Effects of Infarcted Myocardium on Regional Blood Flow Measurements to Ischemic Regions in Canine Heart

ROBERT H. MURDOCK, JR., AND FREDERICK R. COBB

SUMMARY The present study assesses effects of acutely infarcted myocardium on apparent microsphere loss as a function of time, determines effects of apparent microsphere loss on blood flow measurements to ischemic regions, and determines to what extent apparent microsphere loss alters interpretation of serial measurements of collateral blood flow. Studies were performed in 35 awake mongrel dogs chronically instrumented with catheters in the aorta and left atrium and an occluder on the proximal circumflex coronary artery. Myocardial blood flow was measured before and 15 minutes after complete occlusion. Dogs were randomly divided into four groups to be killed at 6 and 24 hours, and 3 and 6 days. In the 3-day group, an additional blood flow measurement was made 24 hours postocclusion. The entire left ventricle was sectioned into 1- to 2-g samples and myocardial blood flow determined. The ratio of preocclusion blood flow in each ischemic sample to mean nonischemic flow was used to calculate apparent microsphere loss and to correct ischemic blood flow in each sample. Significant apparent microsphere loss occurred in epicardial layers at 24 hours and in epi- and endocardial layers at 3 and 6 days; maximum loss at each interval was 22.3, 19.4, and 22.2% respectively. Absolute blood flow corrections for ischemic myocardium were small, range —0.035 to 0.083 ml/min per g. Changes in flow to ischemic regions between 15 minutes and 24 hours were comparable before and after correction for apparent microsphere loss. Although infarction resulted in significant apparent microsphere loss, effects on ischemic blood flow measurements were very small and consequently did not prevent interpretation of serial blood flow measurements after infarction in animals killed at 3 days. Circ Res 47: 701-709, 1980

THE radioisotope labeled microsphere technique has been used to assess regional myocardial blood flow during a variety of conditions (Domenech et al., 1969; Fortuin et al., 1971; Becker et al., 1973; Cobb et al., 1974; Marcus et al., 1975; Capurro et al., 1977), including serial blood flow measurements after acute myocardial infarction (Rivas et al., 1976; Cox et al., 1975; Bishop et al., 1976; Hirzel et al., 1976). Recent reports have suggested that infarced myocardium may effect microsphere loss which introduces errors that prohibit assessment of collateral blood flow measurements at intervals greater than 24 hours (Capurro et al., 1979). Jugdutt et al. (1979) concluded that the loss of microspheres from infarcted myocardium is a limitation of the technique which results in uncertain accuracy of the blood flow values. Other investigators have not observed significant microsphere loss (Pantely et al., 1978). The interpretation of microsphere loss is based on the observation that, in the absence of myocardial infarction, regional myocardial blood flow is relatively homogeneous in the left ventricle, i.e., the ratio of blood flow in circumflex to anterior descending coronary regions equals one (Cobb et al., 1974), but after infarction the ratio of preoclusion regional blood flow in infarcted to noninfarcted regions decreases (Capurro et al., 1979; Jugdutt et al., 1979). Although reduction in the ratio of preoclusion regional blood flow in infarcted to noninfarcted myocardium has been interpreted as resulting from microsphere loss, other investigators have concluded that the reduction in the ratio can be explained by tissue swelling resulting from increased fluid content and/or inflammatory cell infiltration after acute infarction (Reimer and Jennings, 1979). These responses to acute infarction would result in an apparent rather than an actual loss of microspheres. The effects of the apparent and/or actual loss of microspheres resulting from infarcted myocardium on regional blood flow measurements have not been assessed.

The purpose of the present study was to assess the effects of acutely infarcted myocardium on apparent microsphere loss as a function of the time after coronary artery occlusion, to determine the effects of apparent microsphere loss on blood flow measurements to ischemic regions, and to determine to what extent these effects alter interpretation of serial measurements of collateral blood flow.
Effects of infarcted myocardium on regional blood flow were assessed by (1) calculating a correction fraction for individual myocardial samples based on the ratio of preocclusion blood flow in ischemic/nonischemic samples and (2) determining the magnitude of the blood flow corrections in samples grouped according to the degree of myocardial ischemia. The studies were carried out in awake, chronically instrumented dogs to avoid the variables of anesthesia and acute surgery.

**Methods**

Complete studies were performed on 35 mongrel dogs weighing 20-30 kg. The dogs were anesthetized with thiamylal sodium (30-40 mg/kg, iv), and underwent a left thoracotomy. Heparin-filled polyvinyl chloride catheters were inserted into the left atrial cavity and the aortic root, and tunneled to a subcutaneous pouch at the base of the neck.

A polyethylene loop, snare-type occluder was placed around the proximal left circumflex coronary artery and tunneled to a subcutaneous pouch on the anterior chest wall near the base of the thoracotomy incision.

Studies were performed 7-10 days after surgery. The pouches were anesthetized with lidocaine and the catheters and snare exteriorized. Phasic and mean aortic and left atrial pressures, and an electrocardiogram of lead 2 or 3 were recorded on a Sanborn 8 channel recorder.

A control myocardial blood flow measurement was obtained prior to occlusion. Myocardial blood flow was determined by injecting carbonized microspheres 9 ± 1 ± SD jum in diameter and labeled with γ-emitting nuclides 51Cr, 141Ce, 85Sr, 125I, 95Nb, and 46Sc. The microspheres were obtained as 1 mCi of each nuclide in 10 ml of 10% Dextran and 0.05% of polysorbate 80 (3M Co.), and diluted in 10% Dextran such that 1.0 ml of the volume injected, contained approximately 3 × 10^9 microspheres. Before each injection, the microspheres were mixed by alternate agitation for at least 15 minutes in an ultrasonic bath (3M Co., model DA0950) and a Vortex agitator. A volume of 1.0 ml of the microsphere suspension was injected into the left atrium over a period of 10 seconds and the atrial catheter was flushed with 5 ml of isotonic saline. Beginning simultaneously with each microsphere injection and continuing for 90 seconds, a reference blood sample was collected from the aortic catheter in counting vials at a constant rate, with a withdrawal pump. Serial injections of the microspheres resulted in no change in heart rate during the interval of injection and no change in aortic or left atrial pressure measured immediately before and after collection of the reference blood sample.

After a control blood flow measurement, each dog was given lidocaine, 2 mg/kg iv, to reduce possible arrhythmias, and 2 mg of morphine sulfate, iv, to reduce pain which might be experienced following coronary artery occlusion.

Occlusion was completed over a period of 30-60 minutes. Morphine sulfate, 10 mg, was administered in increments of 2 mg every 5-10 minutes during the period of occlusion. The dogs were divided into four groups of 7, 14, 8, and 6, and were killed at 6 and 3 days, 24 and 6 hours postocclusion respectively. Blood flow measurements were made 15 minutes after occlusion in each group and 24 hours after occlusion in the 3-day group. All dogs were anesthetized with sodium thiamyl and the hearts fibrillated with concentrated potassium chloride. The hearts were removed immediately and placed in formalin solution for 3 days.

The hearts were then cut into four rings from base to apex (Cobb et al., 1974; Rivas et al., 1976) (Fig 1). The base ring was cut into four circumferential regions from the anterior, septal, posterior, and lateral walls. Anterior and posterior sections were cut from the apex ring. Each of the two inner rings was divided into six circumferential regions: anterior, septal, posterior, posterior papillary, lateral, and anterior papillary. Each region was then divided into four transmural layers weighing approximately 1–2 g. The radioactivity in each reference blood and tissue sample was measured in a Packard Gamma Scintillation Spectrometer, using window settings selected to correspond with the peak energies of each radioactive nuclide. Blood flow per tissue sample was determined using the counts per ml per minute for the blood samples and

![FIGURE 1](image-url) **FiguRE 1** This schematic illustrates the technique for sectioning the left ventricle. The atrial tissue and right ventricle were removed, as indicated by the stippled and lined area. The left ventricle was sectioned into four transverse rings. Each ring was sectioned into circumferential regions, i.e., anterior (A), septal (S), posterior (P), posterior papillary (PP), lateral (L), and anterior papillary (AP). Each circumferential region in rings 1, 2, and 3 was divided into four equal epicardial and endocardial layers.
the counts per minute for the tissue sample. Blood flow was calculated using the following formula:

\[ Q_m = Q_r \cdot C_m / C_r \quad (1) \]

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

The effects of microsphere loss and/or tissue swelling, henceforth referred to as an apparent microsphere loss, on individual samples of the ischemic region were determined by calculating the ratio of preocclusion blood flow for ischemic to nonischemic samples in the same transmural layer. Percent apparent microsphere loss was calculated using the following equation:

\[ \% \text{ Apparent microsphere loss} = \frac{\text{MCF} - \text{CFI}}{\text{MCF}} \times 100 \quad (2) \]

where \( \text{CFI} \) = preocclusion blood flow in individual samples of the region subjected to ischemia, and \( \text{MCF} \) = mean flow in nonischemic samples from the same layer. If \( \text{CFI} \) is greater or less than \( \text{MCF} \) in Equation 2, the \% apparent loss appears as a negative or positive value, respectively. A positive value indicates a loss and a negative value an apparent gain. Since the infarction process should affect the microspheres injected before and after ischemia in a similar fashion, the apparent loss of all microspheres present in the sample should be comparable. The following equation was used to correct ischemic blood flow measurements in each sample in the ischemic zone.

\[ \text{MBF}_c = \text{MBF}_U \cdot \left( \frac{100}{100 - \% \text{ microsphere loss}} \right) \quad (3) \]

where \( \text{MBF}_c \) = corrected blood flow value, \( \text{MBF}_U \) = uncorrected blood flow value, and \% microsphere loss equal the percent loss from Equation 2. The ischemic blood flow corrections were expressed in ml/min per g. The group analyses of microsphere loss and blood flow correction were analyzed by grouping the samples from epicardial and endocardial layers, respectively, as a function of the degree of ischemia.

Studies were carried out in an additional group of seven animals not included in the preceding analyses to determine whether 9 ± 1 μm microspheres as compared to 15 μ ± 1 SD microspheres are preferentially lost from normal myocardium. Each animal was prepared in the same manner as previously described except that a snare was not implanted on a coronary artery. On the initial study day, regional blood flow was measured by simultaneous injection of approximately three million 9-μm and three million 15-μm microspheres labeled with different radioisotopes. Three days later, regional myocardial blood flow was measured again by simultaneous injection of 9- and 15-μm microspheres. Each animal was killed within 5 minutes of the final injection, and regional blood flow was measured as previously described.

**Data Analysis**

Apparent microsphere loss and the effects on blood flow were analyzed by grouping the samples according to blood flow ranges. In each blood flow range, mean apparent microsphere loss was expressed as a percent loss and blood flow correction in ml/min per g. An intuitive t-test was performed on each value for each group of dogs. A mean value was determined to be significantly different from zero when the value was greater than 2 SE from zero. Comparisons between groups killed on different days were performed by a one-way analysis of variance. The changes in collateral blood flow between 15 minutes and 24 hours after occlusion were analyzed by Student's t-test. The comparison of blood flow measured by simultaneous injection 3 days and 5 minutes prior to injection was performed by Student's t-test.

**Results**

Table 1 lists the mean regional blood flow values ± 1 SEM obtained in 11 dogs not subjected to coronary occlusion. Measurements were obtained during quiet resting conditions. This table illustrates that, with the exception of the anterior region in layer 1, \( P = 0.01 \), there were no significant differences in blood flow in circumferential regions in a given myocardial layer. The exception resulted from low blood flow values in the anterior region of layer 1 in 3 dogs.

Tables 2 and 3 tabulate the apparent microsphere loss, % ± 1 SEM, and blood flow correction, ml/min per g ± 1 SEM as a function of blood flow ranges. Tables 2 and 3 list mean data from epicardial layers 1 and 2, and endocardial layers 3 and 4, respectively. There was no significant apparent microsphere loss at 6 hours. However, there was a small but significant apparent gain of 9.3 and 10.4% in the 0.21–0.35 and 0.36–0.50 ml/min per g blood flow range, respectively. Significant microsphere loss was present at 24 hours in epicardial samples with flow below 0.35 ml/min per g with maximal loss of 22.3% in the most ischemic range. At both 3 and 6 days, there was significant loss in the epicardial and endocardial samples. Maximum loss at 3 days was 19.4%. At 6 days, maximum loss was 22.2%. In general, the greatest apparent microsphere loss occurred in the region with greatest reduction in blood flow. In most regions, significant apparent microsphere loss was associated with statistically significant but very small corrections in blood flow to the ischemic regions, range –0.063 to 0.083 ml/min per g.

Comparison of measurements between the four groups killed on different days, by analysis of variance using the Scheffe test for two group compari-
sons, demonstrated significant differences, $P < 0.05$, between the epicardial samples from the groups killed at 6 hours vs. 24 hours (0.1-0.2 and 0.2-0.35 range), 6 hours vs. 3 days (0.10-0.20 range), and 6 hours vs. 6 days (0.1-0.2 range). The remaining comparisons between groups did not reach statistical significance.

Table 4 gives the mean blood flow values in layers 1-4 of myocardial samples from nonischemic regions at 15 minutes and 24 hours after coronary occlusion from the 3-day group. Mean blood flow values were less at 24 hours in each layer, but achieved statistical significance only in epicardial layer 1, $P = 0.04$. The uncorrected and corrected ischemic blood flow values at 15 minutes and 24 hours grouped according to ischemic blood flow ranges are illustrated in Table 5 and Figure 2. Each ischemic blood flow value was modified for apparent microsphere loss based on factors which corrected preocclusion blood flow in each ischemic sample to the mean nonischemic region blood flow. Collateral blood flow increased significantly in each ischemic blood flow range in which blood flow 15 minutes after occlusion was less than 0.50 ml/min per g, each $P < 0.01$. Correction of ischemic blood flow measurements for apparent microsphere loss resulted in relatively small corrections in absolute blood flow. The relative changes in collateral blood flow in each ischemic range between 15 minutes and 24 hours was comparable before and after correction. Because the blood flow corrections were small relative to the change in collateral blood flow, the effects of apparent microsphere loss did not prevent interpretation of significant increments in blood flow to ischemic regions in the dogs killed at 3 days.

Table 5 gives the measurements of regional blood flow obtained by simultaneous injection of 9- and 15-μm microspheres 3 days and 5 minutes prior to sacrifice in a group of animals that was not subjected to coronary artery occlusion. There was close agreement between the measurements of regional blood flow by simultaneous injection of 9- and 15-μm microspheres 3 days before the dogs were killed, i.e., $1.20 \pm 0.11$ and $1.13 \pm 0.11$, respectively, and 5 minutes prior to sacrifice, i.e., $1.14 \pm 0.09$ and $1.19 \pm 0.09$, respectively. The slight differences in average blood flow were statistically significant on each day. The average blood flow values measured by microspheres of the same size on different days were not significantly different. These data demonstrate no preferential loss of the smaller 9-μm microspheres from normal myocardium during a 3-day period after injection.

### Table 1  Regional Myocardial Blood Flow in Transmural Layers 1-4

<table>
<thead>
<tr>
<th>Layers</th>
<th>Septal</th>
<th>Posterior</th>
<th>Posterior papillary</th>
<th>Lateral</th>
<th>Anterior papillary</th>
<th>Anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi 1</td>
<td>0.70 ± 0.08</td>
<td>0.64 ± 0.06</td>
<td>0.68 ± 0.06</td>
<td>0.63 ± 0.05</td>
<td>0.58 ± 0.05</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>0.76 ± 0.09</td>
<td>0.75 ± 0.07</td>
<td>0.76 ± 0.07</td>
<td>0.72 ± 0.07</td>
<td>0.70 ± 0.06</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>0.79 ± 0.09</td>
<td>0.83 ± 0.07</td>
<td>0.78 ± 0.07</td>
<td>0.75 ± 0.06</td>
<td>0.74 ± 0.06</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td>Endo 4</td>
<td>0.71 ± 0.07</td>
<td>0.76 ± 0.05</td>
<td>0.66 ± 0.04</td>
<td>0.72 ± 0.04</td>
<td>0.76 ± 0.05</td>
<td>0.71 ± 0.05</td>
</tr>
</tbody>
</table>

Mean blood flow values ± SEM in transmural layers 1-4 from circumferential left ventricular regions in a group of dogs not subjected to coronary artery occlusion.

### Table 2  Relationship of Apparent Microsphere Loss and Blood Flow Correction to the Degree of Ischemia in Epicardial Layers 1 and 2

<table>
<thead>
<tr>
<th>Blood flow ranges (ml/min per g)</th>
<th>0-0.10</th>
<th>0.11-0.20</th>
<th>0.21-0.35</th>
<th>0.36-0.50</th>
<th>0.51-0.75</th>
<th>0.76-1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hour % Loss</td>
<td>-4.5 ± 7.6</td>
<td>-8.3 ± 5.5</td>
<td>-9.3 ± 3.0*</td>
<td>-10.4 ± 4.3*</td>
<td>-5.0 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>Flow corr.</td>
<td>-0.001 ± 0.002</td>
<td>-0.009 ± 0.006</td>
<td>-0.023 ± 0.007*</td>
<td>-0.035 ± 0.016*</td>
<td>-0.016 ± 0.036</td>
<td></td>
</tr>
<tr>
<td>24 hour % Loss</td>
<td>22.3 ± 10.7*</td>
<td>17.7 ± 2.9*</td>
<td>16.8 ± 7.6*</td>
<td>8.8 ± 6.4</td>
<td>1.2 ± 4.3</td>
<td>6.4 ± 4.9</td>
</tr>
<tr>
<td>Flow corr.</td>
<td>0.029 ± 0.017</td>
<td>0.038 ± 0.008*</td>
<td>0.083 ± 0.035*</td>
<td>0.062 ± 0.045</td>
<td>0.024 ± 0.031</td>
<td>0.078 ± 0.055</td>
</tr>
<tr>
<td>3 day % Loss</td>
<td>19.4 ± 5.4*</td>
<td>10.3 ± 4.4*</td>
<td>10.8 ± 4.2*</td>
<td>3.1 ± 4.7</td>
<td>3.5 ± 2.8</td>
<td>-3.3 ± 7.4</td>
</tr>
<tr>
<td>Flow corr.</td>
<td>0.018 ± 0.005*</td>
<td>0.024 ± 0.009*</td>
<td>0.048 ± 0.016*</td>
<td>0.031 ± 0.027</td>
<td>0.030 ± 0.017</td>
<td>-0.020 ± 0.058</td>
</tr>
<tr>
<td>7/28</td>
<td>12/43</td>
<td>12/36</td>
<td>11/25</td>
<td>13/40</td>
<td>5/7</td>
<td></td>
</tr>
<tr>
<td>6 day % Loss</td>
<td>22.2 ± 2.9*</td>
<td>16.2 ± 3.1*</td>
<td>8.0 ± 4.91</td>
<td>-3.0 ± 6.6</td>
<td>-12.5 ± 4.3*</td>
<td></td>
</tr>
<tr>
<td>Flow corr.</td>
<td>0.017 ± 0.003*</td>
<td>0.035 ± 0.007*</td>
<td>0.027 ± 0.015</td>
<td>-0.006 ± 0.026</td>
<td>-0.063 ± 0.022</td>
<td></td>
</tr>
<tr>
<td>5/23</td>
<td>6/30</td>
<td>6/18</td>
<td>3/7</td>
<td>6/21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apparent microsphere loss in % ± SEM and blood flow correction in ml/min per g in samples from layers 1 and 2 grouped according to the degree of ischemia 15 minutes after coronary artery occlusion; negative values indicate apparent microsphere gain.

* $P < 0.05$. Ratios = number of dogs contributing to each particular value; total number of myocardial samples. The values were obtained by first calculating an average % loss and flow correction for each dog and then obtaining the averages of both values for the group.
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presented an alternative explanation for the reduc-

tion in the ratio after infarction. These investigators

observed that, following myocardial infarction the

blood flow could be accounted for by tissue swelling

resulted from a combination of tissue edema which

accounted for approximately 40% of the apparent

microsphere loss. These investigators

observed an increase of microspheres in the lungs

initially and by contraction of the infarcted myo-

Cardium later. In recent studies, Jugdutt et al.

(1979) presented evidence that the apparent loss

resulted from a combination of tissue edema which

accounted for approximately 40% of the apparent

loss and true microsphere loss. These investigators

observed an increase of microspheres in the lungs

and regional lymph nodes in dogs which developed

myocardial necrosis but not in dogs which did not

Discussion

In previous studies from this laboratory (Rivas et al., 1976) and others (Cox et al., 1975; Bishop et al., 1976; Hirzel et al., 1976), the radioisotope-la-

beled microsphere technique has been used to as-
sess serial changes in blood flow to ischemic regions after acute coronary artery occlusion. Recent investig-

tors have observed that, following myocardial infarction, the ratio of preoclusion blood flow in infarcted:noninfarcted regions was less than one (Capurro et al., 1979; Jugdutt et al., 1979; Reimer and Jennings, 1979). Since the ratio of blood flow in circumflex to anterior descending coronary artery region in the absence of infarction is approximately one (Cobb et al., 1974), it was concluded that the infarcted myocardium effected absolute and/or app-

parent microsphere loss. Other investigators have not observed microsphere loss (Pantely et al., 1978). In recent studies, Reimer and Jennings (1979) have presented an alternative explanation for the reduction in the ratio after infarction. These investigators observed that 4 days after myocardial infarction the

water content of the infarcted tissue increased by

25% and inflammatory cell elements increased in

proportion to the reduced ratio. They observed that 28 days after infarction, when the edema and in-

flammatory response had subsided and necrotic myocardial tissue had been replaced by scar tissue, the ratio of preocclusion infarct/normal region blood flow was increased 3-4 times. The latter studies demonstrate the striking effect that changes in cellular content may have on microsphere con-

tent per gram of infarcted tissue, and that as the infarcted tissue is resorbed, large numbers of micro-

spheres remain in the infarcted zone. Finally, they observed that the weight of the myocardium per-

fused by the circumflex coronary artery (circumflex area weight : total left ventricular weight) increased

in animals subjected to infarction of the circumflex area as compared to normals. When they assumed no microsphere loss and used the ratio of infarct to normal region blood flow to calculate a correction factor for tissue swelling, the circumflex area weight corrected for tissue swelling: left ventricular weight was comparable to that observed in infarcted ani-

mals. Reimer and Jennings (1979) concluded that changes in the ratio of infarct to normal region blood flow could be accounted for by tissue swelling initially and by contraction of the infarcted myo-

cardium later. In recent studies, Jugdutt et al. (1979) presented evidence that the apparent loss resulted from a combination of tissue edema which accounted for approximately 40% of the apparent loss and true microsphere loss. These investigators observed an increase of microspheres in the lungs and regional lymph nodes in dogs which developed myocardial necrosis but not in dogs which did not

Table 3  Relationship of Apparent Microsphere Loss and Blood Flow Correction to the Degree of Ischemia in

Endocardial Layers 3 and 4

<table>
<thead>
<tr>
<th>Time</th>
<th>% Loss</th>
<th>Flow corr.</th>
<th>% Loss</th>
<th>Flow corr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hour</td>
<td>-3.6 ± 7.3</td>
<td>0.00 ± 0.002</td>
<td>-3.6 ± 7.3</td>
<td>0.00 ± 0.002</td>
</tr>
<tr>
<td>24 hour</td>
<td>3.9 ± 5.3</td>
<td>0.003 ± 0.001</td>
<td>15.5 ± 3.9*</td>
<td>0.010 ± 0.003*</td>
</tr>
<tr>
<td>3 day</td>
<td>7.9 ± 4.7*</td>
<td>0.008 ± 0.002*</td>
<td>17.9 ± 4.7*</td>
<td>0.008 ± 0.002*</td>
</tr>
<tr>
<td>6 day</td>
<td>11.6 ± 5.1*</td>
<td>0.022 ± 0.011*</td>
<td>23.9 ± 6.4*</td>
<td>0.027 ± 0.016*</td>
</tr>
</tbody>
</table>

Apparent microsphere loss in % ± SEM and blood flow correction in ml/min per g in samples from layers 3 and 4 grouped according to the degree of ischemia 15 minutes after coronary artery occlusion; negative values indicate apparent microsphere gain. * P < 0.05. Ratios = number of dogs contributing to each particular value: total number of myocardial samples. The values were obtained by first calculating an average % loss and flow correction for each dog and then obtaining the averages of both values for the group.

Table 4  Nonischemic Flow Values

<table>
<thead>
<tr>
<th>Layer</th>
<th>Flow Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 minutes</td>
<td>1.15 ± 0.13</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.86 ± 0.06</td>
</tr>
</tbody>
</table>

Mean blood flow values ± SEM in ml/min per g in layers 1-4 in samples from the nonischemic region at 15 minutes and 24 hours after occlusion in the group of dogs killed at 3 days.
TABLE 5  Changes in Collateral Blood Flow between 15 Minutes and 24 Hours

<table>
<thead>
<tr>
<th>Ischemic region flow ranges</th>
<th>0.0-0.10</th>
<th>0.11-0.20</th>
<th>0.21-0.35</th>
<th>0.36-0.50</th>
<th>0.51-0.75</th>
<th>0.76-1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncorrected</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>0.042 ± 0.004</td>
<td>0.159 ± 0.005</td>
<td>0.273 ± 0.007</td>
<td>0.433 ± 0.009</td>
<td>0.585 ± 0.012</td>
<td>0.853 ± 0.024</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.172 ± 0.045</td>
<td>0.437 ± 0.055</td>
<td>0.525 ± 0.060</td>
<td>0.588 ± 0.051</td>
<td>0.694 ± 0.053</td>
<td>0.814 ± 0.053</td>
</tr>
<tr>
<td>( P )</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Corrected</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 minutes</td>
<td>0.054 ± 0.007</td>
<td>0.190 ± 0.010</td>
<td>0.320 ± 0.021</td>
<td>0.469 ± 0.026</td>
<td>0.628 ± 0.024</td>
<td>0.849 ± 0.030</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.209 ± 0.055</td>
<td>0.488 ± 0.055</td>
<td>0.598 ± 0.065</td>
<td>0.619 ± 0.049</td>
<td>0.743 ± 0.062</td>
<td>0.788 ± 0.071</td>
</tr>
<tr>
<td>( P )</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Mean blood flow values ± SEM for uncorrected and corrected ischemic region flows at 15 minutes and 24 hours after occlusion in samples grouped according to ischemic blood flow ranges in the group of animals killed at 3 days. \( P \) values represent comparison of 15 minutes to 24 hours data.

The present study supports the observations by Capurro (1979), Jugdutt (1979), and Reimer and Jennings (1979) that acutely infarcted myocardium does effect the content of microspheres per gram of tissue weight. The present study does not address the question of whether the reduction in the ratio of preocclusion blood flow in infarcted to noninfarcted regions represents actual loss of microspheres from the infarcted myocardium or tissue swelling, or a combination of the two processes. However, the net effect of this process is an apparent, if not an actual, microsphere loss. Capurro et al. (1979) observed no change in microsphere loss in endocardial regions after 24 hours but did observe additional loss in epicardial regions after 24 hours. Jugdutt et al. (1977) reported that the ratio of preocclusion infarct to nonischemic region blood flow was comparable at 12-24, 48, and 96 hours and that the inner to outer ratios of the infarcted and noninfarcted regions were comparable. In the present study, significant apparent microsphere loss was present in 24 hours in epicardial regions and in endo- and epicardial regions at 3 and 6 days. The significant apparent loss in the epicardial region but not in the endocardial region at 24 hours may have been influenced by greater cellular infiltration and greater edema in the outer zones of the ischemic region in the early hours after infarction. In previous studies from our laboratory (Rivas et al., 1976)

![Figure 2](image-url)  
**Figure 2** Mean blood flow ± SEM at 15 minutes and 24 hours after occlusion in dogs killed at 3 days. Samples have been grouped according to ischemic bloodflow ranges; mean value represents uncorrected (u) and corrected (c) measurements.
it was observed that, at 24 hours postocclusion, the increment in collateral blood flow was greater in the epicardial regions. Kloner et al. (1974) have demonstrated that the greatest degree of cell swelling occurs when blood flow is increased to an acutely injured area. In general, the apparent microsphere loss was greatest in the most ischemic regions, the regions which would be expected to sustain the greatest degree of myocardial infarction. It is possible that as the edema subsides and tissue absorption occurs an interval of no apparent microsphere loss will precede the time of apparent microsphere loss on blood flow measurements to the ischemic regions.

In the present study, 9 ± 1 μm microspheres were used, and the maximum apparent microsphere loss was approximately 22%, which is comparable to the 19% average loss value reported by Jugdutt et al. (1979) using microspheres of comparable size. Capurro et al. (1979) used 15 ± 5 μm microspheres and observed a maximum loss of approximately 30% in the center of the ischemic region, average blood flow 0.11 ± 0.03 ml/min per g tissue. Calculation of apparent microsphere loss based on comparison of preocclusion blood flow values in individual ischemic regions to a mean blood flow value in nonischemic regions will result in calculation of a loss in certain samples because of the innate variability of blood flow in individual samples relative to a mean value. The calculated values for apparent microsphere loss thus may be greater than the actual effects of acute infarction. Calculation of microsphere loss based on comparison of preocclusion blood flow in ischemic samples to within ± 1 SD of mean flow to the nonischemic region resulted in a maximum apparent loss of 13.9% in the 24-hour group, 9.5% in the 3-day group and 13.2% in the 6-day group.

Capurro et al. (1979) concluded that the microsphere loss from infarcted myocardium introduced error which prohibited quantitative assessment of collateral blood flow measurements at intervals greater than 24 hours. Jugdutt et al. (1979) concluded that microsphere loss is a limitation of the technique which resulted in uncertain accuracy of blood flow values. The major purposes of the present study were to determine the effects of apparent microsphere loss on blood flow measurements to the ischemic regions and to determine to what extent these effects altered interpretation of serial measurements of collateral blood flow. The ratio of preocclusion blood flow in individual ischemic to nonischemic regions was used to derive a correction factor that was used to calculate the volume correction factor which was used to calculate the volume correction of each ischemic blood flow measurement obtained prior to apparent microsphere loss, i.e., 15-minute blood flow measurements. Application of the correction factor resulted in statistically significant but very small absolute blood flow corrections in most regions in which the apparent microsphere loss was also statistically significant. In the dogs killed at 3 days, blood flow was measured at 24 hours and at 15 minutes after occlusion. Both corrected and uncorrected collateral blood flow increased in regions in which blood flow was less than 0.5 ml/min per g of tissue. The relative changes in blood flow measurement to the ischemic regions between 15 minutes and 24 hours were comparable before and after correction. The magnitude of the blood flow correction will determine the minimum change in collateral blood flow that can be interpreted. Because the effects of correction were small relative to the change in collateral blood flow, the effects of apparent loss did not prevent interpretation of significant increases in ischemic blood flow values in this group of animals. It is appropriate to
apply a correction factor to blood flow measurements obtained prior to the onset of microsphere loss, i.e., less than 6 hours after occlusion, but once apparent loss begins, the correction factor will overcor rect ischemic blood flow values. In the present study, the correction of the 24-hour blood flow measurement may have been excessive, since events that effect apparent microsphere loss have already begun. In any event, blood flow corrections were small in the present study so that uncorrected and corrected blood flow values at 24 hours were similar.

The use of radionuclide-labeled microsphere technique for measuring sequential changes in blood flow depends on permanent trapping of the microsphere in the coronary vasculature of normal myocardium. Although initial studies reported approximately 1% (range 0.0-2.4%) of the 8- to 10-μm microspheres passed through the coronary circulation after injection (Buckberg et al., 1971; Utley et al., 1974), several investigators have described additional shunting and/or loss of microspheres from normal myocardium following the initial trapping of microspheres. Consigny et al. (1979) reported that, in open-chested dogs, 3-4% of 9 ± 1 μm microspheres were shunted across the left ventricular myocardium following initial injection and that an additional 2% were shunted by 1 hour. Fan et al. (1979) observed in open-chested dogs that 3.3 and 8.1% of 9 ± 1 μm microspheres were collected in the coronary sinus during normal and systemic acidotic conditions, respectively. These investigators observed a lower myocardial blood flow value in the endocardium and midmyocardium using 9-μm as compared to 15-μm microspheres and reasoned that as much as 10% and 28% of the 9-μm microspheres may be shunted during normal and acidotic conditions, respectively. Crystal et al. (1979) reported 4% shunting of 9-μm microspheres following the initial injection in open-chested dogs. The degree of shunting was increased by increasing coronary perfusion pressure after initial trapping of the microspheres, as well as during the injection of microspheres. A mean coronary perfusion pressure of 177 ± 1 mm Hg was associated with an 8% shunt. The degree of shunting was a function of microsphere size, since no shunting occurred with 25 ± 5 μm microspheres at normal or increased coronary perfusion pressures. We thus reasoned that, if significant shunting and/or loss of microspheres occurred from the normal myocardium of intact dogs, smaller microspheres may be lost preferentially. To test this hypothesis, we carried out studies to ascertain whether there was preferential loss of 9 ± 1 μm as compared to 15 ± 1 μm microspheres from normal myocardium during a period of 3 days following microsphere injection. The 9- and 15-μm microspheres were mixed and injected simultaneously 3 days and 5 minutes prior to sacrifice. The average blood flow measured by 9- as compared to 15-μm microspheres was slightly higher at 3 days and slightly lower 5 minutes prior to sacrifice, (Table 6).

The ratio of 9- to 15-μm blood flow values measured 3 days prior to sacrifice was greater rather than less than 1, as would be expected if greater loss of the small microspheres had occurred. Although these small differences in blood flow measured by simultaneous injections did reach statistical significance, there was in fact very close agreement between the simultaneous measurements both at 3 days and 5 minutes prior to sacrifice. In addition, average blood flow measured by microspheres of the same size 3 days and 5 minutes prior to sacrifice were not significantly different. In these studies on awake animals, there was no preferential loss of 9-μm microspheres as compared to 15-μm microspheres from normal myocardium during a 3-day period following microsphere injection.

The results of the present study support previous observations that acutely infarcted myocardium affects the content of microspheres per gram of tissue and consequently results in an apparent, if not an actual, microsphere loss. The apparent microsphere loss resulted in statistically significant but very small changes in absolute blood flow measurements in the ischemic region in dogs killed at 1, 3, and 6 days. In animals killed at 3 days, the apparent microsphere loss had minimal effects on interpretation of increases in collateral blood flow, since the corrections for loss were very small relative to the changes in collateral blood flow to the ischemic regions. Simultaneous injection of 9- and 15-μm microspheres 3 days and 5 minutes prior to sacrifice demonstrated no preferential loss of the smaller microspheres from normal myocardium during a 3-day period.

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