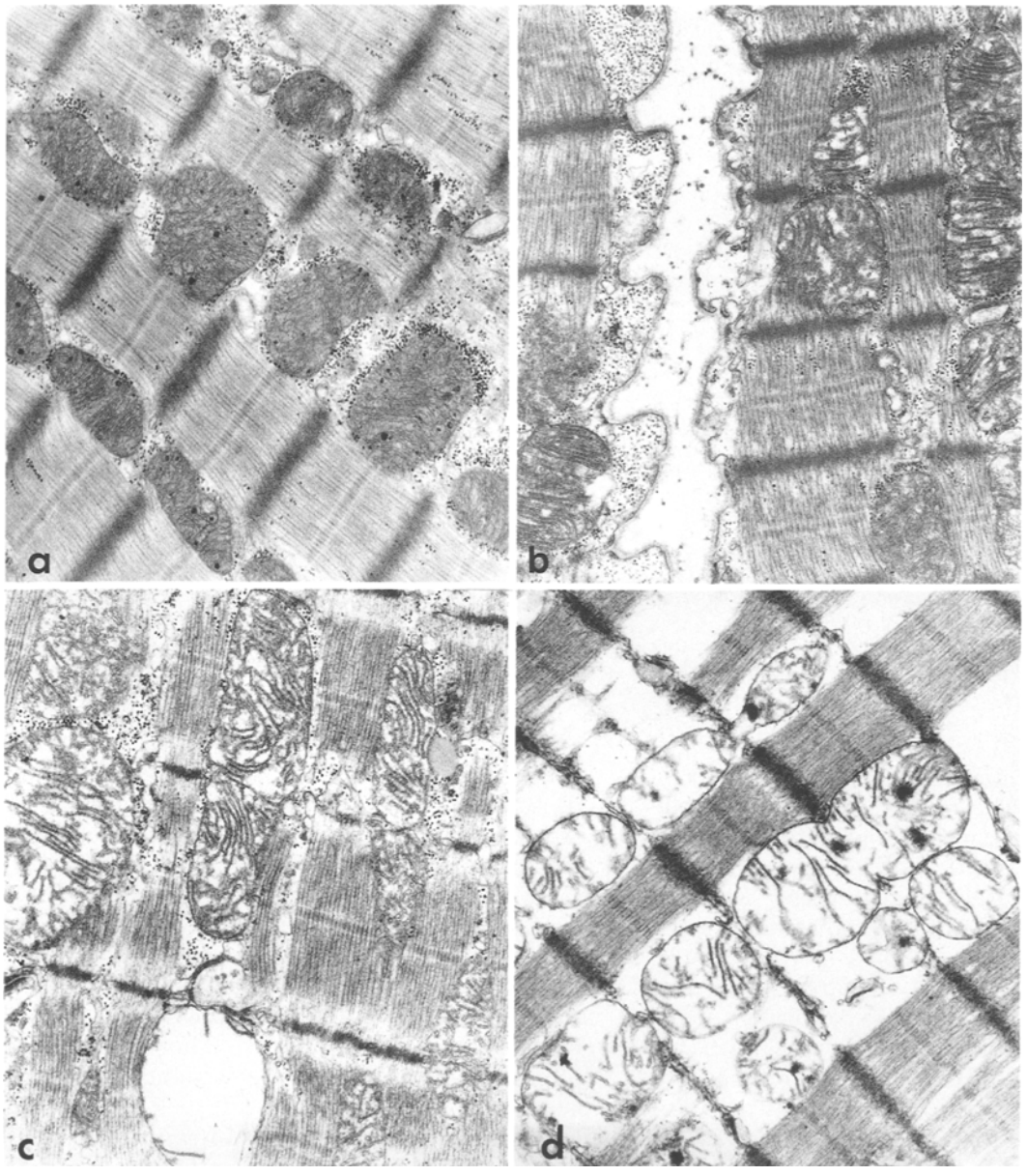

Fig. 1. Ultrastructure of left ventricular subendocardium during pure ischaemia at 25° C. **a** Immediately after aortic cross-clamping; **b** after 60 min; **c** after 120 min; **d** after 180 min. Final magnification ×19600

ischaemia (16.6±2.0%, Fig. 1d), 180 min after St. Thomas-perfusion (14.5±0.6%, Fig. 2c) and 300 min after HTK-perfusion (13.9±1.0%, Fig. 3c).

V_{VMf} (Fig. 4) after 30 min of pure ischaemia (64.8±0.7%) is significantly lower than at the onset ($p<0.05$). After St. Thomas- and HTK-perfusion the values have significantly decreased to 67.3±1.5% and 73.1±1.1% after 120 min and 60 min compared with the onset ($p<0.05$).

Minimal values are reached after 180 min of pure ischaemia (59.6±1.8%, Fig. 1d), 180 min after St. Thomas-perfusion (61.8±0.9%, Fig. 2c) and 360 min after HTK-perfusion (62.6±1.7%, Fig. 3d).

S_{VMi} (Fig. 5) after 60 min of pure ischaemia (1.22±0.03 $\mu m^2/\mu m^3$, Fig. 1b) shows a significant difference ($p<0.05$) compared with values at the onset. 240 min after perfusion with St. Thomas-solution S_{VMi} is reduced to 1.25±0.04 $\mu m^2/\mu m^3$ ($p<0.05$, Fig. 2d). 120 min after HTK-perfusion S_{VMi} is significantly lower (1.33±0.03 $\mu m^2/\mu m^3$, Fig. 3b) than immediately after the end of perfusion.

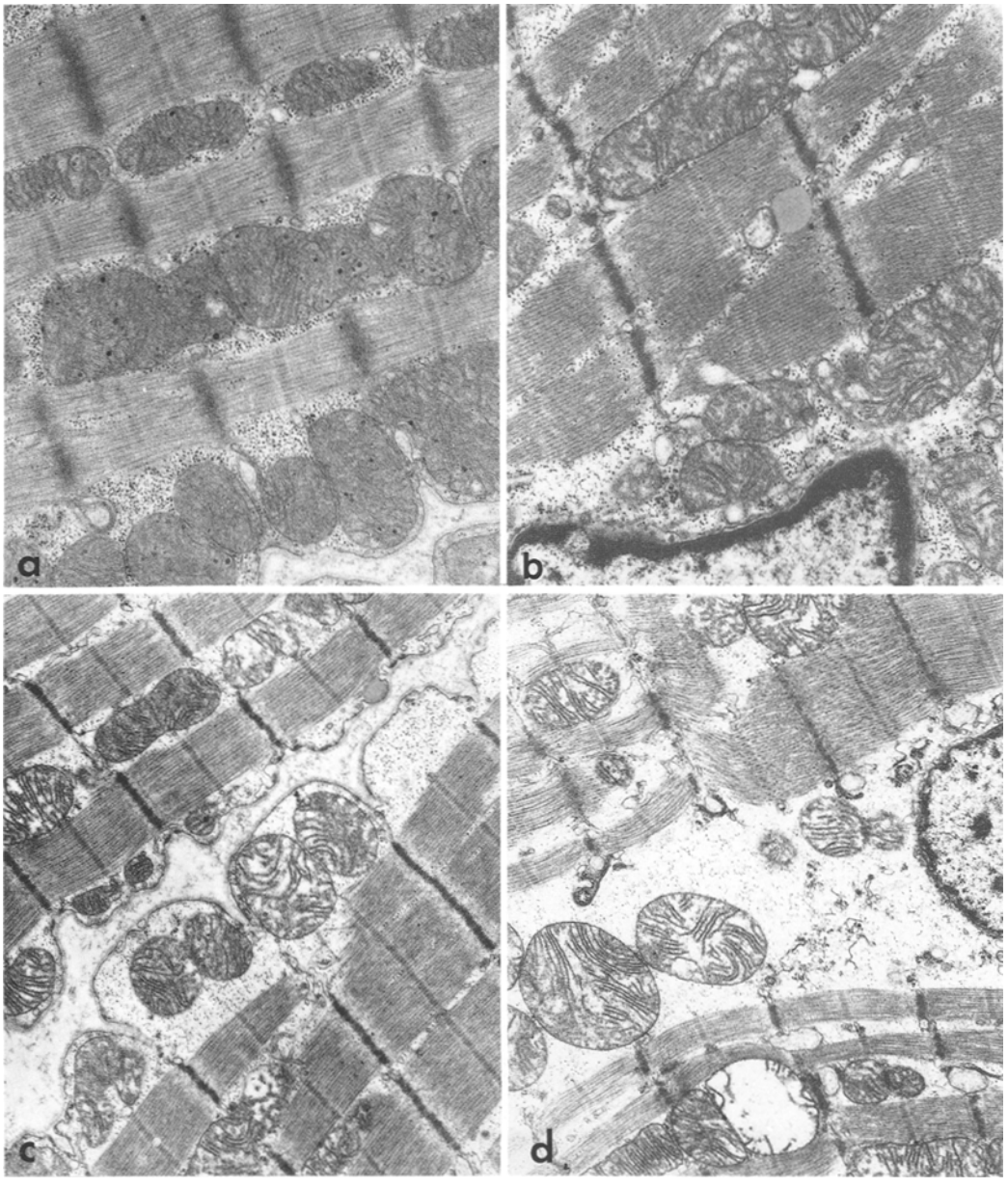

Fig. 2. Ultrastructure of left ventricular subendocardium following 4 min St. Thomas perfusion at 25° C. **a** Immediately after the end of coronary perfusion; **b** after 120 min global ischaemia; **c** after 180 min global ischaemia; **d** after 240 min global ischaemia. Nuclear chromatin shows advanced clumping. Final magnification ×19600

The surface density of mitochondria divided by the volume density of myofibrils (S_{VMi}/V_{VMf}, Fig. 5) during pure ischaemia and during ischaemia following St. Thomas- or HTK-perfusion does not show any significant changes.

$S_V ratio_{Mi}$ (Fig. 6) after 30 min of pure ischaemia ($5.7 \pm 0.1\ \mu m^2/\mu m^3$) is significantly lower ($p < 0.05$) than immediately after aortic cross-clamping. 120 min after coronary perfusion with St. Thomas-solution $S_V ratio_{Mi}$ is $5.9 \pm 0.2\ \mu m^2/\mu m^3$ (Fig. 2b), which is significantly lower than at the onset of ischaemia ($p < 0.05$). The $S_V ratio_{Mi}$ 120 min after perfusion with HTK solution ($6.6 \pm 0.2\ \mu m^2/\mu m^3$, Fig. 3b) shows a significantly lower value than immediately after the end of perfusion ($p < 0.05$).

Minimal values of $S_V ratio_{Mi}$ indicating an extreme swelling of mitochondria are reached after 180 min of pure ischaemia (5.1 ± 0.2, Fig. 1d), 240 min after St. Thomas-perfusion (5.5 ± 0.1, Fig. 2d), and 360 min after HTK-perfusion ($5.1 \pm 0.1\ \mu m^2/\mu m^3$, Fig. 3d).

Although mitochondrial changes during global

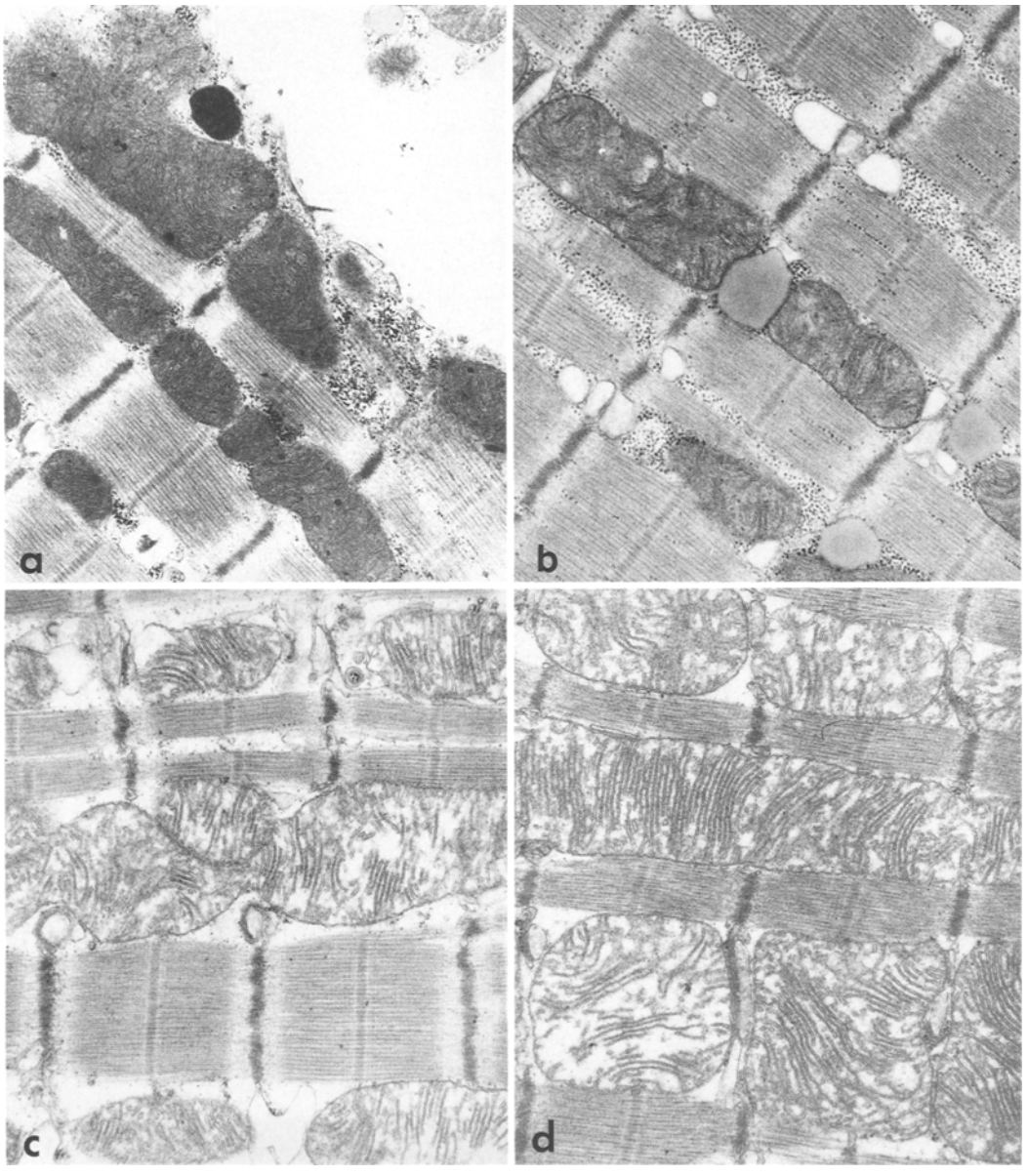

Fig. 3. Ultrastructure of the left ventricular subendocardium following 11 min HTK-perfusion at 25° C. **a** Immediately after the end of coronary perfusion; **b** after 120 min of global ischaemia; **c** after 300 min of global ischaemia; **d** after 360 min of global ischaemia. Final magnification ×19600

ischaemia at 25° C occur at very different times depending on the method of cardiac arrest there is a close correlation of $S_V ratio_{Mi}$ with structural changes of this organelle. Definite ranges of $S_V ratio_{Mi}$ can be related to certain mitochondrial changes (Table 2): –

Clearing of the matrix structure is observed (Figs. 1a, 3b) when $S_V ratio_{Mi} = 6.6 \pm 0.1\ \mu m^2/\mu m^3$; Local losses of matrix material and fragmentation of cristae (Figs. 2b) occur at an $S_V ratio_{Mi} = 5.8 \pm 0.1\ \mu m^2/\mu m^3$. Cristolysis and considerable loss of matrix structure (Figs. 1c, 2c, 3c) become obvious at an average $S_V ratio_{Mi}$ of about $5.6\ \mu m^2/\mu m^3$ and AMD are generally found in combination with nearly complete loss of matrix structure and cristolysis (Fig. 1d) and an $S_V ratio_{Mi}$ lower than $5.5\ \mu m^2/\mu m^3$.

Discussion

The morphometric results at the onset of ischaemia (Figs. 4, 6) agree only partly with reported morphometric data on the working myocardium (David 1979; Schaper et al. 1985; Canale et al. 1986).

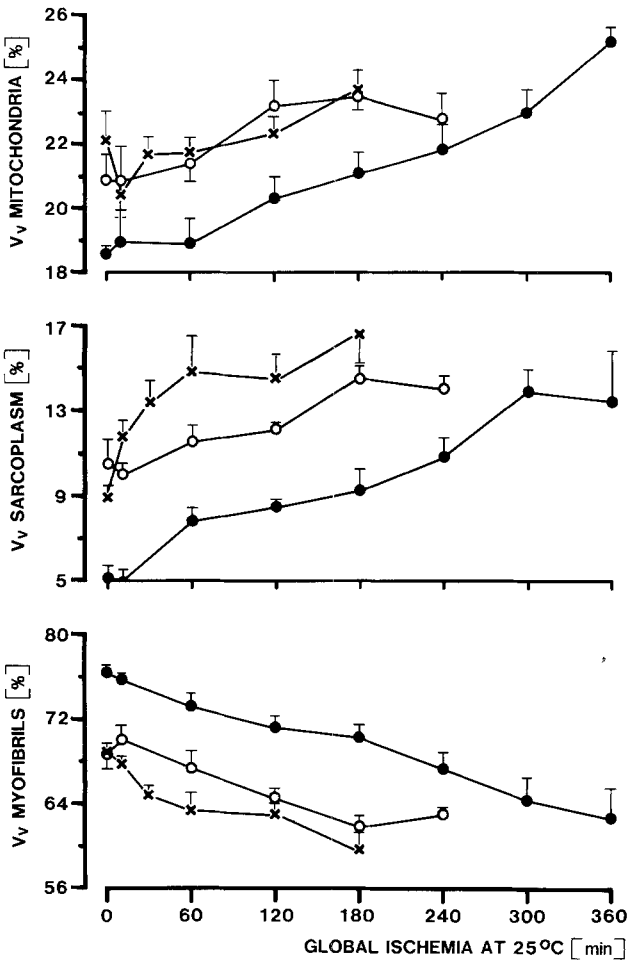

Fig. 4. Volume densities of mitochondria (V_{VMi}), sarcoplasm (V_{VSp}) and myofibrils (V_{VMf}) in percent of the myocardial cells as reference space in the course of ischaemia at 25° C following different methods of cardiac arrest, ×—× pure ischaemia; o—o St. Thomas; ●—● HTK. $\bar{x} \pm$SEM, $n=6$

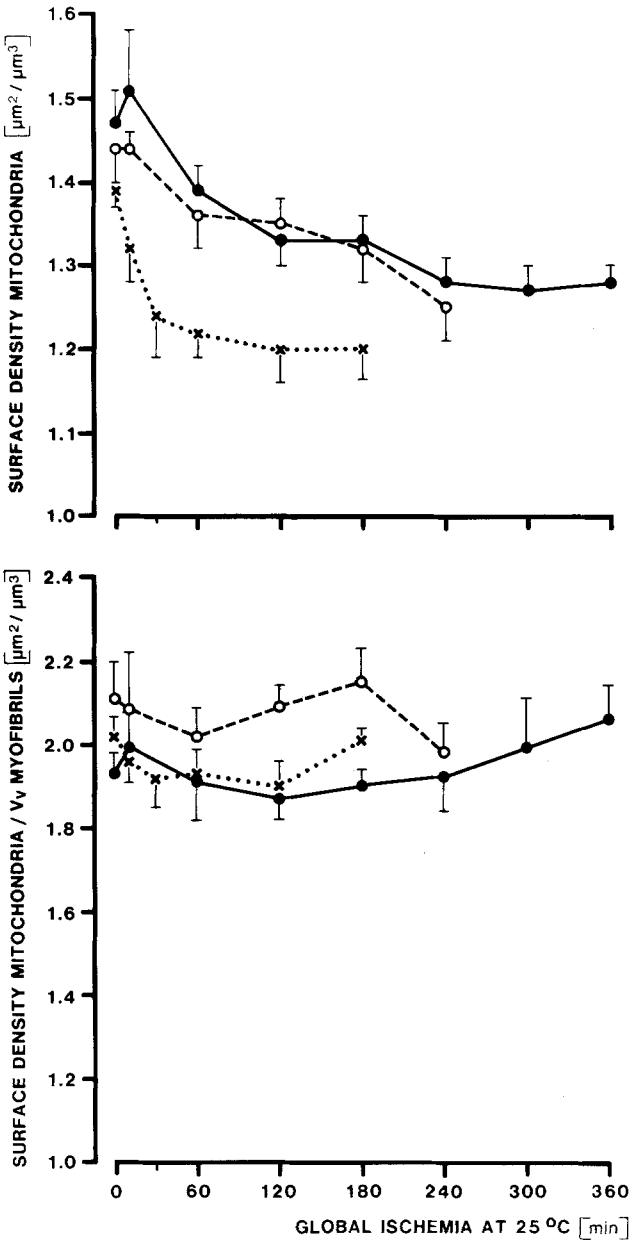

Fig. 5. Surface density of mitochondria (S_{VMi}) and surface density of mitochondria related to volume density of myofibrils (S_{VMi}/V_{VMf}) during global myocardial ischaemia at 25° C after different forms of cardiac arrest, ×—× pure ischaemia; o—o St. Thomas; ●—● HTK. $\bar{x} \pm$SEM, $n=6$

There are not only differences in the results in different species but even within one species there is a wide range of reported values, for example, from 13% (Trump et al. 1976) to 26% (McCallister et al. 1978) for V_{VMi} in dog hearts. The wide range of reported values for V_{VMi}, V_{VSp}, V_{VMf} and $S_V$ratio$_{Mi}$ is partially due to a natural biological inter- and intra-individual variation but can also be aggravated by differences in pretreatment, excision of samples, fixation technique and sampling statistics.

In order to compare the changes induced by ischaemia with the different forms of protection particular attention must be paid to reducing the variation caused by other factors as much as possible. Optimal sampling should compensate for the variance between individual animals. The range of variation in the measured results per test field requires that an appropriate balance between the number of animals, the number of tissue samples per animal and the number of test fields was chosen (see Materials and methods) as determined by calculations for efficiency according to Gunderson and Osterby (1981). Our sampling also compensates for the additional inhomogeneities often found during ischaemia (Schnabel et al. 1987b).

Many factors from fixation to microscopy can

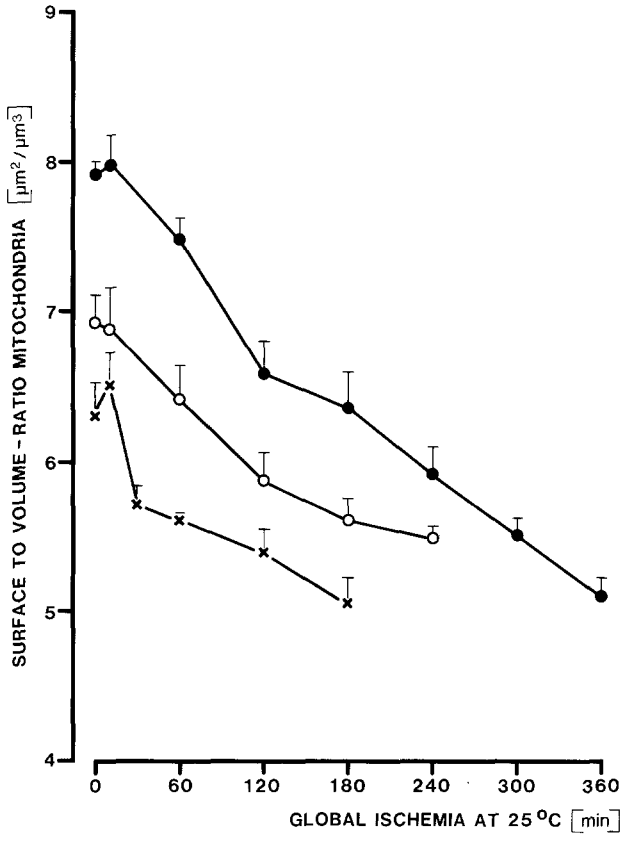

Fig. 6. Surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) in the course of global myocardial ischaemia at 25° C following different methods of cardiac arrest, ×—× pure ischaemia; o—o St. Thomas; ●—● HTK. $\bar{x} \pm SEM$, $n=6$

influence the final appearance of cardiac muscle cells, and therefore also may affect morphometric variables (Canale et al. 1986). For example, it has been shown that the use of discontinuous dehydration and embedding procedures may result in a reduction of cell volume (Tranum-Jensen et al. 1981). The composition of the fixative and particularly the osmolality and ionic composition have important influence on the volume of cells and organelles (Hayat 1981; Tranum-Jensen et al. 1981). A fixative which is not adjusted to the tissue osmolality will cause alterations of cellular volume (Tranum-Jensen et al. 1981).

The effective osmolality of our fixative as well as of the solutions for postfixation and washing procedures is about 440 mosmol/kg according to Hayat (1981). This osmolality does not enhance swelling of cells even in ischaemic myocardium, in which tissue osmolality is increased (Tranum-Jensen et al. 1981).

Fixation of cardiac muscle cells may be performed either by immersion or perfusion. During immersion fixation the quality of the tissue preservation is dependent on the diffusion of the fixative from the outer to the inner portions and thus on the size of the tissue blocks. In contrast, during perfusion fixation all areas of the myocardium are fixed equally (Hayat 1981).

Comparisons of the effects of immersion and perfusion fixation on morphometric parameters have given inconsistent results. In the feline right ventricle papillary muscle immersion fixation was found to result in higher V_{VMi} and lower V_{VMf} than perfusion fixation (Marino et al. 1983). However, other studies have reported that there are no differences in volume densities in the canine left ventricle working myocardium after fixation by immersion or perfusion (Schaper et al. 1985; Schmiedl et al. 1987).

Table 2. Correlation of qualitative structural alterations occuring in two thirds of mitochondria with the surface to volume ratio of mitochondria determined at the same time of ischaemia at 25° C ($\bar{x}$, $n=6$)

	Pure ischaemia		Cardioplegia with			
			St. Thomas solution		HTK solution	
	time (min)	$S_V ratio_{Mi}$ ($\mu m^2/\mu m^3$)	time (min)	$S_V ratio_{Mi}$ ($\mu m^2/\mu m^3$)	time (min)	$S_V ratio_{Mi}$ ($\mu m^2/\mu m^3$)
Clearing of matrix structure	10	*6.5*	30	*6.7*	120	*6.6*
Loss of matrix material	30	*5.7*	120	*5.9*	240	*5.9*
Fragmentation of cristae	30	*5.7*	120	*5.9*	240	*5.9*
Cristolysis	60	*5.6*	180	*5.6*	300	*5.5*
Amorphous matrix densities	180	*5.1*	300	*5.5*	450	*5.1*

The pretreatment of the heart also influences the structural preservation of the myocardium. Our results show that there is mitochondrial and cellular swelling after aortic cross clamping or coronary perfusion with St. Thomas-solution and subsequent fixation by immersion. In contrast, after coronary perfusion with HTK-solution and fixation by immersion there is no oedema of mitochondria or cells (Figs. 4, 6). The HTK-solution can therefore be regarded as suitable for perfusion before fixation (Schnabel et al. 1987c). Up to now many approaches have been considered suitable for the pretreatment before fixation, such as infusion of ice-cold modified Karnovsky's fixative into the left ventricle during partial occlusion of the aorta (Greve et al. 1988), normothermic ischaemic arrest by cross clamping of the aorta and subsequent perfusion fixation (McCallister et al. 1978), or isolated Langendorff-perfusion with an oxygenated Tyrode's solution before perfusion fixation (Marino et al. 1983).

The qualitative structural changes during ischaemia have been shown by other authors to depend on the method of cardiac arrest (Schaper et al. 1986; Schnabel et al. 1987a); Gebhard et al. 1989. Studies on morphometric parameters during pure ischaemia at 35° C (McCallister et al. 1978) and during ischaemia after cardioplegia with calcium antagonist containing solutions (Riede et al. 1982) have also been reported.

A comparison of the different methods of cardiac arrest at comparable times of ischaemia shows, on the basis of the results presented here, that there are no statistical differences in the course of V_{VMi}, V_{VSp} and V_{VMf} during pure ischaemia or after St. Thomas perfusion (Fig. 4). However, after 60 min of pure ischaemia $S_V ratio_{Mi}$ is considerably lower than 60 min after St. Thomas-cardioplegia (Fig. 6).

Thus, during prolonged ischaemia of more than 1 h St. Thomas-solution does not prevent mitochondrial and cellular swelling in comparison with pure ischaemia after aortic cross clamping.

180 min after HTK-perfusion V_{VMi} and V_{VSp} are significantly lower, V_{VMf} and the $S_V ratio_{Mi}$ significantly higher than during pure ischaemia or after St. Thomas-perfusion ($p<0.01$). This shows that in the course of ischaemia cellular and mitochondrial oedema are effectively postponed by HTK-perfusion compared with pure ischaemia or St. Thomas-perfusion, whether absolute times or ATP-concentrations are taken for comparison (Schaper et al. 1979, 1986; Schnabel 1983, 1987a).

In the present study the reference space was the total volume of myocardial cells (Weibel 1979). During ischaemia myocytes show a more or less pronounced swelling dependent on the method of cardiac arrest (Schaper et al. 1986; Schnabel et al. 1987a). Since this ischaemic swelling of myocardial cells increases the volume of the reference space, V_{VMi} cannot be considered as a reliable parameter of the extent of mitochondrial oedema although it has been so used in other studies (e.g. McCallister et al. 1978; Goldstein and Murphy 1983).

V_{VSp}, however, cannot serve as a direct parameter for changes in cell volume because it depends not only on changes in this variable but also on changes in mitochondrial volume (Fig. 4, Greve et al. 1988; Schmiedl 1988).

During the reversible phase of ischaemia when myofibrilolysis is not seen, the total volume of myofibrils would be expected to remain constant. This assumption is supported by the observation that the volume of myofibrils per unit volume of myocardial tissue remains constant until myofibrilolysis is observed (data not given). It should be emphasized that the total volume of myocardial tissue remains constant after cardiac arrest since swelling of myocardial cells reduces the volume of the interstitial space and vice versa. Thus V_{VMf} is inversely correlated with the extent of cellular oedema and V_{VMi}/V_{VMf} and V_{VSp}/V_{VMf} are estimators of mitochondrial and sarcoplasmic swelling, respectively up to the point when myofibrolysis occurs.

Assuming a constant form for outer mitochondrial membranes during ischaemia, the relative decrease of S_{VMi} in the course of ischaemia is caused by swelling of the muscle cell which is the reference space (Fig. 5). Division of a stereological density by V_{VMf}, which may be taken as an indicator of the extent of cell oedema, as described above, would then indicate absolute changes of a certain structure, in this case, the surface density of mitochondria. The ratio S_{VMi}/V_{VMf} is nearly constant in the course of ischaemia, up to the onset of myofibrilolysis, independent of the method of cardiac arrest (Fig. 5) indicating that there is no significant loss of mitochondrial outer membranes during ischaemia, and that swelling of mitochondria does not increase outer membrane surface area.

In order to arrive at a measurement of mitochondrial swelling which is independent of the behaviour of other structures and is applicable over the entire range of ischaemia we used the surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) which is determined by size and shape of the mitochondria only. Because of the importance of this ratio a few remarks on the mathematical relations to be expected under various circumstances may

be of some use. In the case of a spherical object, for example, since $V=4\pi r^3/3$ and $S=4\pi r^2$ where r = mean radius:

$S/V=S_V\text{ratio}=3/r$ (r = mean radius).

When such spherical structures are subject to swelling and remain spherical simple mathematical transformations give:

$S_1/S_2=(V_1/V_2)^{2/3}$,
$S_{V1}\text{ratio}_{Mi}/S_{V2}\text{ratio}_{Mi}=(V_1/V_2)^{-1/3}$,

and more generally we can write:

$S=kV^{2/3}$
$S_V\text{ratio}_{Mi}=kV^{-1/3}$

where k is a proportionality constant.

Alternatively, if swelling occurs without changes of the surface area (ellipsoids tend to become spheres) we obtain the following equation:

$S_{V1}\text{ratio}_{Mi}/S_{V2}\text{ratio}_{Mi}=(V_1/V_2)^{-1}$.

And thus, more generally:

$S_V\text{ratio}_{Mi}=kV^{-1}$

Generally, then, the relation between V and $S_V$ratio$_{Mi}$ can be written:

$\ln S_V\text{ratio}=\ln k-X\cdot\ln V$

where X = variable exponent shown in the exemplary cases above to vary between 1 and 1/3 and in practice, of course, may be anywhere between.

Changes of the ratio V_{VMi}/V_{VMf} obviously estimate changes of V as mentioned above and this term may be then substituted in the above equation. Linear regression analysis of all values from the various measurements here presented resulted in the equation:

$\ln S_V\text{ratio}_{Mi}=0.80-0.9\ln(V_{VMi}/V_{VMf})$ ($r=-0.887$)

($p<0.002$; rank correlation coefficient test of Spearman, Sachs 1978; limits of confidence (95%): $0.8455<\varrho<0.9217$).

An inverse linear relationship can thus be shown between $S_V$ratio$_{Mi}$ and the mean mitochondrial volume. The empirically determined exponent 0.9 indicates that swelling of mitochondria during the investigated phase of ischaemia occurs without major changes of the mitochondrial surface area indicating that the mitochondrial volume increase is achieved by rounding off and/or smoothing of the surface.

$S_V$ratio$_{Mi}$ is independent of the reference space and reacts sensitively to changes in mitochondrial shape and size (Mall et al. 1986). For the evaluation of mitochondrial swelling during ischaemia this parameter has been used by few authors (Greve et al. 1988; Schmiedl 1988).

For all methods of cardiac arrest the correlation of the $S_V$ratio$_{Mi}$ with other mitochondrial changes occuring in the course of ischaemia shows that there is a general correlation between this variable and the further structural alterations seen during global ischaemia (at 25° C) independent of the method used to induce cardiac arrest (Table 2). Furthermore, there is a close correlation between the $S_V$ratio$_{Mi}$ and the ATP-concentration in the left ventricular myocardium during global ischaemia (Schmiedl et al. 1988). For example, in the canine heart an $S_V$ratio$_{Mi}$ of nearly 5.75 $\mu m^2/\mu m^3$ corresponds to a left ventricular ATP-concentration of 4 µmol/gww; an $S_V$ratio$_{Mi}$ of about 5.5 $\mu m^2/\mu m^3$ corresponds to an ATP-concentration of 3 µmol/gww.

Thus, during global ischaemia, the $S_V$ratio$_{Mi}$ correlates strongly with both the occurrence of definite qualitative structural changes of mitochondria and with the ATP-concentration within the defined range in left ventricular myocardium. It can be concluded, therefore, that in myocardial ischaemia $S_V$ratio$_{Mi}$ estimates the degree of mitochondrial preservation or alteration adequately.

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