Relationship between Blood Flow to Ischemic Regions and Extent of Myocardial Infarction

Serial Measurement of Blood Flow to Ischemic Regions in Dogs

Frank Rivas, M.D., Frederick R. Cobb, M.D., Robert J. Bache, M.D., and Joseph C. Greenfield, Jr., M.D.

SUMMARY

This study was designed to measure early sequential changes in blood flow to ischemic regions after acute coronary occlusion and to determine the relationship between blood flow and the extent of subsequent myocardial infarction. Initial studies were carried out on five dogs which verified using radioisotope-labeled microspheres, 7-10 μm in diameter, to measure changes in blood flow in small myocardial regions after acute coronary artery occlusions. Studies then were carried out on 11 awake dogs chronically prepared with indwelling catheters in the aorta and left atrium and occluders on the left circumflex coronary artery. Microspheres were injected via the left atrial catheter 45 seconds and 2, 6, and 24 hours after complete circumflex coronary occlusion. Six days later myocardial blood flow and the extent of histological infarction were determined for multiple samples from four transmural layers of the entire ischemic zone. Average blood flow to the circumflex region was 0.25 ± 0.03 (SE) 0.39 ± 0.05, 0.39 ± 0.04, and 0.53 ± 0.07 ml/min per g at 45 seconds, and 2, 6, and 24 hours, respectively. When samples from each transmural layer were grouped according to increasing ranges of blood flow, the extent of infarction in each layer was inversely related to blood flow. When samples in the same range of blood flow were compared, the extent of infarction in endocardial samples exceeded that in epicardial samples. These data indicate that the relationship between a given measurement of regional blood flow after acute coronary occlusion and the extent of subsequent myocardial infarction varies in different transmural layers and is a function of the time after occlusion that blood flow is measured.

UNDER BASAL conditions the myocardium extracts near-maximum amounts of oxygen from the arterial blood. The metabolism of the myocardium is predominantly aerobic and the capacity for anaerobic metabolism is limited during ischemia. The metabolic integrity of the myocardium is critically dependent on adequate blood flow. The heart is thus extremely vulnerable to disease processes or experimental interventions that reduce myocardial blood flow. It follows that blood flow to a region of ischemic myocardium should be a major determinant of the extent of injury or infarction. That blood flow to an ischemic region after acute coronary occlusion does in fact reduce the extent of myocardial injury is shown by the finding that the infarcted region is usually smaller than the area perfused by the occluded vessel.

However, a quantitative relationship between blood flow after acute coronary occlusion and the extent of subsequent myocardial infarction has not been established. Previous investigators have reported a wide range of flow values after acute coronary occlusion in experimental animals, depending on the experimental model and the technique used to estimate flow. Considerable controversy also remains concerning the time course of changes in blood flow to an ischemic region after acute coronary occlusion. Use of backflow techniques to study both anesthetized and unanesthetized animals generally has shown no significant increment in blood flow to ischemic regions after coronary occlusion for periods from hours to 3 days. Studies using clearance of inert gases have demonstrated significant early increments in flow to ischemic regions in unanesthetized but not in anesthetized animals.

The objectives of our present study were (1) to measure early sequential changes in blood flow after acute coronary artery obstruction and (2) to determine the relationship between blood flow and the extent of subsequent myocardial infarction. The purpose of these measurements was to test the hypothesis that blood flow is the principal determinant of the extent of infarction. It was postulated that a close relationship between blood flow and amount of infarction would exist if other variables that influence the metabolic state of the myocardium remained constant during the course of the study. Accordingly, experiments were carried out in chronically prepared unanesthetized dogs to avoid the variables introduced by anesthesia and acute surgery. It was anticipated that if the relationship between blood flow and the extent of infarction was close, then the technique may provide a suitable method to predict the extent of acute infarction.

Methods

These studies were carried out on 20 mongrel dogs of both sexes weighing 25-30 kg. The dogs were anesthetized with...
sodium thiamyl (30–40 mg/kg, iv) and ventilated with a respirator (Harvard model 607). A left thoracotomy was performed via the 4th left intercostal space. A polyvinyl chloride catheter with outer diameter (o.d.) of 3 mm was inserted via the left internal mammary artery into the arch of the aorta for the purpose of measuring aortic pressure and obtaining reference samples of arterial blood. The ventral pericardium was incised and a polyvinyl chloride catheter, 3 mm in o.d., was inserted into the left atrial chamber via the atrial appendage and secured with a purse-string suture for injection of radioactive microspheres and measurement of left atrial pressure. Both catheters were filled with heparin. The left circumflex coronary artery was isolated and dissected 1–2 cm from its origin and a polyethylene loop snare-type occluder was positioned around it proximal to any branch. The catheters and occluder were tunneled to the base of the neck and placed in a subcutaneous pouch. The chest was closed, the pneumothorax was evacuated, and the dogs were taken off the respirator and allowed to breathe spontaneously.

Postoperatively, each dog was checked daily for signs of infection and trained to lie quietly on a laboratory table. A minimum period of 7 days was allowed for recovery from the operative procedure before study. At the time of study all dogs were free of fever or other signs of infection. Mean hematocrit was 44 ± 5.0 (sd); range, 38–52. The snare and catheters were exteriorized from the subcutaneous pouch. The chest was closed, the pneumothorax was evacuated, and the dogs were taken off the respirator and allowed to breathe spontaneously.

Regional myocardial blood flow was determined by injecting carbonized microspheres 7–10 μm in diameter and labeled with gamma-emitting nuclides 44Sc, 85Sr, 141Ce, and 48Ca. The microspheres were obtained as 1 mCi of each nuclide in 10 ml of 10% dextran and 0.05% of polysorbate 80 (3M Co.). This stock solution was diluted in 10% dextran so that 1.5 ml, the volume injected, contained approximately 4 × 10^6 microspheres. Before each injection, the microspheres were mixed and then injected via the 20-gauge needle and blood flow in each sample was determined as described below.

Sequential measurements of myocardial blood flow then were carried out in another group of 11 dogs by injecting microspheres 45 seconds and 2, 6, and 24 hours after complete occlusion of the left circumflex coronary artery. Immediately after the 45-second measurement was completed, a bolus injection of lidocaine (2 mg/kg, iv) was given to minimize early arrhythmias. After completing the 45-second measurement and repeated 10–15 minutes later to minimize any discomfort resulting from the coronary artery occlusion. This procedure was followed in each dog so that any effect on the relationship between blood flow and subsequent infarction would be constant. With this procedure, four of 15 dogs developed ventricular fibillation and were excluded from the study. Six days later the dogs were anesthetized with sodium thiomidyl (30–40 mg/kg, iv) and ventilated with a respirator, and a left thoracotomy was performed via the intercostal space below the previous thoracotomy incision. The region of the left coronary artery just distal to the circumflex coronary artery snare was dissected free and cannulated with 20- and 26-gauge needles. The 26-gauge needle was attached to a pressure transducer and 15 ml of Evans blue dye were then injected via the 20-gauge needle at pressures equal to aortic pressure.

Immediately after the injection the heart was removed, weighed, and placed in 10% buffered formalin for a 3-day period to facilitate sectioning. The lumen of the proximal circumflex coronary artery was carefully examined to verify the presence of a total occlusion by the snare. The great vessels, atria, right ventricle, large epicardial blood vessels, and visible epicardial fat were dissected from the left ventricle. The average weight of the left ventricle was 107 ± 7 g. The left ventricle was then sectioned, from base to apex, into four transverse sections of equal thickness, as previously reported and illustrated in Figure 1. The two central sections were divided into six circumferential regions, i.e., anterior, septal, posterior, posterior papillary muscle, lateral, and anterior papillary muscle. The blue-stained area in the two central sections corresponded to the posterior,
Cr, where \( Q_m = \text{myocardial flow (ml/min)} \), \( Q_r = \text{reference ml/min} \) was calculated by using the formula
\[
Q_m = \frac{Q_r C_m}{C_r}
\]

with a digital computer for contaminant activity of each nuclide. The counts/min recorded in each window from each myocardial and reference blood sample were window settings selected to correspond to the peak energies of each isotope. Each circumferential region in rings 1, 2, and 3 was divided into four equal transmural layers. Regions in ring 4 were divided into equal epicardial and endocardial layers.

The blue area defined the posterior regions of rings 1 and 4. Each region then was subsectioned into four equal transmural layers, each weighing 1–2 g. In this study, the epicardial layers will be defined as layers 1 and 2, with layer 1 being the outermost. The endocardial layers will be defined as layers 3 and 4, with layer 4 being the innermost. The sum of the weights of these samples stained blue represents the area perfused by the circumflex coronary artery.

Each myocardial sample was placed in a counting vial and 10% formalin was added to preserve the tissue for later histological studies. The myocardial and reference blood samples were counted in a gamma spectrometer at optimum window settings selected to correspond to the peak energies of each nuclide. The counts/min recorded in each window from each myocardial and reference blood sample were corrected with a digital computer for contaminant activity contributed by the accompanying isotopes and for background activity. Flow to each area of myocardium in ml/min was calculated by using the formula
\[
Q_m = \frac{Q_r C_m}{C_r}
\]

where \( Q_m = \text{myocardial flow (ml/min)} \), \( Q_r = \text{reference blood flow (ml/min)} \), \( C_m = \text{counts/min in myocardium} \), and \( C_r = \text{counts/min in reference blood flow} \). Myocardial blood flow (ml/min) was divided by the sample weight and expressed as ml/min per g.

After the blood flow measurements had been obtained, samples were prepared for histological section by recombin- ing the four tissue samples from each circumferential region in the precut transmural sequence. Histological sections were obtained from each region containing the four samples and stained with hematoxylin and eosin. Thus, the estimate of myocardial infarction was determined in each of approximately 32 tissue samples weighing 1–2 g from each dog studied. A minimum of two sections was taken at different depths in each tissue block and the average extent of infarction was determined. The percentage of infarcted myocardium in each rectangular tissue sample was determined with the use of grid markers inked on the side using a millimeter ruler so that the tissue section was divided evenly into 12.5–25% regions.

Each histological section was analyzed by two independent observers. The extent of infarction in each sample represents the average reading. If the extent of infarction determined by the two reviewers differed by more than 10% for a given sample, the histological section was re-reviewed and an average value calculated. This degree of observer variability occurred for approximately 5% of the readings. Infarcted myocardium was characterized by partial or complete cellular dissolution, extensive inflammatory cell infiltrate, and loss of normal cell architecture. Thus, 6 days postinfarction intact and infarcted myocardial tissues were clearly delineated and easily analyzed by use of routine hematoxylin and eosin stains. By this procedure blood flow and the extent of myocardial infarction were determined for multiple small samples of the entire region subjected to ischemia.

Student's t-test for paired data was used to compare sequential changes in blood flow. The coefficients of correlation were calculated by standard regression analysis.

Table 1A lists the average ± SEM of the measurements of blood flow obtained after left circumflex coronary occlusion by simultaneous injection of the four isotopes. The average difference ± SEM of these blood flow measurements is given in Table 1B. Blood flow to nonischemic layers, i.e., the anterior region, varied from 1.36 ± 0.10 to 1.66 ± 0.11 ml/min per g, with the mean blood flow difference between simultaneously measured flows ranging from 0.09 ± 0.02 to 0.12 ± 0.03 ml/min per g. Blood flow to the ischemic regions represented by the posterior, lateral, and anterior papillary muscle regions varied from 0.02 ± 0.01 to 0.56 ± 0.12 ml/min per g. Blood flow differences between simultaneously measured flows were small in the ischemic region, ranging from 0.01 ± 0.001 (SEM) at lowest flow rates to 0.06 ± 0.02 ml/min per g at highest flow rates. Thus, the variability in the distribution of the microspheres that occurred during ischemia resulted in only small differences in the computed blood flow. Changes in blood flow of 0.10 to 0.014 ml/min per g (mean difference ± 2 SEM), respectively, would represent statistically significant differences in regions with blood flow values ranging from 0.50 to 0.10 ml/min per g. This degree of variability was observed when
TABLE 1  Blood Flow Measurements after Left Circumflex Coronary Artery Occlusion Using Four Isotopes Injected Simultaneously

<table>
<thead>
<tr>
<th>Layer</th>
<th>Anterior</th>
<th>Posterior</th>
<th>Posterior papillary</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average flow (ml/min per g ± SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Layer 1</td>
<td>1.36 ± 0.10</td>
<td>0.56 ± 0.12</td>
<td>0.26 ± 0.06</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Layer 2</td>
<td>1.62 ± 0.10</td>
<td>0.56 ± 0.15</td>
<td>0.09 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Layer 3</td>
<td>1.66 ± 0.11</td>
<td>0.53 ± 0.14</td>
<td>0.04 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Layer 4</td>
<td>1.55 ± 0.10</td>
<td>0.46 ± 0.10</td>
<td>0.02 ± 0.01</td>
<td>0.09 ± 0.02</td>
</tr>
</tbody>
</table>

B. Average of flow differences (ml/min per g ± SEM)

<table>
<thead>
<tr>
<th>Layer 1</th>
<th>Layer 2</th>
<th>Layer 3</th>
<th>Layer 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.005</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>0.12 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0.003</td>
<td>0.01 ± 0.005</td>
</tr>
<tr>
<td>0.10 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.01 ± 0.003</td>
<td>0.01 ± 0.002</td>
</tr>
<tr>
<td>0.09 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.002</td>
<td>0.01 ± 0.002</td>
</tr>
</tbody>
</table>

Myocardial blood flow values obtained when four different isotopes were injected 30 seconds after left circumflex coronary artery occlusion. Measurements in part A represent average blood flow values ± SEM. Data in part B represent the average of the differences in blood flow ± SEM between the simultaneous blood flow measurements.

simultaneous injections were used and thus was obtained when hemodynamic states were identical. It is likely that variability between serial injections would be greater.

The mean hemodynamic values for 11 dogs before and at the time blood flow measurements were obtained after complete left circumflex coronary occlusion and are listed in Table 2. Before occlusion heart rate was 86 ± 5 beats/min, aortic pressure was 99 ± 3 mm Hg, and left atrial pressure was 4 ± 1 mm Hg. Heart rate increased after 20-30 seconds of occlusion and remained increased at 2, 6, and 24 hours. Sinus tachycardia was the predominant rhythm until approximately 6 hours after occlusion. At 6 hours after occlusion, premature ventricular contractions increased in frequency and initiated periods of ventricular tachycardia. Ventricular tachycardia was the predominant rhythm 24 hours after occlusion. Mean aortic pressure did not vary from control at 45 seconds and at 2 and 6 hours, but was decreased (P < 0.01) 24 hours after occlusion. Mean left atrial pressure increased significantly from control at 45 seconds and 2 and 6 hours, but was not different from control at 24 hours.

The percent of the left ventricle perfused by the circumflex coronary artery and the percent of the left ventricle and left circumflex region that subsequently became infarcted in each dog are tabulated in Table 3. The area perfused by the circumflex artery was infarcted after complete occlusion. This value represented 19 ± 6% (range, 4-33%) of the mass of the left ventricle. The blue-stained region was at best an approximation of the circumflex region since the collateral vasculature at the time of injection was different from that present at the time of occlusion. Although the blue-stained area encompassed the infarcted region, it may have been larger or smaller than the region perfused by the circumflex artery at the time of occlusion.

Mean blood flow to the anterior region did not change significantly during the course of the study. Mean blood flow to the left circumflex coronary region was 0.25 ± 0.03, 0.39 ± 0.05, 0.39 ± 0.05, and 0.53 ± 0.07 ml/min per g at 45 seconds and at 2, 6, and 24 hours, respectively. Blood flow to the circumflex region increased significantly between 45 seconds and 2 hours and between 6 and 24 hours but remained unchanged between 2 and 6 hours. Linear regression analysis between the percent of the circumflex region that was infarcted and the average blood flow resulted in correlation coefficients of -0.63, -0.87, -0.88, and -0.66 at 45 seconds and 2, 6, and 24 hours. Linear regression analysis between the percent of the circumflex region which was infarcted and the ratio of the circumflex region blood flow to anterior region blood flow for each interval resulted in correlation coefficients of -0.73, -0.81, -0.91, and -0.89. There were no significant correlations between heart rate, aortic pressure, or left atrial pressure and the percent of either the circumflex region or total left ventricle infarcted; each r < 0.6.

Sequential measurements of blood flow and the percent of subsequent myocardial infarction in transmural layers 1-4 from the entire circumflex region are listed in Table 4. Significant transmural gradients in blood flow are present with a progressive decrease in flow from layers 1-3.

TABLE 2  Hemodynamic Measurements

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean left atrial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont 45 sec 2 hr 6 hr 24 hr</td>
<td>Cont 45 sec 2 hr 6 hr 24 hr</td>
<td>Cont 45 sec 2 hr 6 hr 24 hr</td>
</tr>
<tr>
<td>86 ± 5</td>
<td>124 ± 5</td>
<td>127 ± 4</td>
</tr>
<tr>
<td>SEM &lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Heart rate, mean aortic pressure, mean left atrial pressure before (Cont), 45 seconds, and 2, 6, and 24 hours after complete left circumflex coronary artery occlusion. Values represent the mean ± SEM. P values compare the values at each intervention to control values. P > 0.05 = NS (not significant).
Table 3

**Left Ventricular Infarction and Blood Flow to Left Anterior Descending and Left Circumflex Coronary Artery Regions**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>LV wt (g)</th>
<th>% LV cir.</th>
<th>% LV inf.</th>
<th>LAD blood flow (ml/min per g)</th>
<th>LCCA blood flow (ml/min per g)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45 sec</td>
<td>2 hr</td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>37</td>
<td>23</td>
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<td>2</td>
<td>94</td>
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<td>60</td>
<td>1.37</td>
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<td>3</td>
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<td>34</td>
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<td>1.32</td>
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<td>20</td>
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<tr>
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<tr>
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<td>89</td>
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<td>12</td>
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<tr>
<td>10</td>
<td>130</td>
<td>36</td>
<td>16</td>
<td>46</td>
<td>1.54</td>
</tr>
<tr>
<td>11</td>
<td>60</td>
<td>29</td>
<td>8</td>
<td>26</td>
<td>0.87</td>
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</table>

**Mean**

<table>
<thead>
<tr>
<th></th>
<th>109</th>
<th>36</th>
<th>19</th>
<th>52</th>
<th>1.18</th>
<th>1.31</th>
<th>1.16</th>
<th>1.20</th>
<th>0.25</th>
<th>0.39</th>
<th>0.39</th>
<th>0.53</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>±7</td>
<td>±1</td>
<td>±3</td>
<td>±6</td>
<td>±0.10</td>
<td>±0.10</td>
<td>±0.08</td>
<td>±0.15</td>
<td>±0.03</td>
<td>±0.05</td>
<td>±0.05</td>
<td>±0.07</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The tabulation of the left ventricular weight (LV wt, in grams), percent of left ventricle perfused by the left circumflex coronary artery (% LV cir.), percent of left ventricle infarcted (% LV inf.), percent circumflex region infarcted (% cir inf.), and mean blood flow measurements (ml/min per g) in the areas perfused by the left anterior descending coronary artery (LAD) and the left circumflex coronary artery (LCCA) 45 seconds, and 2, 6, and 24 hours postocclusion. *P* values compare the blood flow values at each interval with the preceding value. *P > 0.05 = NS (not significant).

Significant transmural differences in the extent of myocardial infarction were present with progressive increments in infarction in layers 1, 2, and 3. Blood flows and percent of myocardial infarction in layers 3 and 4 were not significantly different. Thus, the transmural distribution of blood flow was inversely proportional to the distribution of myocardial infarction. Blood flow in each layer increased between 45 seconds and 2 hours and between 6 and 24 hours but remained unchanged between 2 and 6 hours. The increments in blood flow after acute coronary occlusion were greater in the epicardial layers than in the endocardial layers, i.e., flow increased an average of 0.42 ml/min per g in layer 1 as compared to 0.14 ml/min per g in layer 4. The percent changes in flows were comparable in each layer.

Table 5 shows the relationship between blood flow at 45 seconds and 2, 6, and 24 hours after occlusion with data grouped in flow ranges of 0.0-0.10, 0.11-0.20, 0.21-0.35, 0.36-0.50, 0.51-0.75, and 0.76-1.00 ml/min per g and the percent myocardial infarction in samples from the four transmural layers in the ischemic area. In each transmural layer the percent myocardial infarction was inversely related to blood flow. For a given range of blood flow the percent of infarcted myocardium in endocardial samples exceeded the percent of infarction in epicardial samples, indicating that the relationship between blood flow and the extent of myocardial infarction varied in different transmural layers of the myocardium. The standard error of each mean value for infarction in a given range of flow is a measure of the variability of the relationship between blood flow and the extent of myocardial infarction. The mean myocardial infarction values ± 2 SEM should predict with 95% confidence limits similar degrees of myocardial infarction in a different group of dogs subjected to comparable experimental conditions. The average standard error of the percent infarction values was ±8%, ±7%, ±6%, and ±7% at 45 seconds and 2, 6, and 24 hours, respectively. The relationship between blood flow 45 seconds and 2 hours after occlusion and the extent of infarction are illustrated graphically in Figures 2 and 3. The infarct values in layers 3 and 4 were combined in the graphs because, with the exception of values in the blood flow range of 0.36-0.50 at 2 hours, the percent of infarction was not significantly different in these layers.

**Discussion**

Myocardial ischemia does not result in homogeneous infarction of the ischemic zone. The ischemic zone contains

Table 4

**Mean Myocardial Infarction and Blood Flow in Transmural Layer from the LCCA Region**

<table>
<thead>
<tr>
<th>Transmural layer</th>
<th>Infarcted myocardium (%)</th>
<th>LCCA region blood flow (ml/min per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 sec</td>
<td>2 hr</td>
</tr>
<tr>
<td>1</td>
<td>19 ± 6</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>46 ± 9</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>70 ± 8</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>76 ± 6</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

Mean percent infarcted myocardium and sequential measurements of myocardial blood flow (ml/min per g) in samples from transmural layers 1-4 of the region perfused by the left circumflex coronary artery (LCCA).
varying amounts of reversibly and irreversibly injured myocardium for a variable period of time after the onset of ischemia. The anatomical criteria for recognition of irreversible, as compared to reversible, cell injury early after ischemia remain controversial. For this reason, in the present study the extent of infarction was determined 6 days after coronary occlusion so that intact and infarcted myocardium for a variable period of time after the onset of ischemia.

The anatomical criteria for recognition of irreversible, as compared to reversible, cell injury early after ischemia remain controversial. For this reason, in the present study the extent of infarction was determined 6 days after coronary occlusion so that intact and infarcted myocardium for a variable period of time after the onset of ischemia. 18-21 The anatomical criteria for recognition of myocardial infarction in the ischemic regions, it was anticipated that selected sampling may not accurately reflect the extent of infarction in a given myocardial region in which blood flow was measured. Thus, the relationship between blood flow and the extent of myocardial infarction was determined by measuring blood flow and the extent of histological infarction in multiple small samples from separate transmural layers of the entire ischemic zone.

Initial studies were carried out to determine the precision of the microsphere technique for measuring blood flow in small myocardial samples under conditions of reduced flow rates. Buckberg et al. 23 observed that if the number of microspheres in certain myocardial samples would be less than 400. To determine whether prohibitively high measurement variability would occur at low flow rates using the microsphere technique, the four microspheres used for measuring organ blood flow would occur when tissue samples contained less than 400. The random variability in the number of microspheres in simultaneously collected reference blood samples increased precipitously. Buckberg et al. 23 observed that if the number of microspheres in certain myocardial samples would be less than 400. To determine whether prohibitively high measurement variability would occur at low flow rates using the microsphere technique, the four microspheres used for measuring organ blood flow; the extent of myocardial infarction in multiple small samples from separate transmural layers of the entire ischemic zone.

Initial studies were carried out to determine the precision of the microsphere technique for measuring blood flow in small myocardial samples under conditions of reduced flow rates. Buckberg et al. 23 observed that if the number of microspheres in certain myocardial samples would be less than 400. To determine whether prohibitively high measurement variability would occur at low flow rates using the microsphere technique, the four microspheres used for measuring organ blood flow in small myocardial samples under conditions of reduced flow rates. Buckberg et al. 23 observed that if the number of microspheres in certain myocardial samples would be less than 400. To determine whether prohibitively high measurement variability would occur at low flow rates using the microsphere technique, the four microspheres used for measuring organ blood flow in small myocardial samples under conditions of reduced flow rates.
Mean blood flow measured for multiple small left ventricular samples using 7- to 10-μm microspheres was approximately 0.80 ± 0.06 ml/min per g during quiet resting conditions. In the present study, mean flow in the region supplied by the anterior descending coronary artery was 1.18 ± 0.10 ml/min per g 45 seconds after left circumflex occlusion. Thus, it is likely that acute coronary occlusion resulted in significant increases in blood flow to the nonischemic regions coincident with significant increases in heart rate from 86 ± 5 to 124 ± 4 beats/min. Blood flow to the circumflex region 45 seconds after circumflex occlusion averaged 0.25 ± 0.03 ml/min per g but was quite variable in different dogs, ranging from 0.12 to 0.52 ml/min per g.

The time course of early changes in ischemic flow after acute occlusion has not been clearly defined previously. Early increments, as well as initial values of ischemic flow, appear to be critically dependent on the conditions of the experiment as well as the technique used to estimate ischemic blood flow. Investigators studying anesthetized open-chest dogs have generally reported no significant increase in flow to the ischemic region in the initial hours after acute occlusion. In intact dogs which were "lightly anesthetized," Rees and Redding observed that 133Xe clearance in an ischemic region increased by approximately 20% within 1-2 hours after occlusion and then decreased between 2 and 6 hours. Pasyk et al., using 133Xe clearance in awake resting dogs, observed progressive increases in clearance rates beginning after occlusion. Clearance rates were equal to or in excess of preocclusion values 20 to 31 hours after occlusion. In the present study, significant increments in blood flow to the ischemic region occurred between 45 seconds and 2 hours and between 6 and 24 hours after occlusion. Blood flow increased by an average of approximately 100% during the 24 hours after occlusion.

In the present study, blood flow was not distributed evenly throughout the ischemic region. There were significant transmural gradients of flow in most regions, with flow decreasing from epicardium to endocardium. Transmural flow gradients were highest in the more ischemic regions. Numerous studies have documented that a variety of interventions which limit coronary flow, reduce coronary perfusion pressure, increase intraventricular pressure, or stimulate the distal vasculature when flow is limited proximally may result in uneven distribution of transmural blood flow measurements were injected simultaneously after left circumflex coronary artery occlusion. The average differences ± SEM between simultaneously measured flows provide an index of the variability of the microsphere technique for measuring blood flow in small ventricular samples and define the change in blood flow which may be detected by this technique. Although the reduced delivery of microspheres to the myocardial region during ischemia resulted in an increased variability of measurement, expressed as percentage differences, such variability resulted in only small differences in the simultaneously measured volumes of blood flow. These data indicate that the microsphere technique can be employed to reliably measure small changes in blood flow to ischemic regions.

Estimates of blood flow after acute coronary occlusion have varied considerably, depending on the measurement technique and conditions of the experiment. An early technique used for surgical preparations quantified the volume of blood flowing retrograde distal to a proximally occluded artery. Retrograde flow after acute occlusion represented approximately 10% of preocclusion flow values. Levy et al., using 82Rb clearance techniques, reported that flow to the ischemic region ranged from 22% to 67% of the flow to nonischemic areas. Rees and Redding, using the 133Xe clearance technique in anesthetized dogs, observed flow values after acute occlusion which ranged from 10% to 25% of preocclusion flows. Pasyk et al., using the same technique in chronically prepared awake dogs, observed flow values in the region supplied by the occluded artery which averaged 45% of preocclusion values. These studies suggest fundamental differences in the level of blood flow to ischemic regions in awake dogs as compared to anesthetized dogs with the chest open. Becker et al., using 15-μm radioactive tagged microspheres and anesthetized dogs, reported that the radioactivity in ischemic regions was 20% of control region radioactivity.

The microsphere technique has proved to be easily adapted to chronic studies. In previous studies in this laboratory on dogs prepared in a manner similar to that of the present study, mean blood flow measured for multiple small left ventricular samples using 7- to 10-μm microspheres was approximately 0.80 ± 0.06 ml/min per g during acute coronary occlusion. These data are presented in tabular form in Table 5.
flow with disproportionate endocardial underperfusion. Moir and DeBra,14 using 82Rb clearance methods, and Griggs and Nakamura,15 using clearance of iodoantipyrine,15,16 observed disproportionate underperfusion of the endocardial region when coronary perfusion pressure or coronary blood flow was reduced. Becker et al.17 using 15-μm radioactive microspheres, observed an endocardial-epicardial (endo/epi) ratio of radioactivity of 0.76 ± 0.30 in the ischemic region after complete coronary artery occlusion. Bache et al.27 observed that ischemia-induced vasodilatation in the presence of a proximal flow-limiting obstruction resulted in redistribution of myocardial blood flow so that selective subendocardial underperfusion occurred independently of changes in either heart rate or aortic or ventricular pressure.

Although previous experimental and clinical studies have demonstrated that the endocardial as compared to epicardial regions demonstrate greater infarction,18,19,25 the relationship between blood flow and the distribution and extent of myocardial necrosis has not been defined. In the present study, blood flow measurements correlated significantly with the subsequent extent of myocardial infarction. Since the area of infarction averaged only 52% (range, 12–84%) of the area perfused by the occluded left circumflex coronary artery, residual inflow after occlusion afforded protection for a considerable but highly variable amount of ischemic myocardium. The extent of infarction was greatest in endocardial layers 3 and 4, with decreasing amounts of infarction in layers 2 and 1. Thus, the transmural distribution of infarction was inversely related to the transmural distribution of blood flow. Infarction in different dogs differed by the degree of extension into layers 1, 2, and 3.

The extent of infarction was greatest in rings 1, 2, and 3, with variable extension in ring 4. Infarction was greatest in the posterior papillary region of rings 2 and 3 and lateral posterior segment of ring 1, intermediate in the lateral segment of rings 2 and 3, and least in the posterior segments of rings 2, 3, and 4 and in the medial posterior segment of ring 1. Histological sections of multiple small myocardial samples from the entire ischemic zone demonstrated that the distribution of infarction was not homogeneous. The boundary zone between noninfarcted and infarcted myocardium was extremely variable. Extension of infarction into layers 1 and 2 did not require total infarction of layers 3 and 4.

The relationship between blood flow and extent of infarction was examined further by grouping the samples for each transmural layer according to blood flow ranges. Within each transmural layer there was an inverse relationship between blood flow and the extent of infarction. Thus, the distribution of flow between rings, circumferential regions within a ring, and transmural layers conformed to the distribution of myocardial infarction as described above. When myocardial samples in the same blood flow ranges in each transmural layer were compared, the extent of infarction was greater in endocardial as compared to epicardial samples. The relationship between blood flow at a given interval after acute coronary artery occlusion and the extent of subsequent myocardial infarction varied in different transmural layers. There are at least two possible explanations for the disproportionate extent of endocardial infarction. As blood flow increased to the ischemic region (Table 5), the number of samples in the low blood flow ranges decreased and the number in the high blood flow ranges increased. There was a greater tendency for samples in the epicardial layers, as compared to samples in the endocardial layers, to shift to higher blood flow ranges, indicating that the increments in blood flow to the ischemic region were preferentially delivered to the epicardial layers. For example, 45 seconds after occlusion there were 44 samples in layer 1 and 66 samples in layer 4 in the flow ranges of 0–0.35 ml/min per g, whereas 24 hours after occlusion, eight samples in layer 1 and 46 samples in layer 4 remained in these flow ranges. Although disproportionate endocardial infarction was still apparent when samples were grouped 24 hours after occlusion, the differences between endocardial and epicardial infarction for comparable blood flow ranges were less striking.

Alternatively, it is possible that factors operating independently of blood flow may have contributed to the disproportionate endocardial infarction. Griggs et al.30 observed that coronary artery occlusion resulted in significant transmural metabolic gradients with concentrations of lactate and pyruvate increasing from outer to inner wall and adenosine triphosphate decreasing from outer to inner wall. The possibility that the transmural metabolic gradients may have resulted from transmural gradients in wall stress as well as blood flow was discussed by these investigators. Although the factors that contribute to the apparent disproportionate endocardial infarction have not been clearly delineated, these data indicate that the transmural location of the myocardial samples is an important determinant of the relationship between blood flow after acute coronary occlusion and the extent of subsequent infarct.

The relationship between measurements of blood flow early after acute coronary artery occlusion and the extent of subsequent myocardial infarction in samples grouped according to blood flow ranges and transmural layers should provide reference data that can be used to evaluate interventions which alter the extent of infarction (Table 5, Figs. 2 and 3). The mean percent infarction ± 2SEM should predict with 95% confidence limits a similar amount of infarction in a different group of dogs subjected to the same experimental conditions. The standard error of each percent infarct value in a given flow range is a measure of the variability of the relationship between blood flow and subsequent infarction. The standard error of each infarct value thus determines the minimum change in infarct size that can be detected in a given flow range and layer. In future studies designed to evaluate the effects of a given intervention on infarct size, the protocol used in the present study will be followed. Blood flow will be measured 45 seconds and 2, 6, and 24 hours after occlusion. The intervention to be evaluated could be initiated after the 45-second, 2-hour, or 6-hour blood flow measurement. The blood flow data obtained before the intervention can be used to predict the expected amount of infarction, using the data provided in the present study. The
blood flow measurements obtained after initiation of the intervention can be compared to the blood flow data observed in the present study. Thus, any alteration in the extent of infarction can be related to changes in blood flow.

Studies by Jennings and Reimer on anesthetized animals indicate that infarction begins first in the papillary muscle areas of the endocardium. As the duration of the occlusion was increased, the initial infarct area progressively increased and involved varying amounts of the epicardium. 

β-Adrenergic blockade with propranolol reduced infarction at the ischemic border or endocardial region, or both and (3) thereby infarction occurred coincidentally with alterations in blood flow. The model employs an awake dog and thus avoids the alterations in the relationship between blood flow and infarction size which may prevent or greatly enhance infarction in these border areas and thus be detected by this technique. Alternatively, if transmural wall tension is an important determinant of the distribution of infarction, interventions which influence the generation of wall tension, such as increases and decreases in aortic pressure, may be expected to alter ischemic injury to a greater extent in the endocardium. It is possible that certain interventions will alter the extent of infarction independently of changes in blood flow whereas other interventions will alter infarction coincidentally with increases or decreases in blood flow to the ischemic region.

By permitting measurement of blood flow at intervals after acute coronary occlusion and determination of the relationship between blood flow and the extent of infarction in separate transmural layers rather than in the total region of ischemia, this model should provide (1) a sensitive technique for evaluating interventions that reduce or extend infarction, (2) data concerning the mechanism whereby intervention alters infarction, i.e., alteration in infarction at the ischemic border or endocardial region, or both and (3) reference data that can be used to determine whether alterations in the relationship between blood flow and infarction occurred coincidentally with alterations in blood flow. The model employs an awake dog and thus avoids the variables introduced by general anesthesia and acute surgery.

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