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Cellular morphometric changes in cat hearts subjected to three hours of regional ischaemia

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Summary. The left anterior descending coronary artery (LAD) was occluded for three hours in seven anaesthetized open chest cats. Seven cats served as sham operated controls. Biopsies were collected from the mid-myocardium of the normal, border and ischaemic zones as defined by fluorescein staining and verified by blood flow measurements with radiolabelled microspheres. In the sham operated hearts the biopsies were taken from the mid-myocardium of the lateral wall of the left ventricle. Fractional volumes of mitochondria, myofibrils and remaining cytoplasm as well as data on the outer mitochondrial membrane were obtained by standard point counting techniques. In the LAD occluded hearts we observed a morphologically distinct lateral border zone characterized by a greater swelling of the mitochondria than in the ischaemic zone. However, in this group a more marked oedema of the cytoplasm and a greater percentage of heavily damaged cells were observed in the ischaemic than in the border zone. The ischaemic zone also had the largest water content. Furthermore, cytoplasmic oedema ocurred in the normal zone of the LAD occluded cats when compared with the sham operated controls. This indicates that LAD occlusion also affects the normally perfused parts of the heart.

Key words: Coronary artery occlusion – Border zone – Morphometry – Cellular oedema – Mitochondrial swelling – Regional blood flow

Introduction

Myocardial infarction is a major cause of morbidity and mortality. A principal clinical goal has therefore been to reduce the myocardial vulnerability to ischaemia, to delay the development of irreversible ischaemic injury, and, finally to reduce the ultimate size of the infarct. A number of reports exists that deals with different drugs which appear to limit infarct size after acute coronary artery occlusion. Such reduction of infarct size has been reported to be achieved by the use of agents such as calcium channel blockers (Downey et al. 1985; Kudoh et al. 1986) and beta blockers (Vik-Mo et al. 1984). Unfortunately, the results reported in the literature have frequently been contradictory (Reimer and Jennings 1984; Yellon et al. 1985). Any infarct size reduction is probably a reduction of the number of cells within the area at risk that develop irreversible ischaemic injuries. In this hypoperfused area (Sládek et al. 1984) a mechanical (Homans et al. 1985; Sakai et al. 1985; Lima et al. 1985), electrophysiological (Ursel et al. 1985), biochemical, and ultrastructural (Hearse and Yellon 1981; Jodalen et al. 1985; Pirolo et al. 1985; Przyklenk and Groom 1983) border zone has been described. The localization, width and extent of cellular injury of this zone has, however, been controversial (Forman et al. 1985; Harken et al. 1981; Hearse and Yellon 1981; Sage 1986; Yellon et al. 1981). A transmural as well as a subepicardial location has been suggested for this zone (Gottlieb et al. 1981; Hearse and Yellon 1981). Furthermore, it has been suggested that the border zone most likely represents a sharp interface, a "border line", between normal and heavily injured cells (Sage 1986; Yellon et al. 1981). However, a true border zone might exist, where cells within a broader zone progress from normal to irreversibly injured ones.

It is also widely accepted that infarct size reduction will depend on the presence of a border zone of tissue to act as a target for the therapeutic intervention. In this study, therefore, we wanted to es-

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tablish whether a border zone exists which constitutes a morphologically and statistically distinct cell population different from the myocytes of the normal and ischaemic myocardium. Further, we have aimed at presenting variables that give evidence for the presence of this particular cell population during myocardial ischaemia. The variables generally used for characterizing ischaemic cell injuries, such as sarcolemmal, nuclear and mitochondrial membrane fragmentation, are signs of severe and irreversible injuries (Jennings et al. 1986; Ludatscher et al. 1984; Reimer and Jennings 1986). Cellular and mitochondrial swelling are signs of less extensive cell injuries (Jennings et al. 1985; Yellon et al. 1981), and might therefore be more favorable variables for measuring cellular alterations in the transition zone of the infarct.

In cat hearts with left anterior descending coronary artery occlusion we have, accordingly, examined mitochondrial, myofibrillar and cytoplasmic volume fractions, as well as the surface to volume ratio of mitochondria and the mitochondrial surface density. We have also studied the influence of acute coronary occlusion on the same variables in the normally perfused area of LAD occluded hearts when compared with sham operated hearts.

Material and methods

Fourteen cats of either sex with an average weight of 3.40 ± 0.61 kg (SEM), were initially anesthetized by sodium pentobarbital i.m. (35 mg/kg). Additional doses of 10 mg sodium pentobarbital were later given i.v. if necessary. The cats were tracheotomized, and ventilated with a gas mixture of 60% N_2O and 40% O_2 containing 5% CO_2 through a positive pressure ventilator (LOOSCO Infant Ventilator M.K. 2, Amsterdam, Holland). Adequate ventilation was controlled by arterial blood gas analysis (Radiometer BMS3 MK 2, Copenhagen, Denmark). A midline thoracotomy and a wide pericardiotomy gave access to the heart. A polyethylene catheter inserted into the left ventricle through the apex, was connected to a Statham P23 ID pressure transducer (Hato Rey, Puerto Rico) for continuous measurement (Hewlett Packard 8805C) of heart rate (HR), left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). Cardiac contractility (dP/dt) was registered by a differentiating unit (Hewlett Packard 8814A Waltham) connected to the pressure channel output. The left atrium was cannulated with a short catheter for injection of radiolabelled microspheres. For collection of reference blood, the abdominal aorta was cannulated via the left femoral artery. A catheter in the left femoral vein was used for infusion purposes. Body temperature was maintained at 37° C by an adjustable heat pad connected to a rectal thermistor, and by coverage of the open chest.

The left anterior descending coronary artery (LAD) was carefully dissected free and just distally to the left main stem junction a 4-0 silk ligature was loosely placed around the artery. After registration of stable haemodynamic variables, the cats were randomized into two groups. In the experimental group (7 cats) the LAD was permanently occluded by tightening the ligature, and in the sham group (7 cats) the ligature was left untightened.

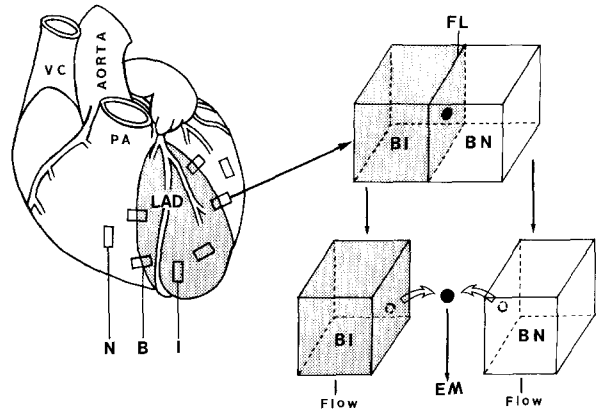

Fig. 1. Two specimens were sampled from the normal (*N*), ischaemic (*I*) zones and four from the border (*B*) zone. Each specimen was divided into two subsamples. Those from the border zone were divided in border normal (*BN*) and border ischaemic (*BI*) subsamples exactly along the fluorescein demarcation line (*FL*). Biopsies for electron microscopy (*EM*) were taken from the midwall portion at the cut surface between the paired subsamples. The subsamples were used in flow and water content measurements

Three minutes before sacrifice, approximately $1\text{–}2 \times 10^6$ microspheres were injected for measurements of regional blood flow. The microspheres had an average diameter of 15.6 ± 0.5 μm (SD) and were labelled with ^{46}Sc, ^{81}Sr, ^{113}Sn or ^{141}Ce, selected at random. The reference blood sample was collected by a constant rate extraction pump (Sage Instruments 351, Cambridge, MA, USA), in two minutes, starting ten seconds before, and terminating fifty seconds after the injection of microspheres, which lasted for sixty seconds. The blood samples were weighed, and the exact withdrawal rate calculated.

Three hours after occlusion or sham operation, 0.8 ml of 10% fluorescein (Fluorescite, Elkins Sinn Inc, Cherry Hill, NJ USA) was injected into the left atrium for visual demarcation of the ischaemic myocardium. Immediately thereafter the cats were killed by perfusion fixation. 50 ml of ice cold modified Karnovsky's fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose and 1.25 mM $CaCl_2$ (pH = 7.4 and vehicle osmolality = 300 milliosmol), was injected into the left ventricle during partial occlusion of the ascending aorta. The hearts were rapidly removed from the animal, the right ventricle and the atria removed, and the left ventricle opened along the posterior septal margin and placed in ice cold modified Karnovsky's fixative.

The hearts were cut under ultraviolet illumination. The normally perfused myocardium, stained by fluorescein, was separated from the non-fluorescent ischaemic myocardium by a sharp demarcation line. In the LAD occluded group (Fig. 1) eight transmural specimens were collected; two from the ischaemic myocardium, four from the lateral margin of the ischaemic zone with the demarcation line in the middle, and two from the non-ischaemic myocardium. In the sham operated cats bright fluorescence was seen in the entire cut surface of the left ventricle, and four transmural specimens were taken from these hearts.

Every specimen was divided into two subsamples, those from the lateral margin of the ischaemic area exactly along the fluorescein demarcation line. The subsamples together with the reference blood sample were counted for γ-emission in a

multichannel counter (CompuGamma 1282, LKB Wallac Company, Turku, Finland), and, regional myocardial blood flow and cardiac output calculated (Heyman et al. 1977). After counting, all subsamples were dried at 73° C for three days, reweighed, and the water content calculated.

Biopsies for electron microscopy were taken from the midwall portion at the cut surface between the paired subsamples of each of the specimens (Fig. 1). For ultrastructural studies of the border zone, we selected the biopsies from the two specimens, from the lateral margin of the ischaemic area, with the largest difference in flow values between adjacent subsamples. The biopsies from the border zone reached maximum 1 mm from the fluorescein line in either normal or ischaemic direction. In sham hearts no evidence of ischaemia was found as judged from the overall bright fluorescence and the myocardial blood flow measurements. Two biopsies, selected at random, were prepared for electron microscopy.

After removing the biopsies from the heart, fixation was continued by immersion in the modified Karnovsky's fixative. The biopsies were postfixed in 1% OsO_4 in cacodylate buffer of the same osmolality, and stained en bloc with uranyl acetate before embedding in Epon. Ultrathin sections stained for fifteen minutes with lead citrate, were used for electron microscopy.

In the seven cats with LAD-occlusion four micrographs (19×24 cm) at magnification of 9600, were taken from both biopsies from all three zones. Four micrographs were taken from both biopsies in all sham operated cats. Altogether, a total number of 224 micrographs were analyzed. Standard point counting techniques were used for measuring fractional volumes according to the Delesse principle (Chalkley et al. 1949; Weibel et al. 1966) by superimposing a grid lattice with a distance of 25 mm between the lines. A total number of seventy intersections of grid lines fall within each micrograph. Intersections of grid lines falling on the mitochondria, the myofibrils or the rest of the cytoplasm were counted in separate channels on a morphometric analyzer (CBM Inc. Model 8032, Santa Clara, CA, USA). Fractional volumes of these main cellular components were calculated as the ratio of points counted for that particular organelle to the total number of points counted in the cell, as shown for mitochondria in this equation:

$$\text{Fractional volume of mitochondria} = \frac{\text{Mitochondrial volume}}{\text{Cell volume}} = \frac{P_{mit}}{P_{cell}}$$

where the cell volume is exclusive of the nucleus, P_{mit} is the number of intersections of grid lines falling on the mitochondria, and, P_{cell} is the total number of intersections falling on the cell exclusive of the nucleus.

Data on the mitochondrial surface was obtained by counting intercepts of the outer mitochondrial membrane with both vertical and horizontal lines in the superimposed grid lattice with correction for the magnification of the micrographs and the distance between the grid lines. Surface density i.e. the ratio of mitochondrial surface to total cellular volume exclusive of the nucleus, was obtained from the equation (Weibel et al. 1966):

$$\text{Surface density} = \frac{S_{V\text{mit}}}{V_{V\text{Cell}}} = \frac{I_{mit}}{a * P_{Cell}}$$

where $S_{V\text{mit}}$ is the mitochondrial membrane area, $V_{V\text{Cell}}$ is the volume density of the cell, I_{mit} is the total number of intercepts, and, a is the distance between the grid lines divided by the magnification of the micrograph. The mitochondrial surface to volume ratio was similarly calculated:

$$\frac{S_{V\text{mit}}}{V_{V\text{mit}}} = \frac{I_{mit}}{a * P_{mit}}$$

To minimize the effects of a possible anisotropy, all morphometric measurements were performed with the lines in the grid lattice both at 0° and 15° to the edge of the micrographs. In the further calculations the mean of the two values obtained at both angles were used. The point counting was performed by two observers separately. The morphometric data were by one of the observers collected blindly. The interobserver variability was less than 5%.

For the statistical analysis of the morphometric data, we used a one way four level nested analysis of variance using the program BMDP8V, and Scheffe's multiple-comparison method to compare the results obtained from each zone in the LAD-occluded hearts. The percentage of heavily damaged cells were quantified in the micrographs, used for the morphometric measurements. According to prior works we regarded cells showing sarcolemmal, nuclear and mitochondrial membrane fragmentation as being severely injured. In micrographs with the same magnification, but with the myocardial cells cut strictly longitudinally, we measured the sarcomere length. The results from these measurements were tested by a one way two level analysis of variance.

We also calculated the percentage water content in each specimen. Both the difference in water content and blood flow measurements were tested by a one way analysis of variance, and, the Scheffe's multiple-comparisons method was used to compare the results between the various groups and zones.

All results from measurements of haemodynamics, blood flow, water content and morphometry, are given as mean values $\pm$SEM. (Significant differences noted for p-values of 0.05 or less).

Results

No significant difference between groups was demonstrated in body weights or pre-occlusion values of HR, LVEDP or dP/dt (Table 1). Pre-occlusion LVSP was significantly higher in sham operated cats (139 ± 4 mmHg) than in cats with LAD occlusion (114 ± 6 mmHg). In the sham operated cats dP/dt increased from 3357 ± 266 mmHg/s to 3821 ± 312 mmHg/s during the 3 h observation time, whereas, HR, LVSP and LVEDP remained unchanged.

LAD occlusion resulted in a sudden decrease in contractility (results not shown in the table), followed by a gradual increase during the three hours of ischaemia from a pre-occlusion value of 3125 ± 290 mmHg/s to 3607 ± 243 mmHg/s three hours post occlusion. Following the LAD occlusion, LVEDP increased from 5.4 ± 0.5 mmHg to 8.7 ± 1.4 mmHg, and remained elevated throughout the ischaemic period. The latter was significantly higher than the LVEDP in the sham group three hours after operation. There was no difference in cardiac output between the two groups measured after 3 h of observation.

Myocardial blood flow (Table 2) in the non-ischaemic regions of hearts with LAD occlusion (1.63 ± 0.17 ml/min/g) did not differ from the flow in the sham operated hearts (1.72 ± 0.28 ml/min/g).

Table 1. Haemodynamic registrations in 7 sham operated and 7 cats with LAD-occlusion. Mean values ±SEM

Groups	Pre-occlusion	3 h post-occlusion	Statistics[a]	
			Within	Between
Heart rate (beats/min)				
Sham	183 ± 10	190 ± 8	NS	NS
LAD occluded	169 ± 12	190 ± 12	NS	
Left ventricular systolic pressure (mmHg)				
Sham	139 ± 4	139 ± 10	NS	$P<0.05$
LAD occluded	114 ± 6	111 ± 6	NS	
Left ventricular end-diastolic pressure (mmHg)				
Sham	3.9 ± 0.7	4.4 ± 0.7	NS	NS
LAD occluded	5.4 ± 0.5	8.7 ± 1.4	$P<0.005$	
dP/dt[b] (mmHg/s)				
Sham	3357 ±266	3821 ±312	$P<0.03$	NS
LAD occluded	3125 ±290	3607 ±243	$P<0.03$	
Cardiac output (ml/min)				
Sham		287 ± 46		NS
LAD occluded		194 ± 19		

[a] Statistics by ANOVA for repeated measurements. Within denotes significant differences within groups at different time points. Between denotes significant differences in preocclusion values between the two groups

[b] dP/dt is the first derivative of left ventricular pressure

Table 2. Regional blood flow and water content in sham operated cats and cats with LAD-occlusion. Mean values ±SEM

Zones	Blood flow (ml/min/g)		Water content[a]	
Sham ($N=7$)	1.72±0.28		74.95±0.60	
LAD occluded ($N=7$)				
normal	1.63±0.17		76.06±0.88	
border normal[b]	1.74±0.16		77.10±0.40	
border ischaemic[c]	0.20±0.06	**	78.63±0.49	
ischaemic	0.06±0.02	**	79.16±0.44	*

[a] $\frac{(\text{wet weight} - \text{dry weight}) \times 100}{\text{wet weight}}$

[b] Specimen taken from the normally perfused side of the fluorescein demarcation line

[c] Specimen taken from the ischaemic perfused side of the fluorescein demarcation line

* Significant different from normal and sham operated values

** Significant different from normal, border normal and sham operated values

In cats with LAD occlusion, ischaemic blood flow was clearly reduced three hours after coronary artery occlusion (0.06±0.01 ml/min/g). The flow was reduced by 96% when compared with normally perfused myocardium. In the ischaemic subsamples from the demarcation line, tissue blood flow was reduced by 88% to 0.20±0.06 ml/min per g, indicating that little normally perfused tissue was included in the ischaemic subsamples of the border zone. In the non-ischaemic subsamples of the borderline no flow reduction was observed (1.74±0.16 ml/min/g). Judged from these results, it is fair to state that the biopsies representing the border zone tissue, were taken from the narrow interface between ischaemic and non-ischaemic myocardium.

Water content (Table 2) in myocardial tissue from sham operated cats was 74.95±0.60% and was not different from the 76.06±0.88% found in the normal zone of LAD occluded hearts. The latter was not significantly different from the 77.10±0.40% found in the non-ischaemic part of the border zone. In the ischaemic zone there was a significant higher water content (79.16±0.44%) than in the normal zone, whereas the increase was not significant in the ischaemic subsamples of the border zone (78.63±0.49%). The increased water content may partly be due to the cellular oedema.

Fractional volumes of mitochondria ($V_{V\text{mit}}$), myofibrils ($V_{V\text{myo}}$) and remaining cytoplasm ($V_{V\text{cyt}}$) in the normal zone of permanently LAD ligated hearts and in sham operated hearts are shown in Fig. 2. In the normal zone of LAD occluded hearts $V_{V\text{cyt}}$ (8.44±0.50%) is significantly higher, and $V_{V\text{myo}}$ (63.65±0.65%) is significantly lower than in the sham operated hearts (2.84±0.32% and 70.10±0.68% respectively), suggesting cellular oedema in the normal zone of LAD occluded hearts. There is, however, no difference between the two groups in $V_{V\text{mit}}$, surface density and mitochondrial surface to volume ratio, as shown in Figs. 2, 3.

The fractional volumes of the main cytoplasmic components in the normal, border and ischaemic zones of hearts in the LAD-occlusion group are illustrated in Fig. 4, and the surface densities as well as mitochondrial surface to volume ratios in Fig. 5. There is a more extensive cytoplasmic oedema in the ischaemic (Fig. 8) than in the border zone (Fig. 7) as suggested by a larger increase in $V_{V\text{cyt}}$ (14.66±0.98% and 11.22±0.70% respectively) but there is no difference in $V_{V\text{myo}}$ (57.79±0.98% and 57.85±0.85% respectively). This was confirmed by the higher water content in the myocardium of these zones (Table 2). $V_{V\text{mit}}$ is found to be highest in the border zone (31.01±0.66%) as compared to the ischaemic

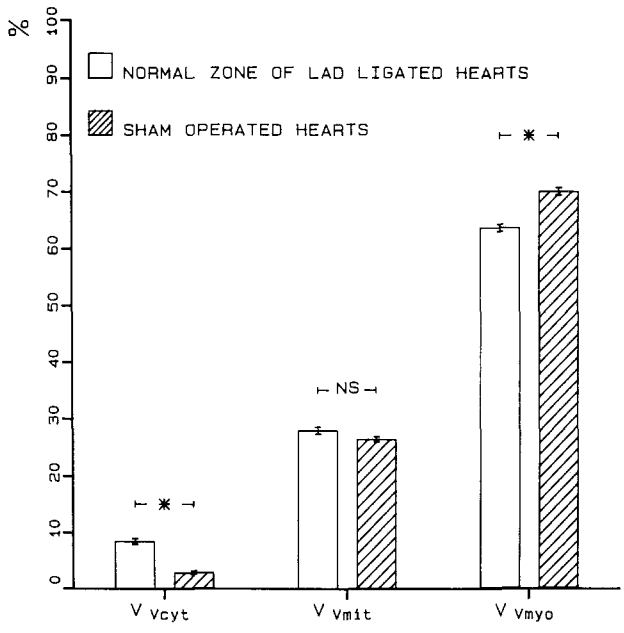

Fig. 2. Fractional volumes (%) of cytoplasm (V_{Vcyt}), mitochondria (V_{Vmit}) and myofibrils (V_{Vmyo}) in normal zone of LAD occluded hearts ($N=7$) vs. sham operated hearts ($N=7$). Mean values ±SEM. *indicates significant differences noted for p-values of 0.05 or less. NS denotes non-significant differences

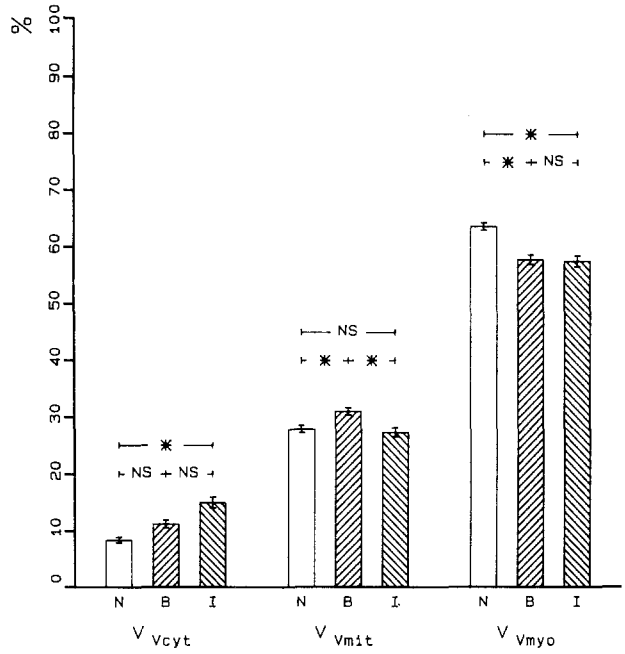

Fig. 4. Fractional volumes (%) of cytoplasm (V_{Vcyt}), mitochondria (V_{Vmit}) and myofibrils (V_{Vmyo}) in the normal (N), border (B) and ischaemic (I) zones in the LAD occluded hearts ($N=7$). Mean values ±SEM. *indicates significant differences with p-values of 0.05 or less. NS denotes non-significant values

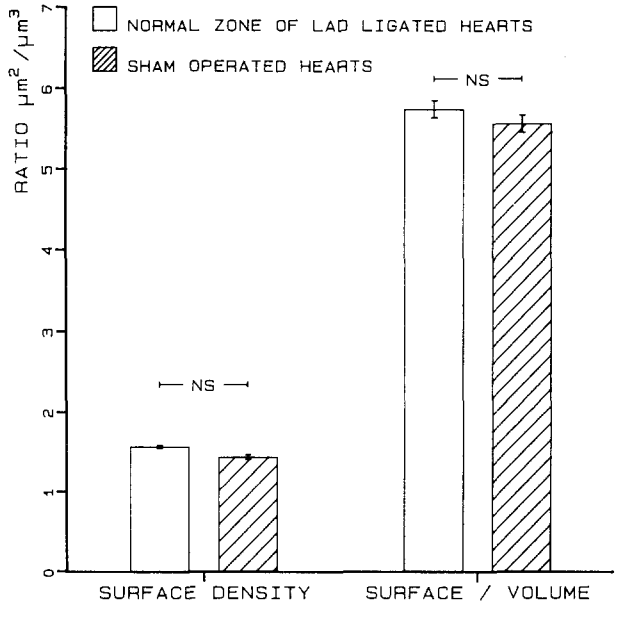

Fig. 3. Mitochondrial surface to volume ratios and mitochondrial surface density in normal zone of LAD occluded hearts ($N=7$) vs. – sham operated hearts ($N=7$). Mean values ±SEM. No significant (NS) differences observed

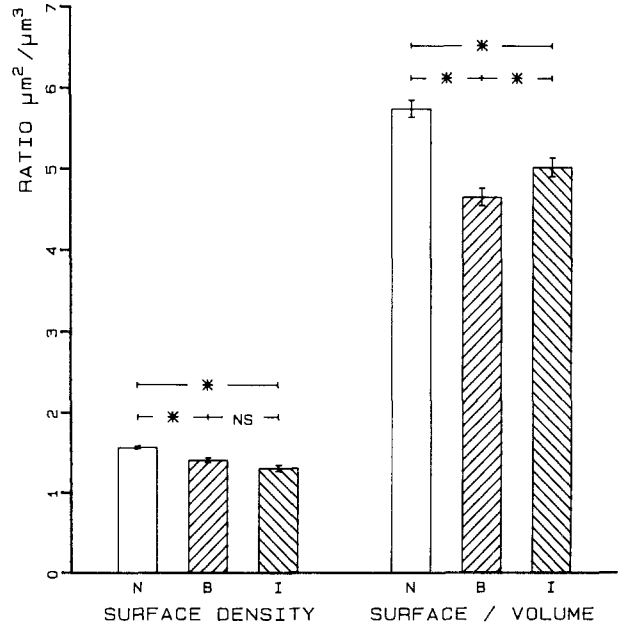

Fig. 5. Mitochondrial surface to volume ratios and mitochondrial surface density in normal (N), border (B) and ischaemic (I) zones in LAD occluded hearts ($N=7$). Mean values ±SEM. *indicates significant differences with p-values of 0.05 or less. NS denotes non-significant values

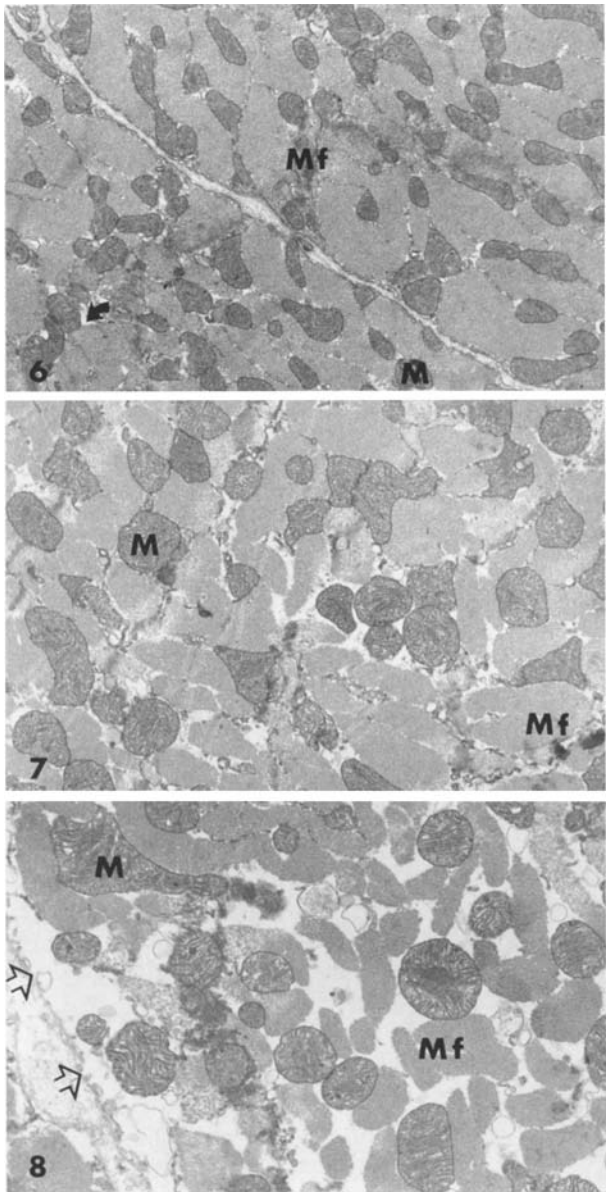

Fig. 6. Normal zone. Well preserved ultrastructure of myocytes 3 hours after LAD occlusion. A moderate cellular oedema was detected by morphometry. M denotes mitochondria, Mf myofibrils and arrow cytoplasm. ×6720

Fig. 7. Border zone. The tissue is characterized by an increasing cellular oedema and pronounced swelling of mitochondria (*M*). Mf denotes myofibrils. ×6720

Fig. 8. Ischaemic zone. There is an extensive damage of the myocytes as shown by a considerable intracellular oedema and a fragmentation of the sarcolemma (*arrows*). M denotes mitochondria and Mf myofibrils. ×6720

(27.45±0.82%) and normal zones (27.86±0.66%), and, the surface to volume ratio of the mitochondria is lowest in the border zone (4.65±0.11 $\mu m^2/\mu m^3$) when compared with the ischaemic (5.02±0.12 $\mu m^2/\mu m^3$) and normal zones (5.74±0.11 $\mu m^2/\mu m^3$), indicating that the border zone is containing the largest mitochondria. The surface density of mitochondria is reduced both in the border (1.56±0.02 $\mu m^2/\mu m^3$) and ischaemic zones (1.30±0.04 $\mu m^2/\mu m^3$).

Table 3. Percent severely injured cells[a] in the sham operated hearts vs. the normal, border and ischaemic zones of the hearts with LAD-occlusion

	Normal	Zones border	Ischaemic
Sham ($N=7$)	2%		
LAD occluded ($N=7$)	1%	27%	53%

[a] Cells with sarcolemmal, nuclear or mitochondrial fragmentation were regarded as severely injured cells. The quantification was performed in the micrographs used for morphometry

We observed no significant difference in sarcomere length between the two groups (2.27±0.07 μm in the sham group and 2.07±0.05 μm in the normal zone of the LAD occluded hearts). Nor did we observe any difference between the three zones in the LAD occluded group (2.12±0.05 μm in the border zone and 2.08±0.05 μm in the ischaemic zone). All values are in accordance with the normal sarcomere length found by Fawcett and McNutt (1969). This indicates that there was no significant difference in contraction state of the myocytes neither between the three zones in the LAD occluded hearts nor as compared with the sham operated hearts.

A larger percentage of severely damaged cells (Figs. 6–8) is observed in the ischaemic zone (53%) than in the border zone (27%) (Table 3). There is no difference in the number of damaged cells between the normal zone of the infarcted hearts (2%) and the sham operated hearts (1%).

Discussion

Despite many reports on the existence and composition of a lateral border zone surrounding the ischaemic area, our understanding of this particular zone is still incomplete. The presence and width of an ischaemic gradient at the lateral boundaries of the ischaemic zone is controversial. Recently, Reimer and Jennings (1986) have claimed that the currently most favoured concept for a border zone in the subendocardium is a sharp lateral interface between the normal and ischaemic tissue, and that applying the concept of a lateral border zone might be a misinterpretation of a mixed population of

normal and necrotic cells. In the subendocardium, however, the concept of a lateral border zone is probably more acceptable (Reimer and Jennings 1986).

The most important finding of the present study was the demonstration of a morphologically and statistically distinct border zone in the midmyocardium with respect to mitochondrial size in the cats following three hours LAD occlusion. Thus, as also reported by Cox et al. (1968), we observed a greater mitochondrial swelling in the border zone than in the ischaemic zone. Using the same model, Jodalen et al. (1985) have reported an accumulation of lipid droplets in the border zone. A preliminary study in our laboratory suggests that the latter covers a width of 1.5–2.0 mm across the border area. These results do not support the concept of a sharp but irregular interface between normal and ischaemic cells, but rather indicate the presence of a border zone with progressive cellular damage. This would implicate that this zone with respect to mitochondrial swelling and lipid droplet accumulation constitutes a morphologically distinct cell population.

The sudden drop in dP/dt and the rise in LVEDP following coronary artery occlusion result from the very early loss of contractile power in the ischaemic region of the left ventricle. This will influence the global mechanical function of the heart. Compensatory mechanisms in the remaining non-ischaemic myocardium account for the gradual restoration of the left ventricular function. LVEDP remained elevated in cats with LAD occlusion. Both in the cats with coronary artery occlusion and in sham operated cats, dP/dt was significantly higher at the end of the experiment than at the beginning. Since this ocurred in both groups, it is probably related to the anaesthetic and experimental procedure.

The percentage of severely damaged cells was quantified in the micrographs used for morphometry. The fact that each of the micrographs usually covered a fraction of the cell only, might have lead to an underestimation of the number of severely injured cells. This also applied to micrographs in which the entire cell was present, as it is evident that only a tiny amount of the total cell volume is being covered by each section. However, this underestimation would probably affect the corresponding zones of both groups to the same extent, and the relative differences between the different zones are rather larger than smaller.

In the present study, we have shown a moderate cellular oedema in the non-ischaemic zone of the experimental hearts as compared with the sham operated hearts. This indicates that the so called normal zone undergoes morphological and functional alterations during the period of LAD occlusion which should be considered when assessing the changes in the border and ischaemic zones.

Species dependent differences in extent and progress of myocardial ischaemic injury due to different collateral blood flow patterns, have been reported (Schaper 1984). In species supposed to have an extensive coronary collateral circulation, like dogs, cats and guinea pigs, a transmural progression of cell death during myocardial ischaemia has been described (Reimer and Jennings 1979; Reimer et al. 1977). Here, we have not studied the presence of a transmural progression of ischaemic damage in the cat hearts. We can, therefore, not exclude that the midmyocardium at the fluorescein demarcation line is also affected to some extent by the transmural progression of ischaemic injury.

When evaluating the presence of a lateral border zone, the results would largely be dependent on the type of variables studied. In our study we have focused primarily on morphometric indices on cellular and mitochondrial swelling. When studying fractional volumes, only relative changes in the volume of different parts of the cell can be detected provided that the contraction state of myofibrils is constant. In this study we observed no changes in the state of contraction by measuring the sarcomere length. By also recording the surface area of a membranous bound compartment (the mitochondria) and relating it to the cellular or its own volume, one might uncover true volume changes providing there is a similar shape of the structures under study. These variables would probably be more sensitive than the extensively used variables, like sarcolemmal, mitochondrial and nuclear membrane fragmentation (Kloner and Braunwald 1980; Kloner et al. 1977). Thus, while we have shown a greater mitochondrial swelling in the border than in the ischaemic zone, the cellular oedema is equal in the two zones. However, the non-different mitochondrial surface density and the non-different fraction of myofibrils, indicate a different distribution of intracellular fluid between the two zones. There is a sarcoplasmic oedema in the ischaemic zone and mitochondrial swelling in the border zone. The qualitatively assessed cell damage in the border zone is, however, less developed than in the ischaemic zone.

The cellular oedema observed in the ischaemic zone of cat hearts, also reported by other authors is probably connected to the accumulation of metabolites and increased osmolality of the tissue as

the blood flow is reduced. The necrotic myocytes loose volume control and the electrolyte distribution becomes similar to that of the extracellular space (Kloner and Braunwald 1980). Furthermore, a reduced matrix density of the mitochondria following swelling of the mitochondria has been reported during cardiac ischaemia (Kloner and Braunwald 1980; Kloner et al. 1977) and cardiac hypertrophy (Anversa et al. 1976). Ischaemia has also been shown to impair mitochondrial function (Wolkowicz et al. 1983). There is some evidence for an accumulation of calcium in the mitochondria during ischaemia (Hagler et al. 1981) and metabolic blockade by iodoacetic acid where also a swelling of the mitochondria has been described (Buja et al. 1985). It has been suggested that mitochondria may have sufficient O_2 to oxidize some lactate and fatty acids during mild ischaemia, and to synthesize some ATP. The cell may thus function by a mixture of aerobic and anaerobic metabolism (Jennings and Ganote 1976). The significance of mitochondrial swelling and calcium accumulation is still not settled, especially not the extensive swelling of the mitochondria in the border zone as shown in the present study.

Our results are indicative of the presence of a border zone in the mid-myocardium surrounding the ischaemic zone during experimental infarction in the cat heart. However, a number of reports reject the presence of lateral border zone surrounding the infarcted area. The latter studies, however, have been undertaken under different experimental conditions and by studying other variables. These basically different results, therefore, probably demonstrate the significance of the experimental model used and the variables under study. This appears to be particularly important when studying morphological ischaemic changes that progress in time and space. Moreover, to achieve more exact knowledge of the spatial cellular composition of the border zone, a careful and time consuming study by serial sectioning across this zone should be needed.

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