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Discrepancies between myocardial blood flow and fiber shortening in the ischemic border zone as assessed with video mapping of epicardial deformation

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Abstract. Myocardial function around the border of ischemia was investigated in eight open-chest dogs using video mapping of epicardial deformation. With this method, 40–60 white markers attached to the left ventricular epicardium were traced in time automatically. Before and 5–10 min after coronary artery occlusion, blood flow and epicardial deformation were determined in 30–40 regions with a spatial resolution of about 5 mm. Epicardial deformation was expressed as subepicardial fiber shortening and surface area decrease during the ejection phase. The latter indicates local contribution to stroke volume. The absolute values of these variables were normalized relative to the central ischemic (= 0%) and remote non-ischemic area (= 100%). The 50% contour line of a variable was defined as its border. The average distance between the borders of perfusion and function was not significantly different from zero, due to considerable variation in this distance both within one heart (± 5.7 mm) and between mean distances for different hearts (± 4.4 mm). The width of the transition zone (distance between the 20% and 80% contour lines) of surface area decrease and subepicardial fiber shortening was significantly larger (20.5 and 15.0 mm, respectively) than those of transmural and subepicardial blood flow (8.5 and 9.5 mm, respectively). The present results demonstrate that in a 20-mm zone around the border of ischemia, major discrepancies are present between perfusion and deformation.

Key words: Myocardial function – Myocardial blood flow – Epicardial deformation – Area at risk – Functional border zone

Introduction

At the border of an ischemic region in the dog heart, the width of the transition zone of blood flow and the content of various metabolic substances is less than 5 mm [16, 38]. In contrast, the transition of regional wall thickening and endocardial and epicardial segment shortening have been found to occur within approximately 10 mm [7–9, 14, 15], even in the pig heart, although its collateral flow is negligible [26].

Ever since the first publication on this discrepancy between myocardial blood flow and shortening in the vicinity of the ischemic border [5, 11] clinical interest has increased, because this discrepancy may hamper correct assessment of the size of the infarcted region or the area at risk, as determined from wall motion modalities using such techniques as two-dimensional echocardiography, ventriculography or nuclear magnetic resonance. Several studies have reported that wall motion measurements poorly estimate infarct size, as assessed by histological techniques or enzyme release in both animals and man [14, 15, 17, 19, 28, 36, 37].

A major problem in the interpretation of these apparent discrepancies is that the spatial resolution of the techniques used (approximately 1 cm) is almost as large as the discrepancies described. It is therefore possible that the discrepancies described until now are a consequence of the limited spatial resolution of the techniques used to measure wall motion. Also, variations in the borderline locations of wall motion and perfusion, both within a heart and between hearts, could explain this discrepancy. Until now, in each heart, motion measurements were limited to one site along the border of ischemia. Consequently, no information is yet available on the variation in borderline location of wall motion and perfusion.

In our laboratory, a video technique has been developed that allows the mapping of epicardial deformation with a spatial resolution of approximately 5 mm over a surface of 15–20 cm^2 and hence the determination of the borderline location of deformation simultaneously at various sites in one heart. With this technique shortening can be measured with an accuracy similar to that using ultrasonic crystals [24].

With the use of the video technique, we investigated to what extent the apparent poor relation between wall motion and blood flow in the ischemic border zone is realistic or due to technical limitations. Epicardial deformation was measured in 30–40 regions around the ischemic border. At these sites, myocardial blood flow was measured with radioactive microspheres. The experiments were performed in open-chest dogs. Measurements of epicardial deformation and myocardial blood flow were performed before and 5–10 min after coronary artery occlusion.

Materials and methods

Animal preparation and measurement of hemodynamic variables. The experiments were performed on eight anesthetized mongrel dogs of either sex and unknown age, ranging in weight from 23 to 40 kg (median: 27). The animals were premedicated with 10 mg fluanisone · kg^{-1} body weight and 0.315 mg fentanyl citrate · kg^{-1} body weight i.m. Anesthesia

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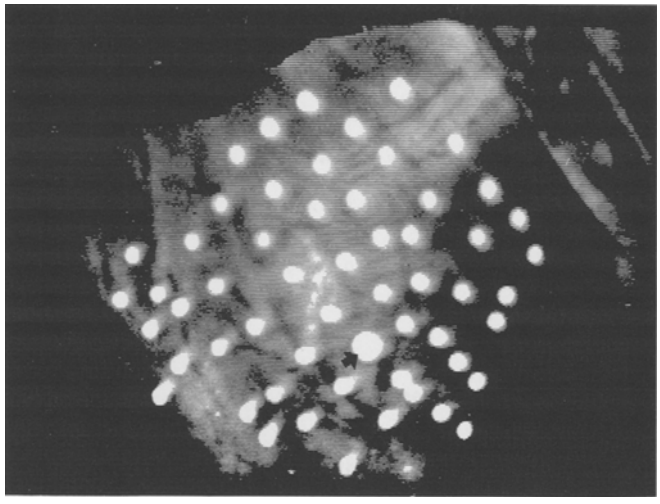

Fig. 1. A video image used for analysis of epicardial deformation. The blood vessel visible in the *right upper corner* of this mirror image is a side branch of the left circumflex coronary artery. The *arrow* indicates the large marker, used as a reference in consecutive frames

was induced with sodium pentobarbital (10 mg · kg^{-1} body weight i.v.) and, after endotracheal intubation, was maintained with oxygen in nitrous oxide (40% O_2/60% N_2O) and a continuous infusion of sodium pentobarbital (2 mg · $kg^{-1} \cdot h^{-1}$ i.v.). Catheters were inserted for the measurement of aortic blood pressure, left ventricular cavity pressure (Millar catheter-tip micro-manometer) and its maximum first derivative as described previously [23, 31]. The electrocardiogram was measured from the limb leads. To assess regional myocardial blood flow, a Silastic catheter was positioned in the left atrium for injection of radioactive microspheres. Arterial reference samples for this determination were taken through a polyethylene catheter inserted into the right brachial artery. Phasic ascending aortic flow was recorded with a sine-wave electromagnetic flow meter (Skalar). The hemodynamic variables were recorded continuously on a multichannel paper recorder (Schwarzer).

Epicardial deformation measurements. This technique has been described in detail previously [24]. In short, 40–60 circular white paper markers (diameter 1.5 mm) were glued to the epicardium of the anterior free wall of the left ventricle with cyanoacrylate (Histoacryl), at 5- to 7-mm intervals, covering a region of 15–20 cm^2. The motion of these markers was recorded via a mirror at a distance of about 2.5 m, using a video camera (Sony AVC 32500 CE) with a 200-mm tele-objective (Canon). To avoid blurring due to heart motion, the heart was illuminated by a xenon flash light (Chadwick-Helmuth), triggered by the 50-Hz video frame pulse. In Fig. 1, a video image thus obtained shows the mirror image of the epicardium with the markers appearing as white spots. The remaining structures are relatively dark. Either the ECG or the aortic flow signal was recorded on one of the audiochannels of the video recorder, enabling synchronization of the video images with the hemodynamic data on the multichannel recorder. After the experiment, the data obtained from the video recorder were digitized by a 4-bit AD converter at a sampling rate of 5 MHz. From each 20-ms frame, a segment of 256×256 pixels was stored in a 2-Mbyte digital video memory designed and built in our laboratory. The memory allowed storage of 64 consecutive frames, representing 1280 ms of video recording time. From the video memory the data were transferred to a minicomputer (PDP 11/73). Markers were detected, the position of their centers of gravity was assessed and, subsequently, traced automatically from frame to frame [24]. Only markers which could be traced throughout the whole cardiac cycle (usually more than 90%) were accepted for further analysis. For each situation two heart beats were analyzed, mainly to compensate for errors in the deformation measurement. In this open-chest preparation, variations in heart rate and blood pressure were always less than 10%. Also, variations in the values of epicardial fiber shortening and surface area decrease during the ejection phase (see below) varied by less than 10%, in both the non-ischemic and ischemic regions.

Analysis of epicardial deformation. To assess epicardial deformation, the positions of the epicardial markers were visualized on a Tektronix graphic display. Deformation was calculated in 30–40 rectangular regions of appropriate size (0.3–1.0 cm^2) which were selected by a computer program. The centers of the regions coincided with the centers of the tissue samples used for measurement of myocardial blood flow (see below). The program provides a regular matrix of rectangular regions with a preset minimal length and width, and with their centers of gravity at a chosen distance from each other. If necessary, the program automatically enlarges a region to the same extent in length and width until this region contains at least three markers – the minimal number used to estimate the deformation of the area under investigation [1]. Sometimes markers were used in this procedure for the calculation of deformation in two adjacent regions. In this situation, the displacement of markers on either side of the markers located at the margin between the two regions was employed in the calculations. This means that, although some regions shared one or two markers, these regions did not actually overlap.

In a previous study [24] the features of this technique have been compared to other methods. In brief, the accuracy of strain measurement in an area of 1 cm^2 was shown to be 0.005 strain unit (equivalent to about 0.5% shortening). This value is comparable to that of ultrasonic crystal measurements and is much better than that which can be obtained by means of two-dimensional echocardiography, as it has an accuracy of 1 mm [18]. The latter accuracy implies a relative error in the measurement of wall thickening as large as 30%. The error due to the projection of the three-dimensional outer surface of the left ventricle on the flat video screen was kept to a minimum by using the 200-mm telelens and was calculated to be less than 0.03 unit for circumferential strain (3% shortening) [24]. Because of the larger radius of curvature in all other directions, including the direction parallel to the epicardial fibers, the error made in measuring the strains in these directions and surface area decrease will be less (less than 0.015 unit, 1.5% shortening). In the present study regional shortening was defined as

$$\text{Shortening}\,(\%) = 100 \times (l_{so} - l_s)/l_{so}$$

where l_{so} and l_s denote the segment length in the reference frame and after deformation, respectively. The end-diastolic frame and the frame at the beginning of the ejection phase were used as the reference for tracings of a deformation variable and shortening during the ejection phase, respectively. In the present study, deformation was quantified by

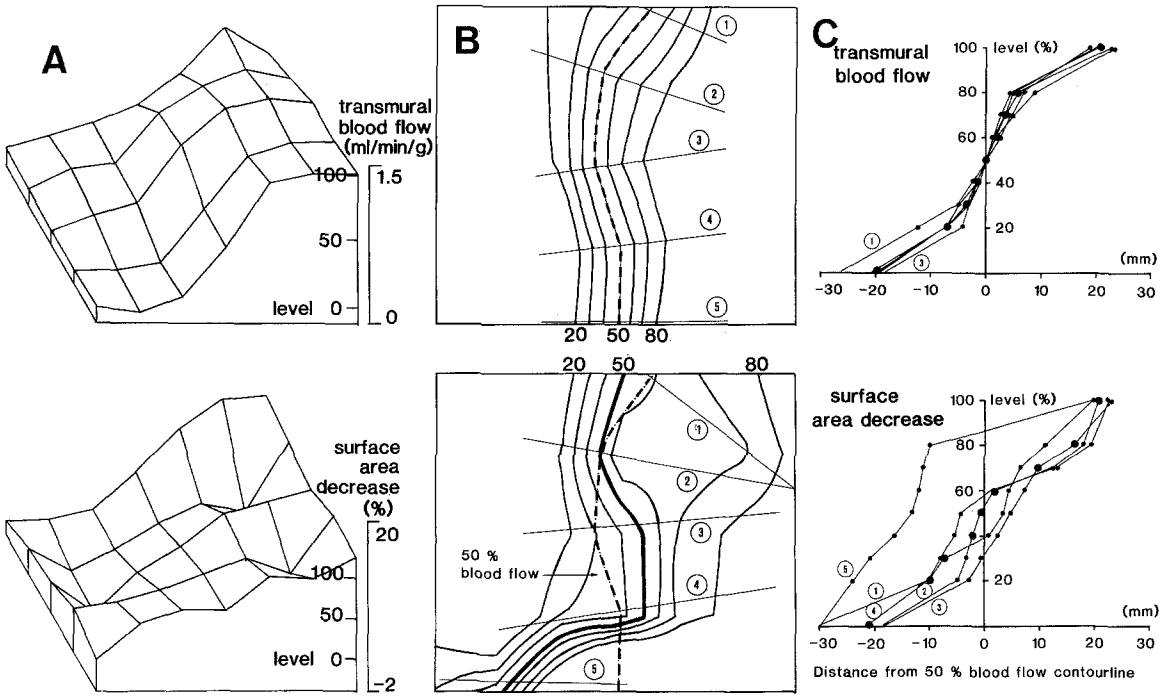

Fig. 2. A Three-dimensional representation of the raw data of transmural blood flow (*upper panel*) and surface area decrease during the ejection phase (*lower panel*) in one experiment. The horizontal plane represents the part of the left ventricular wall under investigation. Indicated are the absolute values of both variables, and the scale for the level in %, which is used for further analysis. **B** 20–80% contour lines of transmural blood flow (*upper panel*) and surface area decrease (*lower panel*), derived from the data presented in **A**. In this experiment the 50% surface-area-decrease contour line (the deformation border) crossed the 50% blood-flow contour line (the perfusion border, the *dotted line* in the *lower panel*) twice. The transition zone (width between 20–80% contour lines) for surface area decrease was clearly wider than that for blood flow. To quantify the transition of blood flow and surface area decrease across the border of ischemia at five locations along the border, the distance was measured between the 50% contour line of transmural blood flow and the contour lines of blood flow and surface area decrease, measured along a line perpendicular to the 50% blood flow contour line. **C** Results of the measurements described in **B**. The level of transmural blood flow (*upper panel*) and surface area decrease (*lower panel*) are plotted as a function of the distance from the 50% transmural blood-flow contour line. The transition of blood flow and deformation thus represented occurs along a more or less sigmoidal curve. Note that all curves of surface area decrease are less steep than those of blood flow, indicating the more gradual transition of the former variable. *Large symbols* indicate the median values for the five measurements of this experiment. Negative distances indicate the ischemic side and positive values the non-ischemic side of the border

the decrease of subepicardial fiber length and surface area during the ejection phase.

Subepicardial fiber shortening was assessed as shortening parallel to the subepicardial fiber direction. This direction was estimated by determining the direction of maximum epicardial shortening during normoxia [22, 25]. The latter direction is less than 10° from the orientation of the fibers in the subepicardial layers, being the outer one-third of the wall [1, 22, 25, 33]. Surface area decrease is the sum of epicardial shortening parallel and perpendicular to the subepicardial fibers, and is closely related to the contribution of a wall section to the stroke volume [1].

In addition to the effects of coronary artery occlusion on deformation during the ejection phase, we also examined its effect on deformation before this phase. To this purpose the values of subepicardial fiber length and surface area at the onset of the ejection phase during occlusion were expressed as a percentage of these values before occlusion.

Determination of regional myocardial blood flow. Myocardial blood flow was determined with radioactively labeled microspheres (isotopes ^{141}Ce, ^{113}Sn, ^{103}Ru or ^{45}Nb; diameter 15 ± 1.5 μm; New England Nuclear), as previously described in detail [21, 31]. The number of microspheres injected into the left atrium was $5 \cdot 10^6$ during normal perfusion and $10 \cdot 10^6$ during coronary artery occlusion. Reference samples were taken from the brachial artery at a rate of 20.7 ml · min^{-1}. After the experiment, the part of the left ventricular wall covered with markers was removed and cut into transmural sections according to a matrix of rectangular samples. The number of sections in both longitudinal and circumferential direction was equal to that used for analysis of epicardial deformation (see below). Subsequently, each transmural section was subdivided into a subepicardial and a subendocardial half. Blood flow was calculated from the gamma counting data of tissue and blood samples. In each sample, transmural blood flow was determined by assessing the average of subepicardial and subendocardial blood flow.

Experimental protocol. During a hemodynamically stable situation, the control measurements of hemodynamic variables, regional myocardial blood flow and epicardial deformation were performed. Thereafter, the left anterior descending coronary artery (LAD) was completely occluded using a tantalium clamp. Markers and the site of occlusion were located so that the occlusion resulted in part of the region covered with markers becoming ischemic; 5–10 min after onset of occlusion, all parameters were measured again. This relatively short period of ischemia was used because

mechanical failure is known to develop within a few seconds after onset of ischemia [22, 25, 29] and to remain stable for hours thereafter [22, 25, 29, 37]. The hemodynamic variables and epicardial deformation were measured simultaneously. The microspheres were injected within 2 min before or after these measurements. At the end of the experiment, the animals were killed by an overdose of the anesthetic. The heart was excised and fixed in 5% formaldehyde for a few days before dissection.

Data analysis. In the present study, myocardial function was defined as fiber shortening or area decrease during the ejection phase. Onset and end of the ejection phase were assessed from the phasic aortic flow tracing, making corrections for the position of the probe and the delay in the electronic circuit [30]. Determination of the ejection phase in the video recordings was possible using the hemodynamic signal registered on both the audiochannel of the video recorder and the multichannel recorder.

In the vicinity of the ischemic border zone, surface area decrease and subepicardial fiber shortening during ejection and subepicardial and transmural blood flow were determined in 30–40 regions. These regions were chosen using a regular matrix of rectangular regions. The outer margin, the number of regions and the form of the matrix were the same as that for dissection of the myocardium for blood flow analysis. The estimated distance between the centers of gravity of the regions of blood flow and epicardial deformation analysis was ± 2 mm (SD). The absolute values of these variables were arranged in rectangular matrices and were visualized three-dimensionally with the use of the MATFUN program, kindly provided by Dr. E.C.J. Eijkman and Dr. P.G.M. Penders of the Department of Medical Physics and Biophysics, University of Nijmegen (The Netherlands). Data on surface area decrease and regional transmural blood flow thus analyzed are presented in Fig. 2A. To enable comparison between the various experiments and between blood flow and deformation variables, the measured values were normalized. The 0% and 100% levels denote the mean value in the central ischemic and the remote non-ischemic region, respectively (vertical axes in Fig. 2A). Discrepancies between the normalized levels of mechanical function and blood flow indicate hyper- or hypofunction relative to blood flow in a particular region.

For a more detailed description of the transition of blood flow and deformation from ischemic to non-ischemic myocardium, the 20%, 30%, 40%, 50%, 60%, 70% and 80% contour lines were calculated by linear interpolation. The maps of blood flow and deformation thus obtained were plotted on a Tektronix graphic monitor and hardcopied (Fig. 2B). The 50% contour lines of blood flow and epicardial deformation are defined as the borders of perfusion and deformation, respectively. In this analysis it is not assumed that a 50% flow reduction induces a 50% reduction in function. The discrete levels are used to quantify the position of the transition curve. Different positions of the 50% levels of blood flow and function, for example, indicate a shift in the transition curves between these variables. The transition zone of these variables is defined as the region between their 20% and 80% contour lines. Because blood flow and epicardial deformation were measured in the same region, the maps could be superimposed. The transition of blood flow and epicardial deformation across the ischemic border was assessed by plotting the distance from a certain contour line of the related variable to the perfusion border. This was done at 5–6 sites (representing the number of samples) along the transition zone (Fig. 2B). The distance between contour lines was measured along lines drawn perpendicular to the 50% blood flow contour line at the center of gravity of the original samples. An example of the results of such an analysis is shown in Fig. 2C, where the level of transmural blood flow and surface area decrease at the five sites analyzed was plotted as a function of the distance to the perfusion border. Positive distances refer to the non-ischemic side and negative ones to the ischemic side of the perfusion border. For each experiment the median curve was determined, as indicated by the large dots in Fig. 2C. In the same way, maps of subepicardial blood flow and subepicardial fiber shortening were obtained and analyzed.

Table 1. Hemodynamic values before and during coronary artery occlusion

Parameter	Value	
	Before occlusion	During occlusion
Heart rate (beats · min^{-1})	110 100–140	125 120–140
End-diastolic left ventricular pressure (mm Hg)	8 3–11	8 3–14
Systolic left ventricular pressure (mm Hg)	100 90–120	100 78–115
Cardiac output (l · min^{-1})	2.0 1.8–2.8	2.1 2.0–2.5
Stroke volume (ml)	20 13–27	17 14–25

$n = 8$. Median values and 95% confidence limits are presented

Statistical analysis. Since the assumption of normal distribution of measured values for a relatively small number of observations is not valid, the data were generally treated according to non-parametric statistics. As a consequence, the data are presented as median values and 95% confidence limits. Intra-individual differences were evaluated for statistical significance by applying the Wilcoxon's matched-pairs signed-rank test (two-tailed probability). A value of $P < 0.05$ was considered to be a significant difference.

Quantification of inter and intra-individual variations of the distance between the borders of blood flow and function, however, is only possible with the use of parametric statistics, as it aims at presenting values for general interpretation. Consequently, these variations were calculated as the standard deviation of the mean distance between and within experiments, respectively. Total variation of a single measurement was calculated as the square root of the sum of intra-individual and inter-individual variance.

Results

Hemodynamic data

After 5–10 min of LAD occlusion only minor changes in systemic hemodynamics were observed (Table 1). Heart rate increased, and systolic left ventricular pressure decreased in

Table 2. Absolute values of blood flow and deformation variables after 5–10 min of coronary artery occlusion in the perfusion areas of the left anterior interventricular (LAD) and circumflex coronary arteries (LCX) before and during occlusion of the LAD

Parameter	Value			
	Before occlusion		During occlusion	
	LAD	LCX	LAD	LCX
Blood flow (ml · min^{-1} · g^{-1})				
Mean transmural	0.56	0.65	0.11[a]	0.67
	0.38–0.65	0.43–0.78	0.03–0.16	0.50–0.80
Subepicardial	0.50	0.59	0.15[a]	0.65
	0.41–0.78	0.40–1.15	0.06–0.25	0.50–0.85
Change during ejection (%)				
Surface area	15	16	1[a]	16
	12–31	13–20	(–2) –2	13–18
Subepicardial	12	12	3[a]	10
fiber length	7–17	9–16	2–3	8–12
Change begin ejection (%)[b]				
Surface area	0[c]	0[c]	18[a]	2
			10–30	0–6
Subepicardial	0[c]	0[c]	12[a]	2
fiber length			7–19	(–3) –4

$n = 8$. Median values and 95% confidence limits are presented
[a] $P < 0.05$ compared to the value before occlusion in the same region
[b] Expressed as percentage increase at the beginning of ejection during occlusion, as compared to the situation before occlusion
[c] By definition

five out of the eight experiments, whereas an increase in end-diastolic left ventricular pressure of 1–3 mm Hg was observed in four experiments. Similarly, no significant changes were observed in cardiac output and stroke volume (Table 1). In these experiments no ventricular fibrillation occurred and ventricular extra systoles were rare, either before or during coronary artery occlusion. Analysis of deformation was confined to heart beats during sinus rhythm.

Blood flow and deformation in central ischemic and remote non-ischemic myocardium

Occlusion of the LAD caused a decrease of transmural and subepicardial blood flow to 5–20% and 10–30% of the normoxic values, respectively (Table 2). The residual blood flow is a result of collateral perfusion, which is well known in dogs [27]. In the non-ischemic area blood flow tended to increase, as compared to the situation before ischemia (Table 2), but this change did not reach the level of significance.

In the central ischemic region, surface area decrease during ejection was approximately zero, indicating that in this region the wall does not contribute to the ejection of blood from the ventricle. The small residual subepicardial fiber shortening during ejection without surface area decrease indicates lengthening perpendicular to the subepicardial fibers [22, 25]. In the central ischemic myocardium, the surface area and fiber length at the start of ejection were increased by 18% and 12%, respectively, as compared with the control situation (Table 2).

In remote normal myocardium surface area and subepicardial fiber length at the start of ejection as well as surface area decrease and subepicardial fiber shortening during ejection were similar to the values in this region before onset of LAD occlusion (Table 2).

Location of the borders of blood flow and deformation

In Fig. 3, the course of the 50% contour lines of subepicardial and transmural blood flow, subepicardial fiber shortening and surface area decrease are depicted for the eight experiments. This figure shows that all these 50% contour lines (defined in Methods as the borders of the various variables) are generally located within 10 mm of each other. In all experiments the borders of perfusion and deformation crossed at least once. At some sites, the distance between the borders of perfusion and deformation was considerable, for example up to more than 10 mm in experiments 1, 3, 4, 5 and 8 (Fig. 3). Overall the distance between the borders of subepicardial fiber shortening and subepicardial blood flow was found to be –0.1 (–6.1 to 2.5) mm, and the distance between the borders of surface area decrease and transmural blood flow was 2.5 (–3.2 to 4.5) mm (median values and 95% confidence limits, see Fig. 5). These distances were not significantly different from zero. As shown in Fig. 5, differences between the location of the transmural and subepicardial perfusion border were minimal. Similarly, minimal differences were observed between the location of the borders of subepicardial fiber shortening and surface area decrease.

From these data, the intra-individual and inter-individual variation of the distance between the borders of perfusion and deformation were calculated. The intra-individual variation (from site to site in the same heart) was 5.7 ± 1.5 mm (mean $\pm$ SD). The inter-individual variation (between mean values of different hearts) was 4.4 mm.

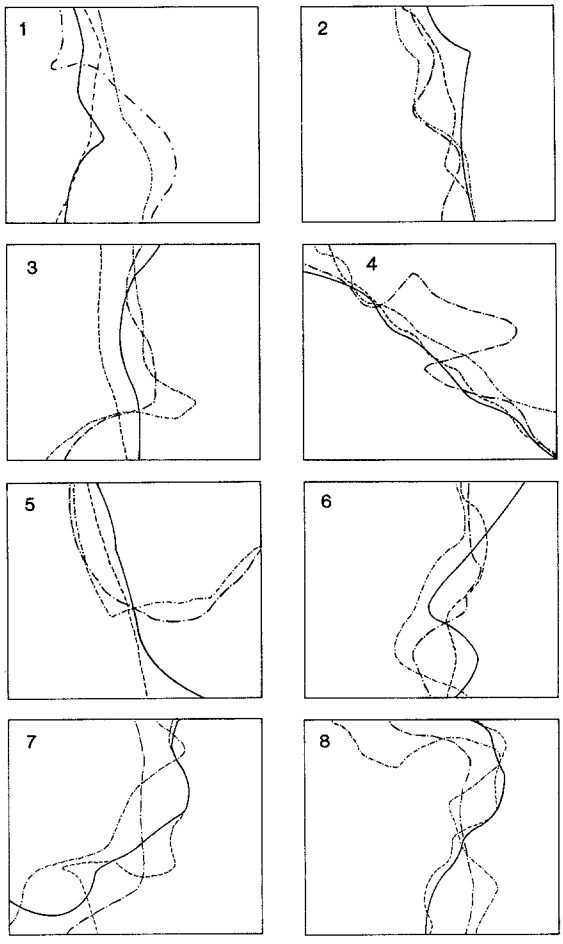

Fig. 3. Course of the 50% contour lines of transmural (———) and subepicardial blood flow (– – – –), surface area decrease (–·–·–) and subepicardial fiber shortening (·····) in the eight experiments. Each *rectangle* represents the total area under investigation (approximately 45 × 35 mm of the epicardial surface under investigation, compare Fig. 2)

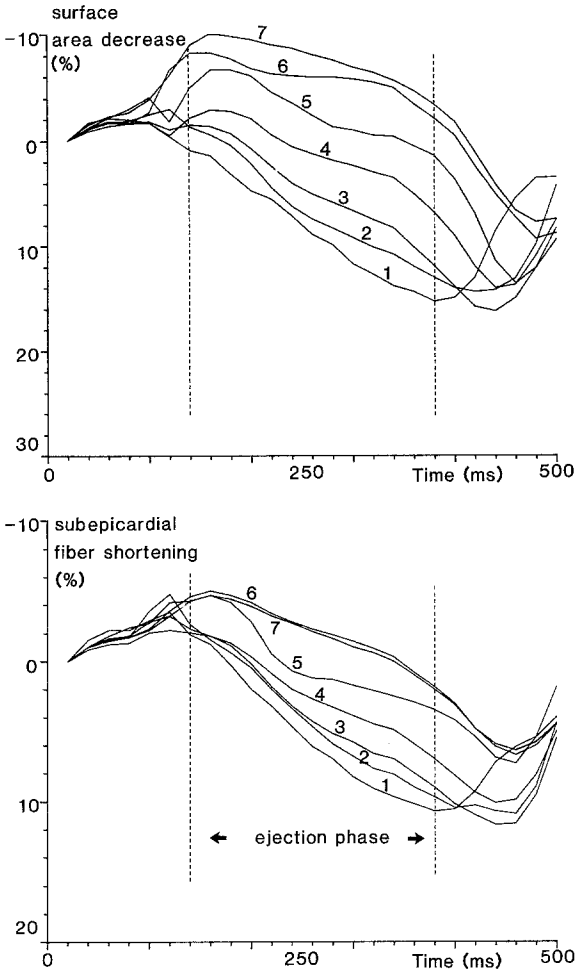

Fig. 4. Tracings of subepicardial fiber shortening (*upper panel*) and surface area decrease (*lower panel*) in a sequence of seven regions oriented perpendicular to the border of ischemia (the middle row of samples in the region represented in Fig. 2A). These tracings are obtained from one heart and are shown as an example. Note that changes in the patterns of both variables occur gradually between regions 1 and 7

Transition of blood flow and deformation from ischemic to non-ischemic myocardium

Tracings of surface area decrease and subepicardial fiber shortening at different sites along a line on the epicardium oriented perpendicularly to the border of ischemia are presented in Fig. 4. In the non-ischemic regions (1 and 2) shortening occurs throughout the whole ejection phase and stops at the end of ejection. In the central ischemic region 7 the myocardium stretches during the first 40 ms of the ejection phase, followed by a phase of little change in surface area or fiber length. Shortening continues during about 100 ms after the end of the ejection phase. The changes in the pattern of subepicardial fiber shortening and surface area decrease occur gradually from the ischemic to the non-ischemic region (cf. tracings 2 – 5 of Fig. 4).

The spatial relation between perfusion and deformation in the border zone is summarized in Fig. 5. This figure was composed from the median curves of the eight experiments, an example of which is shown in Fig. 4. The upper panel of Fig. 5 shows the data for surface area decrease and transmural blood flow, the lower panel the data for subepicardial fiber shortening and subepicardial blood flow. The transition curves all appeared to have a more or less sigmoidal shape, with the steepest part close to the 50% level. By definition the curves for blood flow cross the distance 0 mm at the 50% level. The width of the transition zone (distance between the 20% and 80% contour lines) was 8.5 (6.7 to 13.2) mm for transmural and 9.5 (6.7 to 14.3) mm for subepicardial blood flow. Like blood flow, deformation changed across the perfusion border in a sigmoidal fashion. The transition zone for deformation was about twice as wide as the one for blood flow, despite the fact that the same sample size was used: 20.5 (11.9 – 23.5) mm for surface area decrease and 15.0 (12.9 – 18.5) mm for subepicardial fiber shortening ($P < 0.05$ compared with transmural and subepicardial blood flow, respectively). The difference in width between the transition zones of surface area decrease and subepicardial fiber shortening was not statistically significant.

Discussion

With the use of techniques to measure regional myocardial blood flow and wall deformation with high spatial resolution, it was shown that along an ischemic area the borders

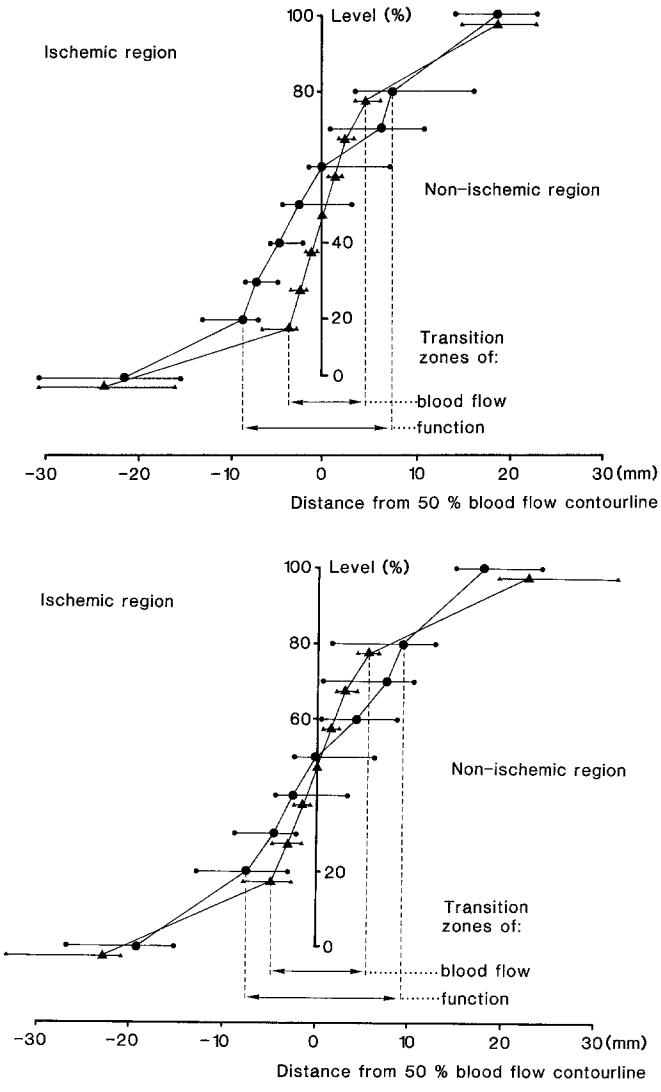

Fig. 5. *Upper panel*: transition of transmural blood flow (▲) and surface area decrease during the ejection phase (●). *Lower panel*: transition of subepicardial blood flow (▲), and subepicardial fiber shortening during the ejection phase (●). Values presented are the median (*large symbols*) and 95% confidence limits (*small symbols*) obtained by pooling the median values of the eight experiments. For further explanation see legend to Fig. 2

of perfusion and deformation grossly coincide. Despite the transmural differences in absolute blood flow values, these borders are located at the same lateral position throughout the wall. However, considerable variation was noticed in the distance between the borders of perfusion and deformation from site to site within a heart and between the mean values of different hearts. Moreover, for deformation the transition zone was found to be approximately twice as wide as for blood flow. Therefore, in the ischemic border zone considerable discrepancies exist between blood flow and deformation, which can not be attributed to technical limitations. These discrepancies limit the estimation of the size of the area at risk or the infarction from the assessment of akinesia.

Comments on the experimental set-up

The results in the present study have been obtained with the use of a video method to measure epicardial deformation. As mentioned in Methods, the spatial resolution of this technique is better than that of most of the techniques commonly in use. One could argue that the 50-Hz sampling rate of the video system results in inadequate temporal resolution. The timing of onset and end of ejection is especially important, because shortening during the ejection phase is used as a measure of regional function. Deformation signals, however, contain barely high-frequency components: generally less than 0.05% of the power is stored in frequency components above 25 Hz, as was shown by analysis of the signals recorded using techniques with high temporal resolution [1, 7, 8]. According to signal analysis theories, a signal can be described unambiguously if the sample frequency is higher than twice the highest frequency in the signal [3]. These conditions are clearly satisfied in the case of video measurement of deformation. Because of the 20-ms sampling rate, the onset and end of the ejection phase could be determined with an accuracy of $\pm$ 5.77 ms (SD). The average rate of shortening at these time intervals was 1.1% and 1.9% per 20 ms, respectively, as determined for surface area decrease in 68 regions of three regionally ischemic hearts. For each situation two beats have been analyzed; therefore, the error in shortening can be calculated to be $(5.77/20\sqrt{2}) \times 1.1\%$ and $(5.77/20\sqrt{2}) \times 1.9\%$, or 0.21% and 0.38%, for the onset and end of ejection, respectively. Thus in the worst case, errors due to incorrect timing of the ejection phase will be far less than 1% of shortening. Therefore, the method is accurate enough to describe the transition of deformation from normal to ischemic myocardium.

Although not addressed in this study, gradients in blood flow also exist within the normally perfused myocardium. In a previous investigation in the same animal model, we observed a gradient in endo-/epicardial blood flow ratio from high values in the posterior-basal regions to values below unity in the anterior-apical regions [21]. This gradient, however, is much less pronounced and distinct than the transition of blood flow at the border of ischemia.

In the present study, values of blood flow and deformation were normalized to the values in the remote non-ischemic (100%) and central ischemic regions (0%). This way of normalization avoids reference to normoxic areas, in which the dynamics and blood flow might change due to hemodynamic alterations as induced by coronary artery occlusion. The normalization disregards the effect of the absolute degree of underperfusion on the transient of deformation across the border of ischemia. However, in this relatively small group of animals, no significant relation was found between width or location of the border zone of deformation and the degree of underperfusion ($P > 0.30$).

The relatively low blood flow values in some normally perfused hearts (Table 1) are most likely due to low demands of the myocardium, since they are associated with low heart rate and blood pressure values. There is no indication for underperfusion before coronary artery occlusion, because the shape of the deformation signals is similar to those in hearts perfused at greater pressure and, hence, higher blood flow values. After all, the coronary circulation has a considerable autoregulation reserve, as shown by the unaltered deformation signals and blood flow at perfusion pressures as low as 50 mm Hg [34].

In the non-ischemic region, no significant increase in blood flow was observed after the onset of ischemia. This finding is in contrast with the approximately 10% increase in previous studies from our laboratory [23, 25]. Gallagher

et al. found no significant increase in non-ischemic blood flow in two open-chest dog studies [7, 8], but did find an increase in conscious dogs [9]. These discrepancies are incompletely understood.

Distance between borders of perfusion and deformation

The gross coincidence of the borders of subepicardial perfusion and fiber shortening is in accordance with the data of Sakai et al. [26] on epicardial segment shortening, as measured with four pairs of ultrasonic crystals.

The similarity in transition of epicardial fiber shortening and surface area decrease is interesting, because the latter variable consists of components of shortening parallel as well as perpendicular to the epicardial fiber direction. In previous studies, it was shown that epicardial shortening perpendicular to the epicardial fibers (which is approximately parallel to the endocardial fibers) is related to shortening of subendocardial fibers [22, 25]. Therefore, surface area decrease may be used as a measure of average fiber shortening in the various layers of a myocardial section. In fact, surface area decrease is closely related to wall thickening, a frequently used measure of transmural wall function. Two assumptions are made in this approach: constancy of volume of a wall section during the cardiac cycle and unchanged radial orientation of the tissue (i.e., absence of shear strains between myocardial layers). Although such strains exist [32], the error in wall thickness assessment due to these shear strains has been estimated to be less than 2% [6].

With the above considerations in mind, the similarity in transition of epicardial fiber shortening and surface area decrease from ischemic to non-ischemic myocardium, observed in the present study, indicates that the borderline location of impaired fiber shortening is similar in the various layers of the myocardial wall. This finding is in agreement with the similarity observed in separate studies on the transition of epicardial [26] and endocardial segment shortening [8] as well as wall thickening [7, 9]. All these studies show that the 50% level of remote non-ischemic function is located between -3 and $+5$ mm from the perfusion border. Similar results were obtained by studies using two-dimensional echocardiography [2, 14, 19] except for the study by Lima et al. [15], who found the 50% function level to be more than 10 mm outside the ischemic area.

The similarity in location of the border of deformation throughout the wall, despite transmural differences in collateral blood flow, may be explained by functional tethering of the epicardial layers by the more severely ischemic subendocardial layers. Due to complete failure of the subendocardial layers, the entire wall stress in a transmural section of the left ventricular wall has to be generated by the subepicardial layers, causing an increased load for the latter layers. Thus the amount of shortening is depressed directly [25, 34].

Variability in the distance between the borders of perfusion and deformation

In the present study, curves of the transition of regional fiber shortening could be composed for several sites in the same heart (Fig. 4). Using this information a significant intra- (5.7 mm) and inter-individual variation (4.4 mm) in the location of the functional border zone with respect to the perfusion border was found. By adding these two variations, it may be concluded that the location of the borderline of perfusion is assessed from the location of the borderline of deformation with a standard deviation of 7.3 mm. Only part of this variation is due to inaccuracies in the measuring techniques and in matching the regions in which blood flow and deformation were assessed. At the 50% blood flow level the number of microspheres per transmural sample was generally 200–400, causing a variation of 6.4% in the measurement of blood flow [21]. Since the mean 50% blood flow value was 0.39 ml $\cdot$ min^{-1} $\cdot$ g^{-1}, the error made will be 0.02 ml $\cdot$ min^{-1} $\cdot$ g^{-1}. The gradient of blood flow was found to be 0.04 ml $\cdot$ min^{-1} $\cdot$ g^{-1} $\cdot$ mm^{-1}, and, hence, the error in determining the perfusion border with the microsphere technique is approximately 0.5 mm. Similarly, an error of 0.01 strain unit (1% shortening) in the deformation measurement implies a standard deviation in the localization of the 50% contour lines of the functional parameters of approximately 2 mm. A similar error is estimated for matching of the localization of the regions used for blood flow and function (see Methods).

When the area at risk is estimated from wall motion measurements, using ventriculography or echocardiography, two borders are present per cross-section measured. The variation in the estimated sector length of the area at risk is therefore $7.3 \times \sqrt{2} = 10.4$ mm. In left ventricles of 100–150 g, the outer radius is approximately 30 mm. If the real area at risk is 30% of the circumference (about 57 mm), this area is estimated with a relative error of $10.4/57 = 18.2\%$ with this type of measurement. Such a variation is large enough to explain a major part of the poor estimation of infarct size or area at risk from wall motion measurements [14, 15, 17, 19, 28, 36, 37].

The cause of the variations in distance between the borders of blood flow and function are not yet clear. Intra-individual variation might be caused by factors such as shape of the ischemic area or the presence of papillary muscles. Inter-individual differences might be due to differences in collateral flow and/or hemodynamic situation. Support for the latter idea is given by preliminary results of Weiss and Marcus [35]. These investigators showed that the extent of regional systolic dysfunction, as assessed with fast CT-scan, is dependent on the value of left ventricular pressure. The limited number of animals included in the present study does not allow conclusions in this respect.

Width of the transition zone of fiber shortening

The width of the transition zone is larger for deformation than for perfusion, despite the similar sample size used for both determinations (Figs. 2, 5). This difference is not caused by averaging the data from a group of hearts or from several sites within the same heart. In Fig. 4 it is shown that at any site the transition in surface area decrease occurs more gradually than that of blood flow. Canine left ventricles of 100–150 g, as used in the present study, have a circumference of approximately 19 cm. With a transition zone of 15–20 mm around the ischemic region, as much as 20% of the ventricular circumference will be included in the transition zone of deformation. The width of this transition zone, as found in the present study, is broader than that in the studies of Sakai et al. [26] and Gallagher et al. [7–9], although the spatial resolution of the technique to measure wall motion is better in the present study. Using the definition of transition

zone as described in Methods, in the study of Sakai et al. [26] the transition zone of shortening was about 10 mm wide. Measuring wall thickening and endocardial segment shortening in the border zone and assuming a sigmoidal transition of these variables, Gallagher et al. [7–9] calculated an epicardial transition zone of about 12 mm wide, after correcting their data for the difference in circumference between endocardium and epicardium. The difference in width found by Sakai et al. and by us may be caused by the fact that their measurements were performed on pig hearts, a species with only limited collateral circulation. The difference between the width of the transition zone of deformation in the studies of Gallagher and colleagues and our study may be explained by the fact that their studies were performed in the perfusion area of the circumflex coronary arteries (LCX). In our study the LAD was occluded. Preliminary observations of Gallagher et al. [10] showed considerably wider transition zones during LAD occlusion than during LCX occlusion.

The difference between our data and those of Sakai et al. and Gallagher et al. is probably not caused by a difference in the duration of ischemia, because all these measurements were performed within the first hour after occlusion, during which no significant shift of the functional border occurs [8]. Moreover, it is known that mechanical failure develops within a few seconds after the onset of ischemia and remains stable for hours thereafter [22, 25, 29, 37].

All studies, including ours, in which local segment or fiber shortening was assessed, demonstrate that the hypokinetic zone in the non-ischemic myocardium adjacent to the perfusion border is narrower than observed in studies employing two-dimensional echocardiography to measure wall motion [14, 15, 19]. This can likely be explained by the limited spatial resolution and accuracy of two-dimensional echocardiography.

Possible explanations for the gradual transition of deformation

The present results show that the transition zone of deformation is almost twice as wide as the transition zone of perfusion, even though the width of the latter might be overestimated because of the sample size of about 7×7 mm. Investigators employing a smaller sample size have reported a transition zone of about 5 mm [16, 38], which is comparable to the width of the transition zone of tissue contents to ATP, creatine phosphate, lactate and glycogen [38]. Due to interdigitation of ischemic and non-ischemic tissue [12, 13] the actual transition of these variables might even be considerably sharper. In this respect the existence of an approximately 20-mm-wide transition zone of deformation indicates that there is a complex relation between the deformation of adjacent ischemic and non-ischemic myocardium. In the transition zone of perfusion, diminished shortening values might be due to interdigitation of non-ischemic with ischemic myocardium [2]. However, in the transition zone of deformation outside the transition zone of perfusion (Fig. 5) such interdigitation is less probable.

Several investigators have attempted to explain the transition zone of deformation on the basis of the structural connections between ischemic and non-ischemic myocardium. Extension of depressed function into adjacent non-ischemic myocardium due to overstretching of sarcomeres in these regions is not likely, because of the limited segment stretching measured at the end of diastole [7–9, 26] or at the onset of ejection (present study). Tethering (mechanical constraint) of normal wall deformation by the ischemic myocardium has also been suggested to be a mechanism. Because of the tight connection between the myofibers, a direct constraining effect of ischemic myofibers on their non-ischemic neighbours, in this way acting as a parallel resistance [37], is feasible. In our preparation, with occlusion of the LAD, this parallel resistance is only present in the subepicardium, whereas in the subendocardium the fibers are oriented perpendicular to the ischemic border. The similarity in location and width of the transition zone of deformation throughout the wall suggests that this kind of tethering does not play a major role in the origin of the transition zone of deformation. An alternative hypothesis about the origin of this zone is a redistribution of wall stress around the ischemic border. Local differences in wall stress influence fiber shortening directly, by changing the load on the myocardial fibers. Calculations using several mathematical models have suggested such a redistribution of stresses [4, 20]. The present study is not conclusive about the importance of stress redistribution in the determination of the width of the transition zone of deformation. However, it urges further investigation to elucidate the cause of the gross discrepancies between perfusion and deformation around the border of ischemia.

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