Myocardial Infarction in the Conscious Dog: Three-dimensional Mapping of Infarct, Collateral Flow and Region at Risk

BODH I. JUGDUTT, M.B., CH.B., GROVER M. HUTCHINS, M.D., BERNADINE H. BULKLEY, M.D., AND LEWIS C. BECKER, M.D.

SUMMARY Myocardial infarcts were examined in dogs to determine the spatial distribution of infarction in the region at risk and the relation between infarction and collateral blood flow. Permanent occlusion of the left circumflex (LC) coronary artery at a constant site was made in 27 conscious dogs that were sacrificed 2 days later. The anatomic region at risk was defined by postmortem coronary arteriography as the volume of the occluded LC coronary bed. The masses of the left ventricle (LV), infarct (I) and risk region (R) were calculated from planimetered areas of weighed “bread-loaf” sections of LV. Infarct size was directly related to the mass of the risk region (I = 0.53 R - 9.87; r = 0.97; p < 0.001). There was no infarction when R was less than about 20 g or 20% of the LV. The infarcts were mainly subendocardial and tapered from base to apex of the LV; 34% of the risk region became infarcted at the base compared with 22% at the apex. In all dogs, a significant rim of noninfarcted myocardium was identified at lateral aspects of the risk region, even at the endocardial surface. Using 9-μ radioactive microspheres, initial postocclusion flow at the margin of the infarct, but well within the risk region, was higher than at the center, and outer flows were higher than inner flows. Postocclusion flow was even higher in the noninfarcted rim within the risk region, but was still significantly less than flow to normal, nonrisk areas. Collateral flows throughout the risk region increased during the first hour after occlusion, and were even higher at 2 days. Epicardially and laterally within the anatomic risk region there is a substantial amount of tissue that does not infarct despite initially reduced blood flow.

THE CONCEPT of infarct size limitation implies the existence of a functional “border zone” in the ischemic region. Although myocardium located in the “border zone” is at risk because of reduced blood flow, it has sufficient flow for immediate survival. Subsequent increases in collateral flow occurring either naturally or after drug treatment provide for ultimate survival of this tissue. In contrast, other areas of the ischemic region have such low flow that necrosis occurs despite therapeutic efforts.

Our goal was to characterize the spatial geometry of myocardial necrosis relative to the anatomic region at jeopardy and the distribution of collateral blood flow after permanent left circumflex (LC) coronary occlusion in untreated conscious dogs. We measured the amount and spatial distribution of myocardium that was located in the bed of the occluded coronary artery but survived infarction over a 2-day period. The amount of noninfarcted myocardium in the occluded bed in untreated dogs served as a baseline for measuring the effectiveness of infarct-limiting therapies.

METHODS

Thirty-two mongrel dogs that weighed an average of 20 kg (range 18–23 kg) were instrumented under general anesthesia through a left lateral thoracotomy. A plastic snare was placed around the LC coronary artery just distal to the first marginal branch. Plastic catheters were placed in the external jugular vein, internal carotid artery and left atrium, and their distal ends were brought out at the back of the neck through a subcutaneous tunnel. Penicillin G (1 million units) and streptomycin (1 g) were given intramuscularly after surgery, and the tubes were flushed daily with 1000 IU heparin. Operative mortality was 9% (three dogs), and 29 dogs survived instrumentation. Experiments were done 10 days later in the laboratory while the dogs were standing in a specially designed sling for support. Morphine (0.25 mg/kg i.v.) was given in two divided doses for sedation and analgesia, and free-flow was established through the catheters. Radioactive microspheres (9 μ in diameter) with Tween-80 added and labeled with one of five different isotopes (125I, 141Ce, 85Sr, 99Nb, 46Sc) were sonicated mechanically for 5 minutes before each injection of 2 × 10⁶ microspheres into the left atrium. Reference arterial blood samples were withdrawn over 2 minutes beginning 30 seconds before each microsphere injection at a constant rate of 2.17 ml/min for calculation of flow by standard techniques.¹ ³ A first injection of microspheres 30–60 minutes after morphine was given was made under resting conditions in all dogs. The dogs were then premedicated with 1 mg/kg xylocaine to suppress ventricular ectopic depolarizations, and 5 minutes later

From the Cardiovascular Division of the Department of Medicine, and the Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland.

Support was provided by grants 1 RO 1 HL-19937-01 and P50-HL-17655-04 from the NIH, PHS, DHEW.

Dr. Jugdutt is a Canadian Heart Foundation Research Fellow.

Address for correspondence: Dr. Bodh I. Jugdutt, Division of Cardiology, Department of Medicine, 6-122A Clinical Sciences Building, University of Alberta, Edmonton, Alberta, Canada T6G 2G3.

the snare was pulled to occlude the LC coronary artery acutely and permanently. Further microsphere injections were made 20 seconds and 1 hour after occlusion. Lead II of the ECG, left atrial and aortic pressures (Statham P23Db) were recorded continuously on a Brush polygraph at either 1 or 25 mm/sec. Ventricular fibrillation developed in two dogs within 30 minutes of LC occlusion and they died.

Two days later, the 27 survivors were brought back to the laboratory for a final microsphere injection and ECG and hemodynamic measurements while fully conscious. The dogs were then sacrificed after a lethal dose of anesthetic, the hearts removed, washed free of blood and weighed.

**Delineation of the Region at Risk**

The coronary arteries were cannulated separately, with a first cannula at the origin of the LC proximal to the site of the occlusion, a second in the main left coronary artery and a third in the right coronary artery. Injections with a barium sulphate gelatin mass containing pigment (Monastral blue, red and green from Dupont Co.) were made simultaneously and under controlled pressure (120 mm Hg) to perfuse separately the bed proximal to the LC occlusion (green), the left anterior descending bed (red) and the right coronary artery bed (blue). The ventricular cavities were packed with gauze swabs and the hearts fixed in 20% formalin to preserve the anatomic detail with cardiac structures in nearly diastolic relationships. The site of the occlusion was indicated by a radiopaque marker in all hearts. Stereoscopic radiographs of the whole hearts were made. Each heart was then cut into five transverse rings of equal thickness (average 1.2 cm, range 1–1.5 cm) from base to apex and these were similarly radiographed without magnification. Color photographs were also taken. One of us examined the coded coronary arteriograms on a stereoscopic viewer and traced the boundaries of the region perfused by the occluded LC vessel on the radiographs of the rings. This was done by following the course of each vessel, radiographically visualized in the whole heart, from ring to ring. In all cases, the occlusion was confirmed and the occluded LC bed filled with contrast material through easily visualized collateral vessels. The viscosity of the injection mass was such that penetration did not occur beyond the precapillary level. Each marked boundary represented an average of that on the top and bottom layers of each slice, and they did not differ by more than 1 mm when viewed stereoscopically. The extent of overlap of the occluded and nonoccluded coronary beds across the boundary was measured in millimeters for each left ventricular (LV) ring on the radiographs; the individual measurements were less than 1 mm on either side in 22 dogs and 1–2 mm in five dogs. Extravasation did not occur. The boundaries of the risk region for each ring were later outlined on coded, unmarked copies of the radiographs by a second observer without the stereoscopic viewer and these markings differed from those of the first observer by \( -1.0 \pm 2.3 \text{ mm (sd)} \) (n = 77 observations). This difference represented 2% of the width of the risk region at the base and 5% at the apex for all dogs.

**Processing of Hearts For Histology and Calculations of Flow and Infarct Size**

The LV rings were then freed of the right ventricle and fatty and valvular tissue and weighed. Outlines of the rings and the infarct, as seen by naked eye, were traced using a transparent plastic sheet and checked by a second independent observer. In order to avoid observer bias, the boundaries of the arteriographic risk region in this study were not made available at the time of sampling for flow and histology or planimetry for infarct size. From each ring, transmural samples (1–3 g) were taken 1) from the center of the non-ischemic left anterior descending artery territory near the anterior papillary muscle, and 2) serially from the region around the posterior papillary muscle, including the center and margins of the infarct and adjoining nonnecrotic tissue (fig. 1). These samples were also mapped on the outlines of the rings and their top surfaces painted with bromochrome. The samples were subdivided into inner and outer halves, which were weighed and counted for radioactivity in a gamma scintillation counter (Packard) in vials containing 10% formalin. All samples from the middle (third) LV rings of all hearts as well as selected samples from the margins and borders of the infarct from other rings were embedded in paraffin. Histologic sections of these samples were made in the same plane as that of the surfaces traced for planimetry and identified by bromochrome. The sections were coded, stained with hematoxylin and eosin, and examined independently for the distribution and amount of necrosis by two of us on two separate occasions. Paired percentage estimates of total necrosis per sample by the two observers were in close agreement (y = 0.97x + 6.0; r = 0.94; n = 130) and differed by an average of 8 ± 8% (sd). The pigments only partially facilitated sampling. The red pigment not only marked the left anterior descending artery (LAD) territory, but also extended along the interventricular septum and penetrated the infarct. Along the lateral wall, green pigment injected into the LC proximal to the occlusion also penetrated the infarct.

**Analysis of Data**

The tracings of the rings were later superimposed on their radiographs and aligned using six markers (the anterior and posterior papillary muscles, the septal wall, and the two junctions with the right ventricle and the cavity outline) and markings of the angiographically defined risk region were copied. The final tracings were coded and planimetered by another unbiased technician for the total area, the area of infarcted myocardium, the area of the risk region, and the area of infarcts in the inner and outer halves of the respective risk regions. The areas for the entire ring, the risk region and infarct, from the top and bottom
 surfaces of each slice, were then averaged. The average areas of infarct and risk region were expressed as ratios of the total area of the rings. The total masses of the necrotic and risk areas were then computed by multiplying the fractions of infarcted myocardium and myocardium at risk by the weight of the rings. From the calculations, the following data were obtained for each LV ring and for the whole heart: 1) total mass of infarcted myocardium; 2) total mass of risk region; 3) percentage of LV mass infarcted; 4) percentage of LV mass at risk; 5) percentage of total risk region infarcted. Similarly, the masses of necrotic and risk regions in the inner and outer halves together with the percent ratios were calculated.

Myocardial blood flows were calculated for the full thickness, inner and outer halves in the ischemic and nonischemic regions by standard methods. In addition, the samples from each ring that were clearly in the center and margins of the necrotic region (at least 2 mm within the endocardial boundary of the infarct) or in the grossly and histologically normal-looking border region at least 2 mm from boundaries of the anatomic risk region on one side and the infarct on the other, were grouped and respective flows calculated for the various time intervals. In selecting samples from this border zone of the risk region, we carefully excluded samples that might have contained necrotic tissue. Flows were expressed as ml/min/g. Coronary vascular resistance (in mm Hg/ml/min/g) was calculated by dividing flow by mean arterial pressure.

The statistical significance of differences was assessed using paired t tests within groups and unpaired t tests between groups. Linear regression analysis was done using the least-squares method for grouped data. A two-way analysis of variance with orthogonal contrast was done on sequential regional flows at the three time intervals after occlusion.

Results

Twenty-seven dogs survived occlusion for 2 days, and only 7% died of ventricular fibrillation within 30 minutes of occlusion.

Gross and Histologic Appearance of Infarcts

Twenty-one of the 27 dogs that survived 2 days of occlusion had measurable infarcts. On inspection, the
infarcts were sharply circumscribed and centered on the posterior papillary muscle, involving adjoining myocardium to various degrees. The majority of medium-sized infarcts had pale centers with a thin (about 1 mm) hemorrhagic-looking rim. The outermost margins of the infarcts were mapped and used to calculate infarct size. Noncontiguous hemorrhagic areas (0.5–1 cm²) were present in the LV basal rings in a few cases and were mapped for histologic examination. These were related to trauma at the site of the LC snare and were therefore not included in the infarct. In five dogs with large infarcts, a larger hemorrhagic rim (2–3 mm thick) surrounded a smaller white central core; these areas were mapped separately, and histology showed that necrosis was more severe in the central core. At the periphery of most infarcts, especially in apical rings, a few small islands of normal-looking tissue measuring less than 1 mm² were present and were mapped whenever possible. However, the error on planimetry of these very small islands did not justify separate analysis of these regions. The average percentage of total histologic necrosis per sample agreed with planimetered necrosis (r = 0.86; slope = 0.95; p < 0.001; n = 130) and differed by less than a mean of 10%. The regional distribution of the infarct in each sample was also similar by gross inspection and microscopy.

About 5 ml of the barium gelatin mass was injected to fill the three coronary beds, and jelling occurred within a few minutes. The LC distal to the occlusion was not injected but filled adequately by collaterals in all hearts. The weight gain by the whole heart after the injection averaged 3.5 ± 1 g (sd) (range 2–6 g). The site of the LC occlusion, just past the first marginal branch, was fairly constant and measured 20–30 mm from the aortic origin of the left coronary artery.

Infarct mass averaged 10.3 ± 8.1 g (sd) (range 0–29 g) in the 27 hearts. The mass of LV averaged 93.6 ± 18.3 g (range 62–136 g). Thus, 10.6 ± 7.6% (range 0–21%) of the left ventricle was infarcted. The mass of the risk region averaged 37.9 ± 14.7 g (range 15–66 g). The risk region, as a percentage of the left ventricle, averaged 39.9 ± 12.9% (range 20–60%). The infarct, as a percentage of the risk region, averaged 22.6 ± 14.0% (range 12–38%). In the six dogs without infarcts, the risk region averaged 20.4 ± 3.7 g (sd) (range 14.6–24.3 g) and amounted to 20% of the left ventricle.

**Relation Between Infarct Size and the Risk Region**

The absolute mass of the infarct (I) correlated closely (r = 0.97; p < 0.001; n = 27) with that of the risk region (R) (fig. 2). This direct relationship, I = 0.53R − 9.87, indicates that the amount of necrosis increases as the size of the risk region increases. The intercept on the horizontal R axis indicates that there is no infarct for a risk region of less than about 20 g, or about 20% of the LV mass (average 93.6 g). The normalized infarct size (I/LV %) also correlated closely with the normalized risk region (R/LV %): (I/LV %) = 0.56 (R/LV %) − 11.87 (r = 0.96; p < 0.001; n = 27). Lesser correlations (p < 0.05) were found between LV mass and infarct size (r = 0.50) or the size of the risk region (r = 0.58) in these dogs.

The ratio of I/R as a percentage is plotted against the mass of the risk region in grams in figure 3. Mathematical analysis of the relation between infarct and risk region indicates that the I/R ratio is related to the mass of the risk region by a negative hyperbola that intersects the R axis at 20 g and whose upper limb is

**FIGURE 2.** The mass of the infarct (I) is directly proportional to the size of the risk region (R). The horizontal axis intercept suggests that there is no infarct for a risk region less than about 20 g in this model. The fact that the slope is 0.5 and significantly less than unity suggests that a significant amount of the risk region does not undergo infarction (i.e., natural salvage). The linear regression for dogs with infarcts only was I = 0.51 R − 8.75 (r = 0.97; SEE = 0.89; p < 0.001).

**FIGURE 3.** The percentage of the risk region infarcted is related to the size of the risk region. The maximum infarct size is about 50% of the risk region for the range of values. The relation for dogs with infarcts only was y = 100 (0.51 − 8.75/x).
asymptotic with a horizontal line through an I/R value of 53%. Thus, the maximum size of the infarct is 53% of the total risk region under natural conditions. The corollary of this observation is that there is a 47% natural salvage of the risk region. The fact that the major portion of the curve has a positive slope indicates that as the size of the risk region increases, the percentage of that region that undergoes necrosis also increases. The linear regression between I/R vs R/LV is I/R = 0.96 R/LV - 15.62 (r = 0.89; see = 6.82; n = 27). Excluding dogs without measurable infarcts, the regression is: I/R = 0.55 R/LV + 3.91 (r = 0.80; see = 4.16; n = 21).

Mapping Collateral Flow and Infarct in the Risk Region

The spatial geometry of the infarct within the risk region in each ring from base to apex of the left ventricle was reconstructed from 22 measurements made in millimeters at seven specific sites within the risk region and infarct in each LV ring. The reconstructed maps are shown in figure 4. Both infarcts and risk regions taper toward the apex of the left ventricle, with the bulk of the infarct in the top three rings. The boundaries of the infarcts and risk regions on the top and bottom surfaces of transverse LV sections were not in vertical alignment due to a rotation away from the septum along the longitudinal axis. This rotation was greatest at the apex and least at the base. In no heart was the infarct completely transmural in all five rings; in eight hearts the infarct extended to the epicardial surface in the two basal rings. The majority of infarcts was greater than 50% of the wall thicknesses and therefore not truly subendocardial. Thus, 55.7 ± 18.2% (SD) of the endocardial half of the risk region underwent infarction in contrast to 18.3 ± 13.3% of the epicardial half of the risk region (p < 0.001). The average maps for each ring (fig. 4) indicate that there is a significant zone of natural salvage both subepicardially and at the lateral margins. The presence of epicardial and lateral rims of noninfarcted tissue was confirmed histologically. For all rings, the lateral margins averaged 7.3 ± 1.6 mm (SD) (range 1-21 mm) on the left side and 6.3 ± 1.5 mm (range 1-16 mm) on the right side of the infarct along the inner contour of the risk region. The percentage of risk region infarcted decreased toward the apex, so that there was relatively greater salvage at the apex than at the base (78.1 ±

**MAP OF INFARCT AND RISK REGION**

<table>
<thead>
<tr>
<th>BASE</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Apex</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFARCT %</td>
<td>34 ± 2</td>
<td>33 ± 2</td>
<td>28 ± 3</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>INFARCT MASS (grams)</td>
<td>4.1 ± 0.4</td>
<td>4.1 ± 0.5</td>
<td>2.9 ± 0.5</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>MASS OF RISK REGION (grams)</td>
<td>12.1 ± 0.9</td>
<td>11.7 ± 1.1</td>
<td>9.8 ± 1.0</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>MASS OF LV RING (grams)</td>
<td>25.9 ± 1.9</td>
<td>26.0 ± 1.8</td>
<td>21.2 ± 1.5</td>
<td>14.6 ± 1.0</td>
</tr>
<tr>
<td>RISK REGION/LV RING %</td>
<td>47.2 ± 1.8</td>
<td>44.1 ± 2.3</td>
<td>46.0 ± 2.8</td>
<td>43.9 ± 3.4</td>
</tr>
</tbody>
</table>

**Figure 4.** The spatial geometry of the infarct in the anatomic region at risk is shown. The 17 points used to reconstruct the map accurately from each left ventricular (LV) ring represent the average data from all hearts with infarcts and were obtained from measurements (in millimeters) made in the risk region. Measurements were made along endocardial and epicardial surfaces at the lateral borders of the risk region and at four equal intervals within the infarct; corresponding measurements from the two surfaces were averaged. Thicknesses were measured along lines drawn between the seven corresponding points on the two surfaces. Within infarcts, thicknesses of the infarct and uninfarcted outer rim were measured. The percentage of the risk region infarcted is given in the stippled area in each ring. There is a subepicardial as well as a lateral rim of uninfarcted myocardium in this model.
16.6% (SD) vs 65.5 ± 10.1%, p < 0.01). Also, in some cases, the apical infarct was intramural and rather patchy. Rarely, the infarct extended to the epicardial surface at the apex. More often, there was neither infarct nor risk region in apical rings.

Although coronary blood flows were measured in all dogs, fewer flows (14 of 21) are reported in the first hour after occlusion in dogs with infarcts and fewer still (nine of 21) at 2 days because ventricular arrhythmias occurred during blood collections (table 1). Immediately after occlusion, collateral blood flow at the margins of the infarct, but well within (≥2mm) the subendocardial boundary, was higher than at the center of the infarct (0.35 ± 0.07 ml/min/g vs 0.21 ± 0.04 ml/min/g, p < 0.001). There was more flow to outer than inner halves of the infarct. In grossly and histologically normal-looking tissue found well within (≥2 mm) the anatomic risk region and away from (≥2 mm) the endocardial boundaries of the infarct, flow was higher than in the margin of the infarct (0.63 ml/min/g vs 0.35 ml/min/g, p < 0.025) but less than that in the center of the nonrisk region more than 20 mm away from the boundary of the risk region (1.26 ml/min/g, p < 0.001). Samples taken from this normal-appearing myocardium just lateral to the infarct may have contained both ischemic LC and nonischemic LC and LAD tissue.

The collateral flow after occlusion in the center of the infarct was lowest in the basal ring, close to the site of the occlusion, and increased toward the apex. Thus, there was less infarct and therefore more salvage toward the apex (fig. 4) because collateral flow was higher. The collateral flows 1 hour after occlusion in the center of the occluded bed for the five rings from base to apex were, respectively, 0.27 ± 0.05, 0.39 ± 0.09, 0.44 ± 0.11, 0.69 ± 0.22 and 0.80 ± 0.21 ml/min/g.

The time-related changes in flow in the center and margin of the infarct are given in table 1. There is a significant increase (paired t test) in flow during the first hour after occlusion in both the center and margin of the infarct. A smaller increase from the 1-hour values occurs during the next 2 days. A slight but non-significant increase in flow in the border region (p < 0.20) occurred during the first hour, while flow in the nonrisk region decreased slightly (p < 0.05). A two-way analysis of variance with an orthogonal degrees-of-freedom breakdown of the time factor sum of squares showed that the flow at 20 seconds was significantly lower than flows at 1 hour and 2 days (table 1).

Changes in hemodynamics and coronary vascular resistance are shown in table 2 for dogs with infarcts. Before occlusion, the average values for heart rate, mean left atrial and mean arterial pressures were 100 beats/min, 5.3 mm Hg and 113 mm Hg, respectively. Heart rate and left atrial pressure increased immediately after occlusion, but arterial pressure did not change. There was no significant correlation (r < 0.50; p < 0.1) between measured hemodynamic parameters and flow or necrosis. Coronary vascular resistance increased after occlusion in the center and margin of the infarct and decreased during the first hour after occlusion and during the 2 days.

In the six dogs with no measurable infarction, the risk regions were small and the myocardial blood flows were high after occlusion. Thus, flow in the center of the occluded bed decreased from 1.19 ± 0.15 (SEM) ml/min/g before occlusion to 0.80 ± 0.08 ml/min/g (p < 0.05) at 20 seconds after occlusion and

---

**Table 1. Myocardial Blood Flow Within the Infarct, the Border Risk Region, and the Nonrisk Region in the Conscious Dogs**

<table>
<thead>
<tr>
<th>Timing</th>
<th>Center of infarct</th>
<th>Margin of infarct</th>
<th>Borders of risk region</th>
<th>Nonrisk region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner</td>
<td>Outer</td>
<td>Trans-mural</td>
<td>Inner</td>
</tr>
<tr>
<td>Preocclusion</td>
<td>0.89</td>
<td>0.79</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>±0.10</td>
<td>±0.09</td>
<td>±0.11</td>
<td>±0.08</td>
</tr>
</tbody>
</table>

**Postocclusion**

<table>
<thead>
<tr>
<th></th>
<th>Inner</th>
<th>Outer</th>
<th>Trans-mural</th>
<th>Inner</th>
<th>Outer</th>
<th>Trans-mural</th>
<th>Inner</th>
<th>Outer</th>
<th>Trans-mural</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 sec</td>
<td>0.15†</td>
<td>0.24†</td>
<td>0.21†</td>
<td>0.29†</td>
<td>0.41†</td>
<td>0.37†</td>
<td>0.57†</td>
<td>0.69*</td>
<td>0.63†</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.04</td>
<td>±0.06</td>
<td>±0.05</td>
<td>±0.07</td>
<td>±0.08</td>
<td>±0.08</td>
<td>±0.08</td>
</tr>
<tr>
<td>1 hour</td>
<td>0.28†</td>
<td>0.36*</td>
<td>0.33†</td>
<td>0.45*</td>
<td>0.64*</td>
<td>0.56*</td>
<td>0.64</td>
<td>0.79</td>
<td>0.72</td>
</tr>
<tr>
<td>(n = 14)</td>
<td>±0.08</td>
<td>±0.07</td>
<td>±0.07</td>
<td>±0.08</td>
<td>±0.08</td>
<td>±0.08</td>
<td>±0.07</td>
<td>±0.07</td>
<td>±0.07</td>
</tr>
<tr>
<td>2 days</td>
<td>0.55</td>
<td>0.69*</td>
<td>0.61</td>
<td>0.57</td>
<td>0.80</td>
<td>0.71</td>
<td>0.88</td>
<td>0.99</td>
<td>0.90</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>±0.16</td>
<td>±0.14</td>
<td>±0.16</td>
<td>±0.16</td>
<td>±0.12</td>
<td>±0.15</td>
<td>±0.13</td>
<td>±0.12</td>
<td>±0.13</td>
</tr>
<tr>
<td>F&lt;sub&gt;14&lt;/sub&gt;§</td>
<td>11.03</td>
<td>21.19</td>
<td>15.59</td>
<td>13.83</td>
<td>27.46</td>
<td>21.07</td>
<td>7.22</td>
<td>9.23</td>
<td>7.48</td>
</tr>
<tr>
<td>p§</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM.

†p < 0.05 from value immediately above (paired t test between consecutive measurements).

‡p < 0.005 from value immediately above (paired t test between consecutive measurements).

§Based on analysis of variance and orthogonal contrast comparing 20-second with 1-hour and 2-day flows in the occluded bed in nine dogs.
was $0.92 \pm 0.07 \text{ ml/min/g} (p < 0.1)$ at 1 hour. The increase in flow during the first hour was significant ($p < 0.01$). The preocclusion flow in the center of the nonischemic region averaged $1.17 \pm 0.09 \text{ ml/min/g}$ and did not change after occlusion. While the heart rate increased after occlusion in these dogs, the mean arterial and the left atrial pressures did not change.

**Relation of Collateral Flow to the Size of the Risk Region and the Infarct**

The amount of collateral flow in the center of the region at risk immediately after occlusion was inversely related to the size of the risk region ($r = -0.80$; $p < 0.001$; $n = 19$) (fig. 5). This center flow represented flow in the center of the infarct in 14 dogs; five dogs had no infarct. In dogs with infarction, collateral flow was also inversely related to the size of the infarct expressed as percentage of the risk region. The $r$ values for the center flows were $-0.83$ ($n = 14$), $-0.80$ ($n = 14$) and $-0.72$ ($n = 9$) at 20 seconds, 1 hour and 2 days after occlusion, respectively; the corresponding $r$ values for the relation between flows at the margins of the infarct, but well within ($\geq 10$ mm) the risk region, and infarct size were $-0.66$, $-0.59$, and $-0.87$, respectively. These $r$ values were significant ($p < 0.005$).

The inverse relation between collateral flow within samples from the middle rings in eight dogs and the percentage of histologic necrosis in those samples is shown in figure 6. Low flows immediately after occlusion (mostly in subendocardial halves) generally correspond to greater subsequent necrosis, while higher postocclusion flows (mostly epicardial halves) correspond to lesser necrosis, and the relation approximates a hyperbola. At 20 seconds, linear correlation coefficients ($p < 0.01$) were similar for the epicardium and endocardium ($-0.58$ vs $-0.58$), but the slope was steeper for the endocardium than for the epicardium ($-0.0078$ vs $0.0028$, $p < 0.001$). At 1 hour after occlusion, the inverse relation tends to become more linear as flows increase. The $r$ values were $-0.73$ ($p < 0.005$) for the endocardium and $-0.52$ ($p < 0.01$) for the epicardium, but the slopes were similar ($-0.0063$ vs $-0.0067$, NS). Also, endocardial samples are displaced rightward relative to epicardial samples, suggesting that for the same level of flow reduction there is more necrosis in the endocardium. This rightward displacement of endocardial points could also be explained on the basis of greater subepicardial edema or greater sphere loss from the subepicardium.

**Discussion**

In this model, an insight into the three-dimensional geometry of the infarct is provided by accurately mapping the entire infarct and risk region from base to
apex of the heart. In animal models used to study the effect of interventions on infarct size, infarct mass is usually expressed as a percentage of LV mass. This approach corrects for variability of cardiac mass and size, but it does not correct for the variations in the size of the anatomic risk region due to variations in coronary anatomy, even when occlusions are made at a constant site. Changes in infarct size resulting from a therapeutic intervention may be missed or require large numbers of animals because of a dilution effect. This effect may be avoided by relating the infarct to the size of the risk region. In this study, the LC was occluded at a fairly constant site just past the first marginal branch, and mortality after occlusion was low. Occlusion resulted in measurable infarcts (average 10.3 g, range 2–29 g) in 21 of the 27 dogs in this study, but six dogs had no measurable infarcts and their risk regions were less than about 20 grams or 20% of the left ventricle. In all dogs, the infarcts averaged 10.6 ± 1.5% (SEM) of LV mass (range 0–21%). With both proximal LC and LAD occlusions in conscious dogs, Bishop et al. also found that some dogs had no measurable infarcts at 4 days. Infarct size in their model ranged from 0–35% (average 15 ± 2%) of LV mass in 25 dogs. Under similar experimental conditions, more proximal LC occlusions might be expected to result in bigger infarcts. Although 2-day and 6-day infarcts may not be comparable, 6-day infarcts with LC occlusion proximal to any branch in the study by Rivas et al. were bigger (p < 0.01) than in our study and averaged 19 ± 3% (range 4–33%) of LV mass in 11 dogs. In our study, mortality was 7%, which is less than in the well-defined model of Jennings et al., where 40 minutes of LC occlusion followed by reperfusion for 2–4 days resulted in 60 out of 123 dogs (49%) surviving LC occlusion and release, and 63 dogs (51%) dying of ventricular fibrillation, mostly 10 minutes after occlusion or at the time of release.

The anatomic region at risk of infarction was accurately defined by postmortem coronary arteriography in our model. Postmortem coronary arteriography, which has been used by various investigators, including Fulton and Schaper, permits clear visualization of the vessels so that their courses can be followed. We used this technique to outline the vascular territories of the vessels and thereby quantify the risk region. The close agreement between different observers in defining the outlines of the risk region on stereoscopic viewing of the arteriograms proved the method reliable and reproducible. The error introduced by the 1.2-mm thickness of the LV rings was less than 1 mm, which was the thickness of the marker used. For the average heart in this study, a 1-mm error at the boundaries of the risk region would have caused a 1.64-g change in its mass, or 1.74% of LV mass. The injections were made simultaneously and under equal pressure. Although pigments are incorporated in the injection mass, gross inspection of pigment distribution did not permit an accurate delineation of the risk region. Because of collaterals, the pigments from the LAD and proximal LC cor-

**Figure 6.** Relationship of collateral flow in infarct samples and the amount of necrosis in the samples 20 seconds (left) and 1 hour (right) after occlusion. Data from the middle ring of the left ventricle in nine dogs are shown. Endocardial flows were lower than epicardial flows and corresponded to greater endocardial than epicardial necrosis. The relationship at 20 seconds is more hyperbolic while that at 1 hour is more linear (see text).
ory arteries entered the area of the infarct. When we viewed the stereoscopic arteriograms, however, penetration of injectate into the infarct region actually helped us to delineate the bed of the occluded LC vessel. Unlike Lowe et al., we did not directly inject pigment or barium into the bed distal to the LC occlusion because we found cannulation of the occluded vessel technically difficult.

Previous investigators have defined the region at risk by other methods and reported values smaller than those we obtained using the arteriographic method. Rivas et al. injected Evans blue dye into the occluded LC bed at pressures equal to aortic pressure. Although the more proximal LC occlusion in their study created bigger infarcts, they found a smaller region at risk than we did: 36 ± 1% of LV mass (range 28–43%) vs 39.9 ± 2.5% (range 20–60%). Thus, as percentages of the risk region, infarcts averaged 22.6 ± 2.7% (range 12–38%) in our study vs 52 ± 6% (range 12–84%) in the study of Rivas et al. Lowe, Reimer, and Jennings obtained results similar to those of Rivas et al., but they also defined the risk region by gross inspection after injecting dye together with neoprene latex, first into the left main coronary artery and then into the previously occluded LC bed using a hand-held syringe. Lowe et al. suggest that the order of injection might have caused the extent of the risk region to be underestimated by excluding "all functionally important collaterals" and "mild ischemic tissue." and defining a "functional circumflex bed." It is not clear whether these investigators attempted to map the entire risk region from base to apex of the heart. In our study, the relative size of the infarct and risk region changes from base to apex. While lower estimates of the risk region in other studies may be related to different methods, the anatomic risk region, as defined by coronary arteriography in our study, appears to be accurate, easily reproducible and reliable. In preliminary studies, we found the use of dye injections in defining the risk region to be inaccurate because the solution penetrates capillaries, overlaps into other coronary beds, and often leaks outside capillaries. When we injected different colored dyes into the two coronary beds simultaneously and maintained equal pressures in the two beds, we saw an intermixing at the boundaries in a few minutes after the injections were made. Thus, the size of the risk region defined by dye injections is influenced by the viscosity of the solution, pressures used during the injection, timing of injections of different beds, and displacement after injection. The use of dyed neoprene latex only circumvents problems of capillary penetration and displacement after injection. With the arteriographic method, on the other hand, inadequate visualization of the vessels due to technically poor injections could theoretically cause either an underestimation or an overestimation of the risk region. In this study, however, epicardial and transmural vessels were clearly visualized in both occluded and nonoccluded beds.

We found a clearly defined border of uninfarcted myocardium both subepicardially and laterally within the risk region. Although subepicardial sparing has been recognized by other investigators, the existence of a lateral border of natural salvage has been questioned. In our model, the boundary of the arteriographically defined risk region was clearly 6–7 mm lateral to the outermost boundary of the infarct at the endocardial surface. Studies using pathologic and histochemical techniques have also shown a morphologic lateral "border zone." In contrast, Hirzel et al., using hand injections of dye to delineate the risk region, found that the lateral extent of the infarct coincided with the edges of the risk region. For reasons indicated above, the angiographic technique may provide a more accurate assessment of the risk region than dye injection. Our observation of uninfarcted myocardium in the occluded bed provides an explanation for the fact that increases in infarct size may occur in both lateral and subepicardial directions. A recent report by White, Sanders and Bloor supports this observation.

The lateral border of uninfarcted myocardium in the risk region is most likely related to higher levels of collateral flow at the periphery than at the center of the ischemic area. We found that collateral flow at the margin of the infarct — but well within the confines of the risk region, making contamination with normal myocardium within the nonrisk region unlikely — was higher than at the center of the infarct. Similarly, Bishop et al. found lower collateral flow in the core of the infarct than in the remainder of the ischemic region, well removed from the nonrisk region. Other studies in anesthetized dogs with a coronary perfusion model have shown a zone of increased collateral flow of 18.3 mm when flow is corrected for admixture of ischemic and nonischemic tissue. Hirzel et al. have not found a lateral zone of increased collateral flow. This disparity may be related to technical factors in Hirzel's model, in which the risk region is perfused through a cannula. Pressures were probably somewhat lower in the coronary bed distal to the cannula than in the normal coronary beds. Some of the radioactive microspheres used to mark the nonischemic beds could have inadvertently entered the risk region through collaterals. This would have led to an underestimation of the size of the risk region and an overestimation of the amount of nonischemic tissue present in samples taken from the periphery of the ischemic region. In our study, although histologically and grossly normal myocardial samples taken from this border region were at least 2 mm away from the boundary of the risk region and had flows intermediate in value between those within the infarct and the center of the LAD region, the possibility of some admixture with nonrisk myocardium could not be excluded. In our study, flows measured 48 hours after coronary occlusion and just before the dogs were sacrificed also showed a gradient across the occluded bed, but the flows in the lateral borders were close to levels in the nonoccluded bed. Using autoradiographic scanning after 14C antipyrine given just before sacrifice, 24 hours after coronary occlusion, Vokonas et al. also showed a gradient of flow from the border
to the center of infarction after branch LC coronary occlusions in dogs; however, they only measured flow 24 hours after coronary occlusion. We measured flows during the first hour and 48 hours after occlusion. Because marked increases in collateral flow occur by 24 or 48 hours after coronary occlusion, the volume of myocardium containing reduced flow in the first hour may be larger than that containing reduced flow 24 or 48 hours after occlusion.

We measured a uniform, significant increase in collateral blood flow within the infarct during the first hour after occlusion from base to apex of the left ventricle. This increase may be important for salvage of initially ischemic myocardium. The increase in flow occurred in both endocardial and epicardial halves of the center and margin of the infarcts. Rivas et al. found a similar increase in collateral flow during the first few hours after occlusion in conscious dogs. On the other hand, Schaper et al. and Bishop et al. found that epicardial flows increased only mildly, while flow to the endocardial core remained unchanged. As in our study, all these investigators: found that collateral flow measured a few minutes before the dogs were sacrificed was much higher than early postocclusion flow. Recently, an average 20% loss of radioactive microspheres has been found to occur differentially from necrotic myocardium beyond 24 hours after occlusion. The effect of this loss would be artifactual reduction of the earlier flow measurements relative to the measurement made just before sacrifice of the animal.

Infarct size was directly related to the size of the risk region. Theoretically, interventions to modify infarct size would be expected to alter the relation between infarct mass and the mass of the risk region by changing the slope of the relation seen in control animals. In addition, the percentage of the risk region infarcted increased as the size of the risk region increased. This was probably related to the decrease in collateral flow per gram of tissue with increasing size of the risk region. The fact that infarct size was so closely related to the size of the risk region (r = 0.97) suggests that there may be less natural variation in collaterals in dogs than is generally thought. Thus, the commonly observed variation in infarct size among dogs may be related more to variation in size of the region at risk than to differences in the density of native collaterals.

Acknowledgments

We are grateful to Patricia Shaw, Alexander Wright, and Anthony Dipaula for technical assistance; to Stephanie Meredith and Rosemary Hopkins for typing the manuscript; and to Dr. Myron Weisfeldt for helpful suggestions. We also thank Clayton Kallman, Robert Burrow and David Mellits for assistance with biostatistics.

References

Myocardial infarction in the conscious dog: three-dimensional mapping of infarct, collateral flow and region at risk.
B I Jugdutt, G M Hutchins, B H Bulkley and L C Becker

Circulation. 1979;60:1141-1150
doi: 10.1161/01.CIR.60.5.1141

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1979 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/60/5/1141.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/