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Morphological changes across the border zone of cat hearts subjected to regional ischaemia

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Summary. The left anterior descending coronary artery (LAD) was ligated for 3 h in six open chest cats. Six biopsies for electron microscopy were collected from the normal, the ischaemic and the border zones as defined by in vivo injection of fluorescein and as verified by blood flow measurements. Across the fluorescein demarcation line, we collected 3 mm long border zone biopsies, extending 1.5 mm in both the normal and the ischaemic direction. Starting from the normal end twenty subsequent areas (165 μm apart) were studied. By examining a total number of 5280 myocytes we observed an abrupt increase in the number of cells with sarcolemmal fragmentation. This point called *border line* is accompanied by an abrupt increase in the number of cells with chromatin clumping or margination, and of cells which are mainly necrotic with a massive cytoplasmic oedema. Morphometric analysis of 576 micrographs indicates a *border zone* on both sides of the border line. The border zone is characterized by a larger fractional volume of mitochondria (extending 1.5–2.0 mm in the normal direction) and by a larger lipid droplet accumulation (extending 1.2 mm or less in the normal direction) than seen in the ischaemic zone. These changes are in the normal part of the border zone accompanied with a moderate cytoplasmic oedema and a fragile sarcolemma with focal disruptions.

Key words: Myocardial infarction – Morphometry – Mitochondrial swelling – Lipid droplet accumulation – Cytoplasmic oedema

Introduction

The border zone phenomenon has been disputed ever since Cox et al. reported it in 1968. The debate has been less intense during the last few years, but the problem has not been solved, and the abated dispute might stem more from confusion than a lack of interest.

Any infarct size reduction is most probably due to a reduced number of myocytes that undergo necrosis in the area at risk. Within the area at risk a morphological and functional border zone has been described (Buda et al. 1986; Gallagher et al. 1986; Gallagher et al. 1987; Hearse and Yellon 1983; Homans et al. 1985; Lima et al. 1985; Przylenk and Groom 1983; Sakai et al. 1985; Ursel et al. 1985). We have previously shown a greater swelling of mitochondria and a larger lipid droplet accumulation in the border zone than in the ischaemic zone of cat hearts subjected to 3 h regional ischaemia (Greve et al. 1988a; Jodalen et al. 1985). The swelling of mitochondria and the accumulation of lipid droplets are variables affected by administration of calcium antagonists and beta-blockers (Greve et al. 1988b; Grong et al. 1986).

Both a transmural and a lateral localization of the border zone and progression of the ischaemic injury have been described (Gottlieb et al. 1981; Hearse and Yellon 1981; Reimer et al. 1977; Yellon et al. 1981). The width and extent of the cellular injury of this zone is, however, controversial (Hearse and Yellon 1981; Axford-Gatley and Wilson 1988; Forman et al. 1985; Reimer and Jennings 1986). To learn more about the spatial cellular composition of the border zone, we have examined ultrathin sections from succeeding areas across this zone, and, in the present study we have attempted to find any sequence in the ultrastructural alterations and to estimate the width of this zone.

Materials and methods

Six adult cats of either sex (2.88 ± 0.10 kg), bred for laboratory use and fasted overnight, were anaesthetized by sodium pentobarbital (35 mg/kg i.m.) and ventilated by a positive pressure

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ventilator (LOOSCO Infant ventilator M.K. 2, Amsterdam, Holland). The anaesthesia was maintained by ventilation with a gas mixture of 60% N_2O and 40% O_2 containing 5% CO_2. Adequate oxygenation was checked by repeated arterial blood gas analyses. The heart was exposed by a midline thoracotomy and a longitudinal pericardiotomy. A polyethylene catheter, inserted into the left ventricle through the apex, was connected to a Statham P23De pressure transducer (Hato Ray, Puerto Rico) for continuous measurement (Hewlett Packard 8805C) of heart rate (HR), left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). *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 and fluorescein. Body temperature was maintained at 37°C by an adjustable heat pad connected to a rectal thermistor.

The left anterior descending coronary artery (LAD) was exposed, and it was loosely encircled by a 4-0 silk ligature just distally to the branching of the left main stem. After allowing the haemodynamic conditions to stabilize for 5 min, LAD was occluded for 3 h by tightening the ligature. To compensate for the blood loss, 0.9% NaCl was infused into the left femoral vein at a low rate during the ischaemic period. Adequate hydration was secured by repeated measurements of haemoglobin and haematocrit. 3 min before sacrifice, approximately $1–2 \times 10^6$ microspheres were injected for measurements of regional blood flow. The microspheres had an average diameter of 15.6 μm and were labeled with ^{46}Sc, ^{81}Sr, ^{113}Sn, ^{141}Ce or ^{153}Gd selected at random. The reference blood sample was collected from the abdominal aorta via the left femoral artery by a constant rate extraction pump (Sage Instruments 351, Cambridge, MA, USA) for 2 min; starting 10 s before the injection of microspheres, which lasted for 60 s. The blood samples were weighed, and the exact withdrawal rate calculated.

Exactly 3 h after LAD occlusion, 0.8 ml of 10% fluorescein (Fluorescite, Alcon Lab, TX) was injected into the left atrium for visualization of the ischaemic myocardium. Immediately thereafter the animals were killed by injection of 50 ml ice cold 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 mosmoles). The fixative was injected into the left ventricle during partial occlusion of the ascending aorta. The hearts were rapidly removed from the animal. The left ventricles were opened along the posterior septal margin and placed in the same ice cold fixative.

The hearts were cut under UV-illumination. The normally perfused myocardium was stained by fluorescein and was separated from the non-fluorescent ischaemic myocardium by a sharp demarcation line. Two transmural samples were collected from the central area of both the ischaemic and normally perfused myocardium in all cats. These samples were divided into two subsamples, and biopsies for electron microscopy were taken from the midwall portion at the cut surface between the paired subsamples. Five samples were collected from the border zone (Fig. 1). Biopsies, 3 mm or longer, for electron microscopy were obtained from the midwall portion of these samples, all extending at least 1.5 mm in both the normal and ischaemic direction from the fluorescein demarcation line. The surrounding epi-, endo- and mid-myocardium were divided exactly along the fluorescein demarcation line into border normal and border ischaemic subsamples.

All subsamples and the reference blood sample from each cat were counted for gamma-emission in a multichannel counter (CompuGamma 1282, LKB-Wallac Company, Turku, Finland), and the regional myocardial blood flow and cardiac output were calculated (Heymann et al. 1977). After counting all subsamples were dried for 3 days, then reweighed and the water content calculated.

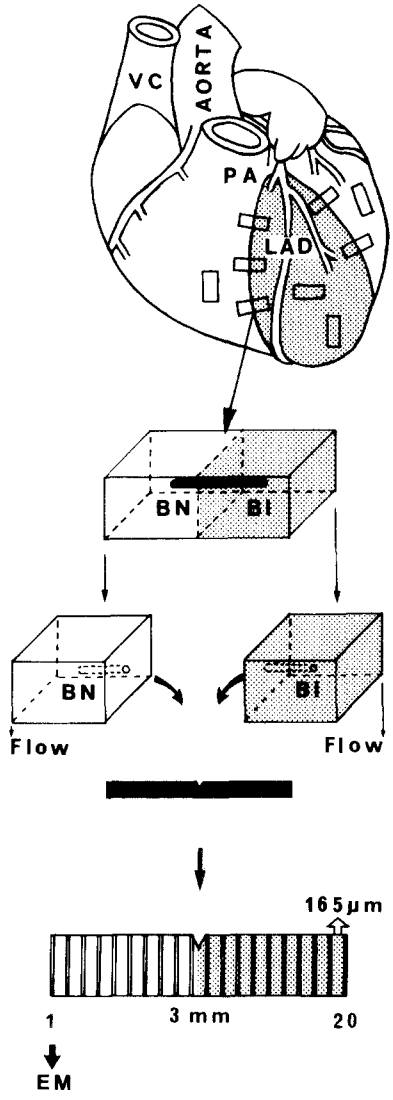

Fig. 1. Samples were collected from the normal, the ischemic and the border zone as shown here. The 3 mm long border zone biopsies were obtained from the mid-myocardial layer across the fluorescein demarcation line extending 1.5 mm into the normally perfused part of the border zone (BN) and 1.5 mm into the ischaemic part (BI). Myocardial blood flow was measured in the adjacent tissue on both side of this line. 10 ultrathin (50 nm) sections for electron microscopy (EM) were cut from 20 subsequent areas (165 μm apart)

Tissue preparation. All biopsies were fixed by immersion for 3 h or longer, then washed in cacodylate buffer (300 mosmol) and postfixed in 1% OsO_4 solution of the same buffer before they were dehyderated in ethanol and embedded in Epon. Ultrathin (50 nm) sections stained for 1 h with uranyl acetate and 15 min with lead citrate, were used for electron microscopy.

Expression of results. From each cat the two biopsies from the border zone with the largest difference in flow values in

adjacent subsamples were selected for ultrastructural studies. The biopsies were orientated in a normal – ischaemic direction and cut from the normal end; starting exactly 1.5 mm from the fluorescein demarcation line which had been marked in the biopsies by a small incision. Ten ultrathin sections (0.05–0.075 μm) were cut from twenty subsequent areas in every border zone biopsy (Fig. 1). Between two adjacent areas 165 semithin sections (1 μm) were cut away. Ultrastructural studies were performed in sections from all areas in the electron microscope at a magnification of 12000 or more. Sections from every second area were used for morphometric analyses. From each area we analysed four micrographs (18*24 cm) of myocytes at a magnification of 9600. Additionally, we collected four micrographs from the corresponding normal and ischaemic biopsies. Altogether 576 micrographs were analyzed.

By inspection in the electron microscope the number of cells with sarcolemmal fragmentation, chromatin clumping or margination, and nuclear membrane fragmentation were counted. When necessary the sections under study were tilted $\pm 60°$ to prove that any disruption of the sarcolemma or nuclear membrane was real. These variables were studied in twenty myocytes and twenty nuclei in each area as well as in all biopsies from the normal and ischaemic zones. A total number of 5280 myocytes were studied for sarcolemmal fragmentation and 5280 nuclei were examined for chromatin clumping or margination and nuclear membrane disruptions.

Standard point counting techniques (Weibel et al. 1966) were used for measuring fractional volumes of mitochondria (V_{Vmit}), myofibrils (V_{Vmyo}), lipid droplets (V_{Vlip}) and remaining cytoplasm including the lipid droplets (V_{Vcyt}), according to the Delesse principle, and as described in extenso elsewhere (Greve et al. 1988a). Except for the measurements of the fractional volume of lipid droplets, a grid lattice with a line distance of 20 mm was superimposed, and approximately 108 intersections of grid lines fell on each micrograph. Data on the mitochondrial surface were obtained by counting intercepts of the outer mitochondrial membrane with the lines of the superimposed grid lattice, correcting for magnification and for the distance between the lines in the grid lattice. Mitochondrial surface density (the ratio of mitochondrial surface to total cytoplasmic volume) and mitochondrial surface to mitochondrial volume ratio were calculated. Lipid droplets are identified as vaguely opaque spherical but non-membrane bound structures. They are usually located between myofibrils and often adjacent to the mitochondria. We have previously confirmed that these droplets contain lipid material by Sudan Black B staining of frozen sections (Jodalen et al. 1985). For calculation of the fractional volume of lipids we used a grid lattice with a distance of 5 mm between the lines. Approximately 1518 intersections of grid lines fell on the micrographs.

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

The length of the sarcomeres was measured in selected micrographs with the same magnification, but with the myocardial cells cut strictly longitudinally. Eighty sarcomeres from the normal zone, eighty from the ischaemic zone and 300 from the border zone were measured.

Differences in measurements of water content, regional blood flow and sarcomere length were tested by a one way analysis of variance. The Scheffé's multiple comparison method was used to compare the results between the zones. Differences in haemodynamic measurements before and after occlusion were tested by a paired *t*-test.

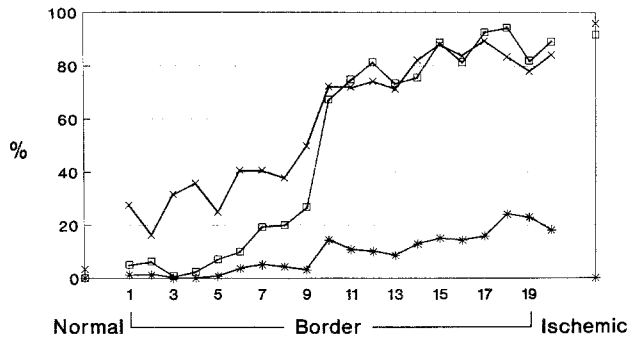

Fig. 2. Percentage of cells with sarcolemmal fragmentation (×), nuclear membrane fragmentation (*), and chromatin clumping or margination (□) in the normal zone, central ischaemic zone and all the areas of the border zone in cat hearts subjected to 3 h regional ischaemia. The border line is set to area 10

Only when ultrastructural studies revealed a sharp increase in proportion of cells with sarcolemmal fragmentation, *border line*, within the border zone biopsies, were these accepted as true border zone biopsies. The areas of the border zone were numbered with regard to this border line. We defined area 10 to be the area where the proportion of cells with sarcolemmal fragmentation rises abruptly and exceeds 50%. Area 9 is the last area used for morphometry on the normal side of the border line and area 11 is the first on the ischaemic side (Fig. 2). When the rise in the number of cells with sarcolemmal fragmentation was less distinct, we defined the border line to the area where it coincided with the increase in proportion of cells with chromatin clumping or margination. In four of twelve border zone biopsies no border line was discovered, and, hence, they were excluded. Since both border zone biopsies from one cat were excluded the corresponding normal and ischaemic biopsies from this cat were excluded too. The border zone was further divided in a normal and an ischaemic part by the border line, and the data were tested by a one way four level nested analysis of variance and Scheffé's multiple comparison test to compare the results obtained from the normal and ischaemic zones as well as the two parts of the border zone biopsies.

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

Results

LAD occlusion induced an increase of LVEDP from 3.50 ± 0.83 mmHg to 6.83 ± 1.20 mmHg 3 h after occlusion (Table 1). Following occlusion of the LAD we also observed a sudden decrease in *dP/dt*, but 3 h after occlusion *dP/dt* (3645 ± 592 mmHg/s) even exceeded the preocclusion value (3270 ± 437 mmHg/s) (Table 1), but the change in *dP/dt* 3 h after occlusion was not significantly different from the preocclusion value. LVSP and HR remained unchanged. Cardiac output was 259.28 ± 21.84 ml/min 3 h after LAD-occlusion.

Table 1. Haemodynamic registration in 6 cats with 3 h LAD-occlusion. Mean values ±SEM

Variables	Pre occlusion	3 h after occlusion	Statistics[a]
HR (beats/min)	175.83±3.67	173.17±7.94	NS
LVSP (mmHg)	110.83±5.97	118.33±8.43	NS
LVEDP (mmHg)	3.50±0.83	6.83±1.20	$P<0.05$
dP/dt[b] (mmHg/s)	3270.83±437.10	3645.83±591.97	NS

[a] Statistics between pre-occlusion values and values obtained 3 h after LAD-occlusion by paired *t*-test
[b] dP/dt is the first derivative of left ventricular pressure

Table 2. Regional water content (%) and blood flow rate (ml/min/g) in cat hearts subjected to 3 h regional ischaemia. Mean values ±SEM

Zones	Blood flow	Water content[a]
Normal	1.91±0.38	74.64±1.00
Border normal[b]	1.93±0.33	76.78±0.78*
Border ischaemic[c]	0.09±0.02*/**	80.00±0.44*/**
Ischaemic	0.12±0.09*/**	78.99±0.54*/**

[a] $\frac{(\text{wet weight} - \text{dry weight}) * 100}{\text{wet weight}}$
[b] Subsample obtained from the normally perfused side of the fluorescein demarcation line
[c] Subsample obtained from the ischaemic side of the fluorescein demarcation line

Statistics by one way ANOVA.
* Significantly different from the normal zone
** Significantly different from the border normal subsample

Myocardial blood flow rate (Table 2) in the non-ischaemic subsamples of the border zone (1.93±0.33 ml/min per g) did not differ from the blood flow in the normally perfused myocardium (1.91±0.38 ml/min per g), whereas the blood flow rate was significantly reduced in the ischaemic subsamples of the border zone (0.09±0.02 ml/min per g) and in the ischaemic myocardium (0.12±0.10 ml/min per g).

There was a significantly higher water content (Table 2) in the ischaemic myocardium (78.99±0.54%) and in the ischaemic subsamples of the border zone (80.00±0.44%) than in the normally perfused myocardium (74.64±1.00%) and the normal subsamples of the border zone (76.78±0.78%). The difference between the two latter zones was also significant. The increased water content in the ischaemic zones may be partly due to the cytoplasmic oedema observed here.

There was no significant difference in the sarcomere length between any zone (normal zone: 2.25±0.23 μm, border zone: 2.31±0.30 μm and ischaemic zone: 2.28±0.26 μm) and the measured sarcomere lengths agree with those reported by Fawcett and McNutt (1969) and by us elsewhere (Greve et al. 1988a, Greve et al. 1988b).

In eight of the twelve border zone biopsies we observed an abrupt increase in per cent myocytes with sarcolemmal fragmentation (Fig. 2). The angles of the slopes, however, differ somewhat between the biopsies. This increase usually runs parallel to an even more abrupt increase in percentage of myocytes with chromatin clumping or margination. Both events appear to coincide with a distinct morphological change from mainly normally appearing cells to mainly necrotic ones, and this switch is usually found close to the macroscopically distinct fluorescein demarcation line (median distance ±600 μm). Three of the border zone biopsies turn out to be localized on the normal side of the morphological border line, whereas one biopsy seems to be localized in parallel with the border line. Hence, four of the biopsies were excluded from the comparison of the border zone biopsies.

The sarcolemmal fragmentation seems to take place in two steps. Focal disruptions of the membrane could be seen either close to a restricted submembranous cytoplasmic oedema or without any other morphologically obvious injury (Fig. 3). In Fig. 2 this is reflected as a limited number (<50%) of myocytes with sarcolemmal disruptions on the normal side of the border line. A more pronounced fragmentation of the sarcolemma is accompanied by a massive cytoplasmic edema (Fig. 4). The nuclear membrane is in the border zone less frequently injured than the sarcolemma. In the ischaemic zone, injured nuclear membranes are hardly ever seen 3 h after LAD occlusion (Fig. 2).

The increase of V_{Vcyt} and decrease of V_{Vmyo} is larger in the ischaemic zone (24.67±1.15% and 52.33±1.23%, respectively) than in the border zone as compared with the normal zone (4.90±0.34% and 71.90±0.67%, respectively) (Table 4). This indicates a larger cytoplasmic oedema in the ischaemic zone. As seen in Fig. 5 and Table 3 the overall trend within the border zone is an increasing V_{Vcyt} and decreasing V_{Vmyo}. The ischaemic part of the border zone has a significantly larger V_{Vcyt} and smaller V_{Vmyo} (19.76±0.70% and 51.86±0.64%, respectively) than the normal part (12.40±0.58% and 59.32±0.70%, respectively) (Table 4).

There is a significantly larger V_{Vmit} in both the normal (28.27±0.33%) and ischaemic part of the border zone (28.34±0.41%) when compared with

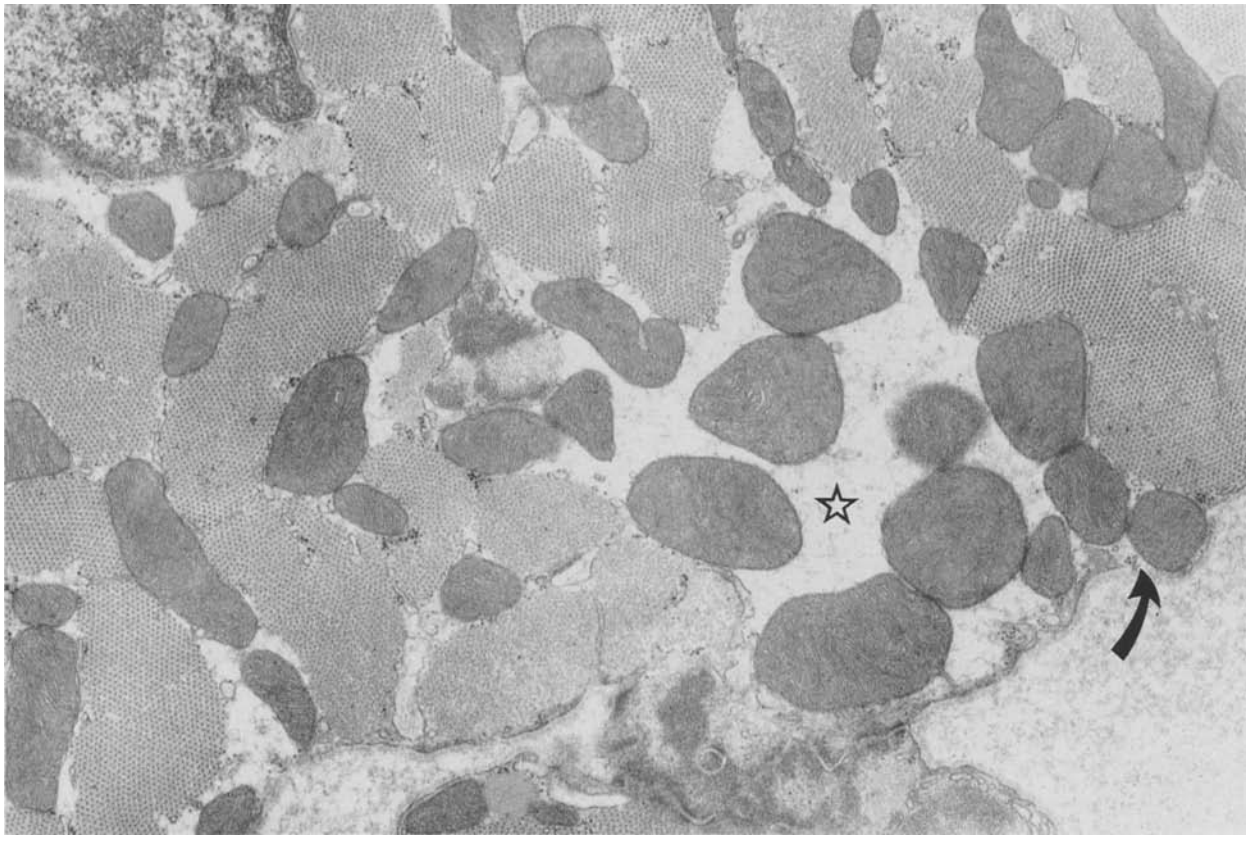

Fig. 3. On the normal side of the morphological border line cells with focal disruptions of the sarcolemma (*arrow*) are observed, here, close to a restricted cytoplasmic edema (*asterisks*) in an otherwise normally appearing cell. The disruptions were controlled by tilting $\pm 60°$. Section stained by uranyl and lead. Magnification $\times 20\,500$

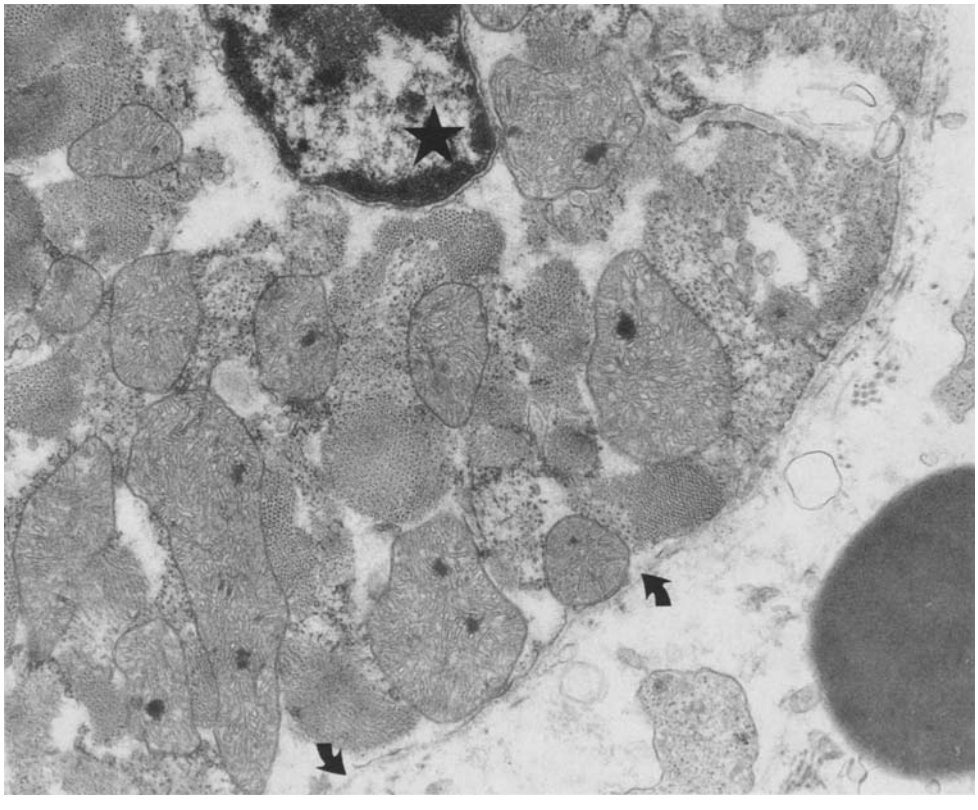

Fig. 4. The ischaemic part of the border line is mainly characterized by a more pronounced sarcolemmal fragmentation (*arrows*) which usually is combined with a massive cytoplasmic oedema and chromatin clumping or margination (*asterisk*). Section stained by uranyl and lead. Magnification $\times 16\,200$

Table 3. Morphometrical analysis of cat hearts subjected to 3 h regional ischaemia. Mean values ±SEM

Zones Areas	(*N*)	V_{Vmit} (%)	V_{Vmyo} (%)	V_{Vcyt} (%)	V_{Vlip} (%)	Surface/ volume ($\mu m^2/\mu m^3$)	Surface density ($\mu m^2/\mu m^3$)
Normal	40	23.20±0.66	71.90±0.68	4.90±0.34	0.39±0.09	8.07±0.25	1.80±0.04
Border							
1	16	26.79±0.85	66.70±1.67	6.45±1.46	0.34±0.09	6.23±0.34	1.62±0.06
3	24	28.75±0.94	63.92±1.44	7.43±0.88	0.55±0.09	5.59±0.25	1.57±0.06
5	28	28.03±0.85	59.17±2.01	12.79±1.46	0.94±0.26	5.58±0.22	1.51±0.05
7	28	28.29±1.03	57.97±1.83	13.73±1.35	0.94±0.28	5.46±0.21	1.49±0.04
9	32	28.80±0.81	55.43±1.32	15.75±1.42	1.28±0.25	5.01±0.17	1.42±0.05
11	28	29.27±0.95	52.43±1.54	18.29±1.61	1.14±0.31	4.51±0.17	1.31±0.04
13	28	28.29±1.00	51.33±1.49	20.38±1.55	1.12±0.29	4.69±0.19	1.28±0.05
15	28	28.71±0.98	53.19±1.28	18.09±1.24	1.00±0.19	4.59±0.16	1.29±0.05
17	24	28.60±0.66	50.51±1.65	20.88±1.78	0.89±0.23	4.42±0.15	1.25±0.04
19	20	29.49±0.95	49.02±1.38	21.48±1.42	1.07±0.32	4.51±0.16	1.31±0.06
Ischaemic	40	23.28±0.63	52.33±1.23	24.67±1.15	0.72±0.20	5.46±0.14	1.23±0.03

N denotes number of micrographs

Table 4. Fractional volumes (%) of mitochondria, myofibrils, cytoplasm and lipids, and mitochondrial surface density ($\mu m^2/\mu m^3$) and surface to volume ratio ($\mu m^2/\mu m^3$) in the normal, border and ischaemic zones in cat hearts subjected to 3 h regional ischaemia. The border zone is divided in a border normal and a border ischaemic part by the border line. Mean values ±SEM

	Normal	Border normal	Border ischaemic	Ischaemic
V_{Vmit}	23.20±0.66	28.27±0.33*	28.34±0.41*	23.28±0.63**/***
V_{Vmyo}	71.90±0.67	59.32±0.70*	51.86±0.64*/**	52.33±1.23*
V_{Vcyt}	4.90±0.34	12.40±0.58*	19.76±0.70*/**	24.67±1.15*/**/***
V_{Vlip}	0.39±0.09	0.68±0.08	1.14±0.10*/**	0.72+0.20
Surf. dens.	1.80±0.04	1.57±0.02*	1.27±0.02*/**	1.23±0.03 */**
Surf./vol.	8.07±0.25	5.74±0.10*	4.59±0.08*/**	5.46±0.14*/***

Statistics by one way four level ANOVA.
* Significantly different from the normal zone
** Significantly different from the border normal part of the border zone
*** Significantly different from the border ischaemic part of the border zone

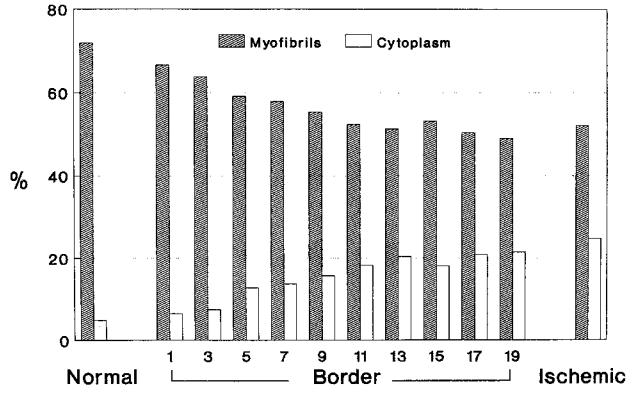

Fig. 5. Fractional volumes (%) of myofibrils and cytoplasm of cells in the normal and ischemic zones, and from every second area of the border zone

the normal (23.20±0.66%) and ischaemic zones (23.28±0.63%) (Table 4). As seen from Fig. 6 and Table 3 the mitochondrial volume fraction is relatively stable throughout the border zone.

Further, there is a larger mitochondrial surface to volume ratio in the normal zone (8.07±0.25 $\mu m^2/\mu m^3$) than in any other zone (Table 4). On the normal side of the border line there is an intermediate value (5.74±0.10 $\mu m^2/\mu m^3$) which is significantly higher than on the ischaemic side of the border line (4.59±0.08 $\mu m^2/\mu m^3$) but not significantly different from the ischaemic zone (5.46±0.14 $\mu m^2/\mu m^3$). The mitochondrial surface density is significantly smaller in both the normal (1.57±0.02 $\mu m^2/\mu m^3$) and ischaemic part of the

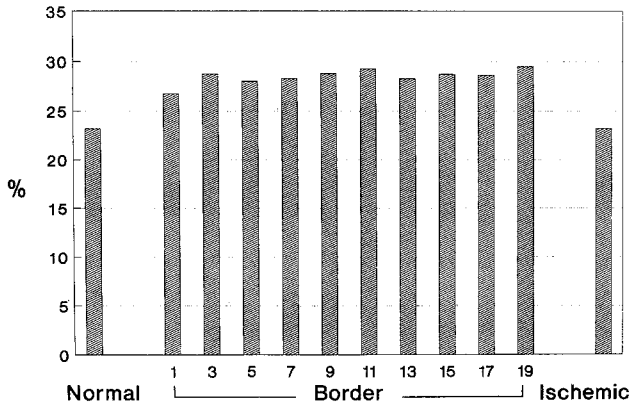

Fig. 6. Fractional volume of mitochondria (%) of cells in the normal and ischaemic zones as well as in every second border zone area

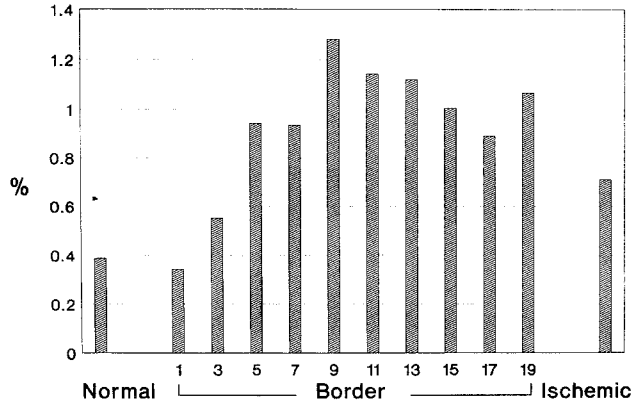

Fig. 8. Fractional volume of lipid droplets (%) in the normal and ischaemic zones as well as in every second border zone area

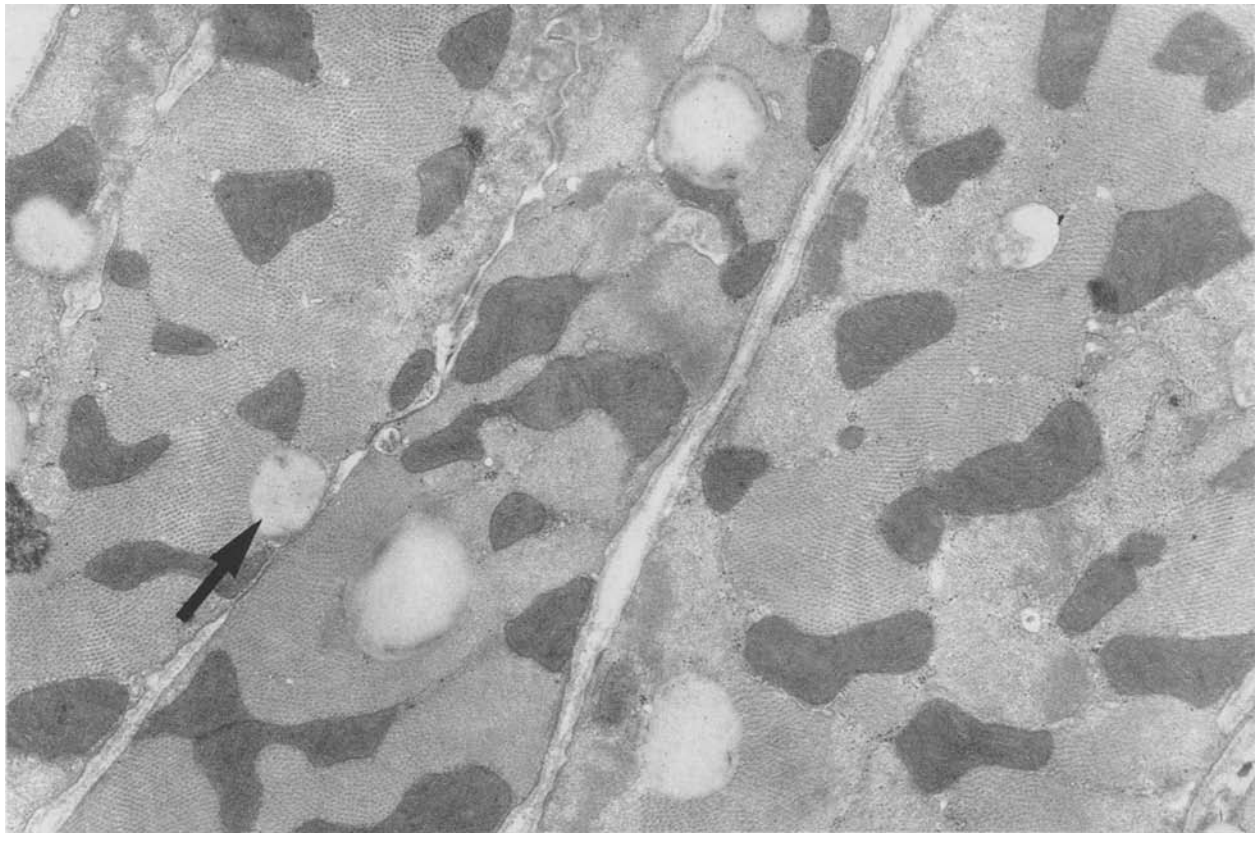

Fig. 7. Within the border zone there is an accumulation of lipid droplets (*arrow*). The micrograph is obtained from the normal part of the border zone. Section stained by uranyl and lead. Magnification × 20500

border zone (1.27 ± 0.02 $\mu m^2/\mu m^3$) than in the normal zone (1.80 ± 0.04 $\mu m^2/\mu m^3$). The surface density is declining throughout the border zone, and the surface density is significantly larger in the normal part than in the ischaemic part of the border zone and in the ischaemic zone (1.23 ± 0.03 $\mu m^2/\mu m^3$) (Table 4).

As seen in Table 4, there is a significantly larger V_{Vlip} in the ischaemic part of the border zone ($1.14 \pm 0.10\%$) than in the normal part of the border zone ($0.68 \pm 0.08\%$), the normal zone ($0.39 \pm 0.09\%$) and the ischaemic zone ($0.72 \pm 0.20\%$) indicating a lipid droplet accumulation here (Fig. 7), but as seen in Fig. 8, V_{Vlip} has its

maximum close to the border line on its normal side.

By examining each biopsy individually the increase in V_{Vlip} and sarcolemmal fragility extends less than 1.2 mm in the normal direction of the border line, whereas the increased V_{Vmit} extends 1.5–2.0 mm in the same direction. The increase of V_{Vmit} and V_{Vlip} and decrease in mitochondrial surface to volume ratio extend also in the ischaemic direction. The extent of the border zone in the ischaemic direction exceeds 1.5 mm.

Discussion

The presence of a border zone has been the subject of argument, but the debate has slowed recently, although the literature remains inconclusive. However, it is clear that in order to reduce infarct size, the therapeutic approach must be directed at the non-necrotic area at risk adjacent to the necrotic area. Many clinical studies indicate that infarct size reduction is possible, and there are strong indications in favour of the existence of a border zone which is qualitatively different from the normal myocardium. It gives rise to ventricular arrhythmias (Buda et al. 1986; Buda et al. 1987; Gallagher et al. 1986; Gallagher et al. 1987; Johnson et al. 1987; Liedtke 1988). The morphological and biochemical evidences of the presence of a lateral border zone is more controversial (Axford-Gatley and Wilson 1988; Gottlieb et al. 1981). A lateral progression as well as a wave front progression of the infarct from the endocardium to the epicardium have been claimed. When studying the midmyocardial part of the ischaemic border at a specific time, a lateral border zone could be attributed both to a lateral and a transmural progression of ischaemic damage. In previous works we have reported the presence of a distinct morphological border zone in the midmyocardium, characterized by a larger mitochondrial swelling (Greve et al. 1988a) and a larger lipid droplet accumulation (Jodalen et al. 1985) than in the ischaemic zone. These findings are confirmed in this study and could not be the result of a mixture of normal and necrotic cells, as one would expect intermediate values from a mixture of two populations. We have also shown that these variables can be manipulated by drug treatment (Greve et al. 1988b; Grong et al. 1986).

Between the normal and ischaemic myocardium there is an irregular interface, which can be visualized macroscopically by a sharp fluorescein line. The accuracy of sampling the biopsies across this line is far less than the accuracy by which successive sections could be obtained from the embedded biopsies. The fluorescein line is, therefore, useful only in assisting sampling of the biopsies. In order to compare the sequences of ultrastructural alteration in the border zone biopsies, it was necessary to number the areas according to a distinct alteration of a morphological variable appearing in most biopsies. Hence, we defined the border line as an abrupt increase in the proportion of cells with sarcolemmal fragmentation, and numbered the areas according to this. Only biopsies revealing this border line were accepted as true border zone biopsies. The morphological border line diverges only with a median distance of ± 600 µm from the incision representing the fluorescein demarcation line. This indicates that after 3 h regional ischaemia the fluorescein demarcation line, as a flow marker, and the morphologically defined border line coincide closely in the cat hearts.

In the present contribution the border line is defined as the abrupt increase in number of cells with sarcolemmal fragmentation and chromatin clumping or margination, whereas the border zone is characterized by mitochondrial swelling and lipid droplet accumulation. Across the border line there is not only a sudden increase in number of myocytes with sarcolemmal disintegration, but also a qualitative change in the extent of sarcolemmal injuries. On the ischaemic side there is usually a widespread fragmentation of the sarcolemma accompanied by a massive cytoplasmic oedema. Focal disruptions of the sarcolemma are combined with – if not a lack – at most a restricted cytoplasmic oedema in the otherwise normally appearing cells, and they are mainly observed on the normal side of the border line. These focal disruptions impede the interpretation of the sarcolemmal damage. In this study no attempt was made to rank the cells with regard to their degree of sarcolemmal disruption as this would demand serial sectioning of individual cells. The focal disruptions might result from the fixation procedure making the myocytes more fragile due to anoxia (Vander Heide and Ganote et al. 1987), hypoxia or metabolic alterations.

We chose to follow 3 mm long biopsies across the fluorescein demarcation line in this study as we have found a lipid droplet deposit covering a width of 1.5–2.0 mm in a preliminary study (Grong et al. 1986). An accurate determination of the width of the border zone by the method used, runs into two difficulties. The width depends on whether the longitudinal axis of the biopsy runs radially or tangentially to the border zone. In addition, any interdigitation of normally perfused and

ischaemic tissue may easily obscure the interpretation of the study (Hearse and Yellon 1981; Janse et al. 1979). However, it would be possible to estimate the maximal width and the sequence of cellular alterations across this particular zone, but the width of this zone might be time related, and we found great interindividual differences. This study also indicates that the width of the border zone depends on the variables studied. However, we have estimated a width of the border zone in both the normal and the ischaemic direction from the border line for the variables studied.

The accumulation of lipid droplets in ischaemic myocardium is well known (Grong et al. 1986; Jodalen et al. 1985; Liedtke 1988; Schwaiger et al. 1987). The V_{Vlip}, exhibits great interindividual differences. In all the hearts, however, there is a characteristic pattern: V_{Vlip} increases in the ischaemic tissue, but even more so in the border zone. The lipid droplets are believed to represent deposits of neutral fat in the cell (Jodalen et al. 1988; Ward and Harris 1984). An increased synthesis of triglycerides from plasma non-esterified fatty acids, redistribution of intracellular lipids as well as a reduced lipolysis of endogenous triglycerides within the ischaemic tissue have been proposed. The fact that the lipid accumulation is higher in the border zone than in the central ischaemic zone indicates metabolic alterations different from those seen in the central ischaemic zone. Also in the ischaemic part of the border zone there is a larger V_{Vlip} than in the central ischaemic zone. Hypothetically, a growth of the infarct in direction of the normally perfused myocardium might explain this larger lipid droplet accumulation. Thus, as the border line moves, the higher level of lipid droplets is a sign of a preceeding border zone.

Ischaemia is reported to induce mitochondrial swelling and, hence, a reduced matrix density of the mitochondria (Kloner et al. 1977; Kloner and Braunwald 1980), and to interfere with mitochondrial function (Wolkowicz et al. 1983). There is evidence of accumulation of calcium in the mitochondria during ischaemia at the expense of energy (Borgers et al. 1987; Hagler et al. 1983). It has been suggested that mitochondria may have sufficient O_2 to oxidize some lactate and fatty acids and synthesize some ATP during mild ischaemia. The cell may thus function by a mixture of aerobic and anaerobic metabolism (Jennings and Ganote 1976). The significance of mitochondrial swelling, calcium accumulation and other metabolic changes is still not settled, especially the extensive swelling of the mitochondria in the border zone.

In the present study we consider relative changes to be more reliable than the absolute values for the mitochondrial surface to volume ratio and surface densities. Even so the interpretation of the results are complicated by the irregular shape, spatial orientation and possible periodicity of the mitochondria. In addition to changes in mitochondrial size, the changes in these ratios could be ascribed to altered shape. The measurements were carried out with the lines of the grid lattice both at 0° and 15° to the edges of the micrographs in order to correct for anisotropy in the plane. A similar correction in the third dimension is not possible since sections are inherently limited to one plane. Since we only consider relative changes, the problem of anisotropy is further reduced by using a large number of micrographs and selecting non-orientated biopsies at random. It is also less likely that the anisotropy changes from one zone to another.

The cytoplasmic oedema observed might be the result of the metabolic alterations or accumulation of waste products, which would both alter the osmotic conditions within the cells or in the extracellular space. In necrotic cells the intracellular electrolyte distribution becomes similar to that of the extracellular space (Kloner and Braunwald 1980). Even though there is a gradual increase in cytoplasmic oedema, the necrotic cells appear to have a more extensive cytoplasmic oedema than the cells with only focal sarcolemmal disruptions. In previous work we have shown that even in the normally perfused myocardium of infarcted hearts, there is cytoplasmic oedema when compared to sham operated hearts (Greve et al. 1988a).

The larger proportion of nuclei with membrane fragmentation in the border zone than in the central ischaemic area, might be related to time and the metabolic alterations within the border zone. The significance of this observation is not clear and in the literature little attention has been given to the nuclei of ischaemic myocytes.

Chromatin clumping and margination are frequently observed in ischaemic tissues, but are usually regarded as reversible changes (Kloner et al. 1983). However, in this study the increase in proportion of cells characterized by such damage, coincides with sarcolemmal fragmentation and the appearance of mostly necrotic cells.

In conclusion we found an abrupt increase in the number of cells in the border zone with sarcolemmal fragmentation, accompanied by a sudden increase in number of cells with chromatin margination or clumping and by the appearance of mainly necrotic cells with a massive cytoplasmic oedema. On the normal side of this border line we observed

a distinct morphological border zone characterized by a larger fractional volume of mitochondria (1.5–2.0 mm wide) and lipid droplet accumulation (less than 1.2 mm wide) than in the ischaemic zone, moderate cytoplasmic oedema and a fragile sarcolemma with focal disruptions. The maximum lipid accumulation is just on the normal side of the border line but extends both in the normal and ischaemic direction.

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