The Loss of Radioactive Microspheres from Canine Necrotic Myocardium

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

SUMMARY To verify whether there is a loss of radioactive microspheres (RM) from regions undergoing myocardial infarction, we injected 7-10 μm RM into the left atrium in 75 dogs before left circumflex (LC) coronary artery occlusion and measured the myocardial RM content when the dogs were killed 6-96 hours later. The preocclusion RM content per gram in the occluded LC bed was not significantly different from that in the nonoccluded left anterior descending (LAD) coronary bed in dogs killed within 6 hours and in those without necrosis and killed later. In contrast, in dogs with necrosis, the relative RM content in the LC bed was significantly reduced, indicating an apparent loss of RM averaging 19% (LC/LAD at 12-24 hours = 0.84; 48 hours = 0.80; 96 hours = 0.80). The percent of apparent RM loss from transmural samples correlated closely with the percent necrosis in the samples (r = 0.93, P < 0.001). Desiccation of samples revealed that edema in the infarct accounted for about 40% of this apparent loss. Most of the remaining 60% appeared to represent true physical loss as evidenced microscopically by decreased RM counts in infarcted tissue, especially in zones of coagulative necrosis. Furthermore, following systemic RM injections and, more importantly, selective intracoronary RM injections into the occluded coronary bed, more RM were found in the lungs and neck lymph nodes in dogs with necrosis than in dogs without necrosis. These results indicate that apparent RM loss from necrotic myocardium is related both to increased tissue water and true physical RM loss and suggest that measurements of flow in infarct regions by RM may be reduced falsely.

Ore Res 45: 746–756, 1979

RADIOACTIVE microspheres (RM) have been used to measure collateral blood flow after coronary artery occlusion (Fortuin et al., 1971; Cobb et al., 1974; Marcus et al., 1975; Schaper and Pasyk, 1976; Rivas et al., 1976; Bishop et al., 1976). Although RM blood flow measurements have been validated (Rudolph and Heymann, 1967; Domenech et al., 1969; Buckberg et al., 1971) and their limitations (Archie et al., 1973; Heymann et al., 1977; Tripp et al., 1977a) studied in acute experiments lasting a few hours, the accuracy of RM flow measurements in regions that have undergone myocardial necrosis when experiments are extended over several days recently has been questioned (Capurro et al., 1979). A reduction in the content of RM injected prior to coronary occlusion has been found in necrotic areas of myocardium relative to nonnecrotic areas, suggesting loss of microspheres (Capurro et al., 1977, 1979; Jugdutt and Becker, 1977). Because of this, flow measurements made in the early hours after coronary occlusion in studies of myocardial infarction may be underestimated.

This study was done: (1) to verify that there is a discrepancy in RM content between necrotic and nonnecrotic myocardium, (2) to determine whether the discrepancy is related quantitatively to necrosis, and (3) to ascertain whether the discrepancy represents a real physical loss or is explained by greater edema in necrotic regions.

Methods

A total of 142 dogs was used in these experiments (groups 1-6).

Verification of Microsphere Loss (Groups 1 and 2)

Ninety-four dogs weighing an average of 20 kg (range, 18-24 kg) were instrumented under general anesthesia through a left lateral thoracotomy. A plastic snare was placed around the left circumflex (LC) coronary artery, just distal to the first large marginal branch. Plastic catheters (polyvinyl chloride no. 13) 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. Antibiotics (penicillin, 1,000,000 U, and streptomycin, 1 g) were given intramuscularly after surgery, and the tubes were flushed daily with heparin.

Ten days later, experiments were done on the 85 dogs surviving instrumentation while they were standing in a sling for support. Morphine (0.5 mg/kg) was given intramuscularly for sedation and analgesia, and free flow was established through the
catheters. Radioactive microspheres (7-10 μm in diameter) with Tween-80 added and labeled with one of five different isotopes (125I, 109Ce, 85Sr, 90Nb, and 46Sc) were sonicated for 5 minutes in a mechanical shaker prior to each injection. In 75 dogs (group 1), several injections, each consisting of 3 × 10^6 RM, were made into the left atrium. The first injection was made under resting conditions 1 hour after the morphine injection. The dogs were then premedicated with lidocaine (1 mg/kg) to suppress ventricular ectopic beats, and the snare was pulled 5 minutes later to acutely and permanently occlude the left circumflex (LC) coronary artery. Additional RM injections were made at 20 seconds and later postocclusion in all, but observations with these are not reported in this study. In 34 of the 75 dogs in group 1, an additional RM injection was made preocclusion: in one, the microspheres were mixed together, in another the injections were made 2 minutes apart, and in 32 dogs the injections were made 20 minutes apart. In another 10 dogs (group 2), a single injection of RM was given before occlusion: 5 × 10^6 to eight dogs (nos. 34, 37, 54, 55, 73, 130, 145, 148) and 20 × 10^6 to two dogs (nos. 56, 174). No RM were given postocclusion in this group.

At various times postocclusion, dogs in group 1 either died or were killed with a lethal dose of anesthetic: 1 hour: 15; 6 hours: 3; 12 hours: 2; 24 hours: 6; 48 hours: 39; 96 hours: 16. The 10 dogs in group 2 all were killed at 48 hours. The hearts were removed immediately, washed free of blood, and the LC, left anterior descending (LAD), and right coronary arteries were injected simultaneously under controlled pressure (120 mm Hg) so as to delineate the occluded LC and the nonoccluded LAD vascular beds and to facilitate tissue sampling. The injectates (2-5 ml) consisted of indiffusible dyes in dextran (Brilliant green and red) in 19 hearts (from dogs killed at 1 hour: 6; 24 hours: 3; 48 hours: 1; 96 hours: 9) and a barium gelatin mass (Jugdutt et al., 1979b) containing pigment (Monastral red, blue, and green, Dupont Co.) in the remaining 66 hearts, which were then packed with gauze to preserve diastolic relationships and fixed in 20% formalin. Weight gain resulting from the injections averaged 3.5 ± 1 (SD) g (range, 2-6 g). Transverse sections of equal thickness (1-1.5 cm) were made from base to apex and photographed. Rings of the left ventricle (LV), freed of the right ventricle, and fatty, valvular, and connective tissues were weighed. The depths of the rings were measured. Transmural blocks of myocardium were taken serially from the center of the occluded LC bed (green) around the posterior papillary muscle, so as to include infarcted tissue when present, and from the center of the nonischemic LAD region (red), around the anterior papillary muscle. The top surfaces of the samples from all 85 hearts were marked with bromochrom and divided into inner and outer halves which were weighed, placed in plastic vials, and counted for radioactivity (multichannel gamma scintillation counter, Packard, model no. 5986). The vials contained 5 ml of 20% formalin to permit later histological examination. Tracings of the top surfaces of the LV rings were made, using a transparent plastic overlay on which the sites of sampling and outer boundaries of grossly visible necrosis were marked. Areas of the traced LV rings and necrosis were planimetered electronically. Areas of top and bottom surfaces then were averaged. The ratios of average areas of necrosis and LV rings were multiplied by weights of the rings to calculate total necrosis as percent LV for each heart.

**Calculation of Apparent Microsphere Loss**

The RM content (radioactive counts per gram tissue) was computed by standard techniques (Rudolph and Heymann, 1967; Domenech et al., 1969; Buckberg et al., 1971). The content of the preoclusion RM was examined in samples from ischemic LC and nonischemic LAD regions. In the 75 dogs receiving pre- and postocclusion RM injections (group 1), "ischemic" samples were defined as those whose immediate postocclusion RM content was less than 50% of the RM content in the nonischemic LAD region. In the next 10 dogs receiving only one injection of RM preoclusion (group 2), "ischemic" samples were taken near the posterior papillary muscle in the center of the occluded LC bed (Jugdutt et al., 1979b). Since seven of these dogs had visible infarcts, their "ischemic" samples encompassed the necrotic tissue. Nonischemic samples were defined as those from the center of the LAD region in both groups of dogs. Single average values of RM counts per gram for ischemic and nonischemic regions were computed by combining individual RM counts for all samples in the two regions. The ischemic:nonischemic (I:NI) ratio of the preocclusion RM was calculated for all hearts. The degree of apparent net loss of the preocclusion RM per heart was calculated as the difference of the I:NI ratio for that RM from unity and expressed as a percent (i.e., RM loss = [1 – (I:NI)] × 100%). The I:NI ratios were compared in the 34 dogs given two preocclusion RM injections. The inner-outer (IN:OUT) wall content of the preocclusion RM was calculated in the 75 dogs in group 1.

**Measurement of Necrosis**

Formalin-fixed samples from 40 hearts from group 1 were embedded in paraffin, sectioned (8 μm) in the same plane as the bromochrome-marked top surfaces, and stained with hematoxylin and eosin. The total amount of necrosis, as percent, in each of 716 coded histological sections was assessed microscopically by two separate observers (BB, GH). Sections were made from 356 of the 854 "ischemic" samples (42%) included in calculations of I:NI ratios in the 40 hearts and were taken from their middle LV rings. There was close interobserver agreement in the paired estimates of total necrosis per sample (slope = 0.97, r = 0.94). The
areas of the samples and of visual necrosis within the samples were measured on the tracings using a mm$^2$ grid, and visual necrosis, as percent, was compared to histological necrosis, as percent, in the same samples. A close agreement was found between visual and histological necrosis (slope = 0.95, $r = 0.86$).

**Microscopic Verification of Microsphere Loss**

In the 10 dogs in group 2, the numbers of microspheres in 8- and 16-μm sections were counted microscopically under high power by two observers (BB, BJ) on two separate occasions. Good intra- and interobserver reproducibility was established. The area of each section was measured using a mm$^2$ grid, and RM count was expressed as the average number of spheres per cm$^2$ of ischemic and nonischemic tissue per dog. In the seven dogs in group 2 that had necrosis, the relative amounts of coagulation-type and contraction band-type necrosis, as percents of the total necrosis in the microscopic sections, were assessed (BB, GH), and the areas of the sections as well as the total number of microspheres were measured. From these data, the regional distribution of RM in normal zones and zones of contraction band necrosis (CBN) and coagulation necrosis (CN) were calculated and expressed as RM/cm$^2$. CBN was identified by its characteristic histological appearance: disrupted myocardial architecture with dense eosinophilic transverse bands within the myocardial cells and surrounding red blood cell infiltration. CN was characterized by hypereosinophilic staining of the myocardial cells with little loss of myocardial cell structure and lack of hemorrhage. Nuclear changes and polymorphonuclear infiltration were similar in both forms of injury at 48 hours (Henderson et al., 1965; Reichenbach and Benditt, 1968).

In two of the dogs in group 2 which were given 20 × 10$^6$ RM preocclusion, the distribution of microspheres as a function of size was assessed. The number of RM of different sizes (<7, 7–8, 8–9, 9–10, >10 μm) in samples from both occluded LC and nonoccluded LAD beds was counted, using a calibrated grid in the eyepiece (in μm). Aliquots (10 ml) of the various solutions used during preparation of the histological sections in these two dogs were counted for radioactivity, then centrifuged, and the residue examined for microspheres.

**Drying Experiments**

In 11 other dogs (group 3) given 3 × 10$^6$ RM preand postocclusion and killed at 48 hours, all the myocardial samples were desiccated in an oven at 105°C for 12 hours to remove tissue water. Weight loss was calculated from weights before and after the process. New I:NI ratios of the preocclusion RM were calculated from the dry weights. In seven more dogs (group 4) also killed at 48 hours, samples were taken from the infarct core and margin as well as bordering normal tissue. These samples were dried similarly to assess the water content in those regions. A 2-mm thick transmural section was taken from the infarcted region of the middle LV ring for histology.

**Examination of Regional Lymph Nodes and Some Other Tissues**

In 22 more dogs (group 5), regional lymph nodes draining the heart (around the aortic arch, left bronchus, the hilum, left thoracic outlet, and neck) and samples of various tissues (epicardial fat, kidneys, spleen, liver, both lungs) were removed immediately after killing the dog at 48 hours. The tissue samples were washed free of blood, weighed, and counted for radioactivity. The preocclusion RM content per gram of tissue was calculated and expressed as a percent of RM content per gram of the nonischemic myocardial tissue. In 11 of these dogs, a second RM was injected immediately prior to killing, and its content in the tissues was compared to that of the preocclusion RM.

**Intracoronary Microsphere Experiment**

In eight other dogs (group 6), 3 × 10$^6$ RM were injected into the left atrium after they had been instrumented, but while they were still anesthetized (sodium pentobarbital 20 mg/kg, iv) and their chests were open. A 25-gauge needle then was inserted into the LC just distal to the large marginal branch and 3 × 10$^6$ RM injected selectively into the LC bed. The needle then was removed and the LC ligated immediately above and below the puncture site in six dogs, whereas in two dogs the hole was merely closed but the LC was not ligated. The dogs were killed 2 days later, and myocardial, node, and tissue samples examined for the content of the two microspheres.

**Statistics**

Paired and unpaired Student’s $t$-tests and linear regression analysis by the method of least squares for grouped data were used. Analysis of variance was used for multi-group comparisons (Fig. 1, Table 2). Values are expressed as means ± SEM.

**Results**

Of the 75 dogs in group 1, a total of 40 developed necrosis which was clearly visible on gross inspection and was confirmed by histological examination of samples from the center of the occluded LC bed in all 40 hearts. The infarcts were located around the posterior papillary muscle and were well circumscribed in all but the apical LV rings. Infarcts averaged 16 ± 2% LV (range, 3–38%). The ischemic LC:nonischemic LAD ratio of the preocclusion RM (I:NI) in transmural myocardial samples was significantly lower in dogs with necrosis than in those without necrosis ($F = 28.3$, $P < 0.001$ by two-way
analysis of variance) (Fig. 1). In the 35 dogs that did not develop necrosis, the I:NI ratio of the preocclusion RM was 1.01 ± 0.03 (SEM) in the 18 dogs killed before 6 hours and did not differ significantly in dogs living 12–24 hours (1.01 ± 0.06, n = 3), 48 hours (1.05 ± 0.01, n = 9), or 96 hours (1.01 ± 0.04, n = 5), the mean value being 1.00 or unity. In contrast, the I:NI ratio was significantly lower than unity in the 40 dogs with necrosis (12–24 hours = 0.84 ± 0.04, n = 5, P < 0.005; 48 hours = 0.80 ± 0.04, n = 25, P < 0.001; 96 hours = 0.80 ± 0.03, n = 10, P < 0.005). The difference between unity and the average value (0.81) of the I:NI preocclusion RM ratio in these dogs with necrosis was 0.19, indicating an average 19% apparent RM loss.

The average amount of apparent RM loss per heart, calculated as percent for transmural tissue samples, correlated closely with the average amount of necrosis (as percent) in these samples for the 40 dogs with necrosis: Percent RM loss = 0.88 × percent necrosis - 32.4, r = 0.93, P < 0.001 (Fig. 2). The intercept of the horizontal axis of the linear regression plot in Figure 2 suggests that RM loss is not significant until necrosis in a heart exceeds about 37% of the ischemic zone. In 30 of these hearts, we also determined the relation between apparent RM loss and necrosis in groups of transmural samples comprising the ischemic zone in individual LV rings (n = 113 rings, 1–5 samples per ring): Percent RM loss = 0.78 × percent necrosis

Figure 1 The I:NI ratio of the preocclusion RM in 75 dogs killed at various time intervals. In 35 dogs without infarction, the average ratio is nearly unity. In 40 dogs with infarction, the average ratio is significantly (P < 0.005) less than unity. By analysis of variance there is no significant difference among the various times within infarct and no-infarct groups, but the difference between groups is highly significant (F = 28.3, P < 0.001).

Figure 2 The relationship between apparent microsphere loss and necrosis in 40 dogs. "Microsphere loss" in transmural samples was calculated as (1 - I:NI) × 100%, and an average value was determined for each dog. There is a close quantitative relationship between apparent microsphere loss and necrosis (r = 0.93, P < 0.001). The threshold for apparent sphere loss (horizontal axis intercept) is 37% necrosis of the ischemic zone.

Figure 3 The I:O preocclusion microsphere ratio in the ischemic left circumflex (LC) and the nonischemic LAD regions for the 40 dogs in group 1 with necrosis. The ratio of microsphere content in inner and outer halves of the left ventricular wall did not differ for LC and LAD regions, indicating uniform apparent RM loss from inner and outer halves of the infarct. Since there was twice as much necrosis in inner than outer halves in the LC region (60 vs. 30%, P < 0.001), twice as much RM apparently was lost per gram of necrotic tissue from outer regions.
To determine whether this apparent RM loss from necrotic regions occurred uniformly from inner and outer regions, we examined the ratio of the preocclusion RM content in the inner and outer halves of the left ventricular wall (IN:OUT) for the ischemic LC and nonischemic LAD regions in the 40 dogs developing necrosis (Fig. 3). Although there was a wide range (0.66-1.70) of individual values for IN:OUT RM ratios in both regions, there was no significant difference in the average ratios in the two regions (1.20 vs. 1.19), suggesting that loss of RM from inner and outer halves of the necrotic region was uniform.

The reproducibility of RM loss within dogs was determined by injecting RM with two different isotope labels before LC occlusion in 34 dogs. There was no significant difference in the LC:LAD RM ratio for the two determinations (Fig. 4). The mean percent difference between the two RM content determinations was only 5.6%. An additional observation was that a few values with 125I deviated from the line of identity (Fig. 4). Since the hearts had been kept in formalin for variable periods of time (1-10 days) before sectioning and counting, the formalin solution was centrifuged and the supernates and residues were counted. The supernate contained several thousand counts of 125I but very few counts (background level) of the other isotope. The residue contained a few hundred counts of the other isotope, but no iodine, and showed only a few microspheres on microscopic examination. This suggests that 125I tended to leave the microspheres more readily than the other radionuclide labels.

To verify whether RM are physically lost from necrotic regions, we examined a total of 436 histo-

Table 1  Number of Microspheres by Light Microscopy in Myocardium from 10 Dogs Given a Single RM Preocclusion (Group 2)

<table>
<thead>
<tr>
<th>Sub-group</th>
<th>Dog no.</th>
<th>RM ratio (I:NI)*</th>
<th>No. of samples</th>
<th>No. of sections</th>
<th>Average no. of microspheres/cm²</th>
<th>No. of samples</th>
<th>No. of sections</th>
<th>Average no. of microspheres/cm²</th>
<th>RM ratio (I:NI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct</td>
<td>34</td>
<td>0.75</td>
<td>22</td>
<td>44</td>
<td>3.7</td>
<td>16</td>
<td>32</td>
<td>4.8</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.77</td>
<td>8</td>
<td>21</td>
<td>3.6</td>
<td>6</td>
<td>16</td>
<td>4.6</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>0.48</td>
<td>8</td>
<td>23</td>
<td>1.9</td>
<td>4</td>
<td>14</td>
<td>4.0</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>0.88</td>
<td>8</td>
<td>22</td>
<td>2.5</td>
<td>4</td>
<td>12</td>
<td>3.0</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>0.74</td>
<td>10</td>
<td>20</td>
<td>8.9</td>
<td>10</td>
<td>20</td>
<td>10.2</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>0.90</td>
<td>10</td>
<td>20</td>
<td>1.4</td>
<td>8</td>
<td>16</td>
<td>1.6</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>0.79</td>
<td>16</td>
<td>32</td>
<td>4.6</td>
<td>8</td>
<td>16</td>
<td>5.7</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean ± se</td>
<td>0.76 ± 0.05</td>
<td>3.8 ± 1.0</td>
<td></td>
<td></td>
<td>4.8 ± 1.0‡</td>
<td></td>
<td>0.77 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No infarct</td>
<td>130</td>
<td>1.06</td>
<td>6</td>
<td>12</td>
<td>2.0</td>
<td>6</td>
<td>12</td>
<td>1.9</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>0.99</td>
<td>10</td>
<td>20</td>
<td>1.6</td>
<td>10</td>
<td>20</td>
<td>1.6</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>1.00</td>
<td>10</td>
<td>20</td>
<td>5.4</td>
<td>6</td>
<td>12</td>
<td>5.4</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean ± se</td>
<td>1.02 ± 0.02</td>
<td>3.0 ± 1.2</td>
<td></td>
<td></td>
<td>3.0 ± 1.2</td>
<td></td>
<td>1.02 ± 0.02†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on radioactive counts.
† Expressed as the average number of spheres per cm² for the full wall. Numbers of spheres in sections of individual myocardial samples were counted. The thicknesses of the sections were 8 μm in all but dogs nos. 73, 156, and 174, in which they were 16 μm. All dogs were given 5 x 10⁷ RM except nos. 156 and 174, which got 20 x 10⁷ RM.
‡ Based on microscopic counts.
§ P < 0.006 vs. ischemic region in infarct group.
|| P < 0.02 vs. infarct group.
logical sections made from 196 samples obtained from the 10 dogs in group 2 given a single RM (5 x 10^6 RM in eight dogs and 20 x 10^6 in two dogs) before LC occlusion (Table 1). At 48 hours, seven of the 10 dogs had visible infarcts whereas three did not. In the infarcted dogs, there were fewer microspheres in the necrotic LC region than in the normal LAD samples (3.8/cm^2 vs. 4.8/cm^2, P < 0.005). In contrast, the average number of microspheres was similar in the center of the occluded LC bed and the nonoccluded LAD bed in the three dogs with no infarcts. These results support the finding that there is microsphere loss from necrotic myocardium. Furthermore, the I:NI ratios of numbers of microspheres were in close agreement with those based on radioactive counts and generated by computer for individual dogs, suggesting that microspheres were not lost from necrotic tissue during the processing of tissue samples for histology.

On the basis of microscopic counts, the average number of RM per cm^2, which closely approximates RM per gram of myocardium, was calculated to be 500 for left atrial injections of 2-3 million RM and is within the range reported by other investigators (White et al., 1978a). Microscopically, the microspheres in samples containing areas of necrosis were distributed mainly in the normal-looking tissue rather than in the necrotic tissue, were mostly within capillaries, and occasionally were seen as clumps of 2-5 in arterioles. Occasionally, a microsphere was seen at the edge of the necrotic region and was surrounded by several macrophages and inflammatory cells. In an attempt to quantify differences in regional distribution of RM in different regions of necrotic samples, we counted the microspheres in normal zones and zones of CBN (Hend-son et al., 1965; Reichenbach and Benditt, 1968) and CN, whose areas were measured in seven hearts in group 2 (Table 2). We found more RM/cm^2 in normal zones than in zones of CBN and CN (6.0 vs. 2.6 vs. 0.5 in transmural samples. These microscopic data indicate that the type of necrosis influences the amount of apparent RM loss, so that there is greater RM loss from zones of CN than CBN. In the samples from the mid LV rings and encompassing the infarcts in the seven dogs in group 2, the average amount of total necrosis was 53% (26% CBN and 27% CN).

To determine whether there was preferential loss of smaller (7-8 μm) compared to larger (9-10 μm) microspheres from necrotic regions, we examined the size distribution of microspheres in all samples from two dogs given 20 x 10^6 RM preocclusion (Fig. 5). In the dog with necrosis (no. 156), there were fewer RM of all sizes within the range of 7-10 μm in the LC region with necrosis than in the normal LC region, but there does not appear to be a preferential loss of smaller microspheres. Microspheres were counted in 16-μm sections in 64 samples (42 LC, 22 LAD), and both dogs were given 20 x 10^6 microspheres labeled with ^41Sc preocclusion.

TABLE 2  Microsphere Distribution in Zones of CN and CBN and Normal Myocardium in the Occluded Bed (Group 2)

<table>
<thead>
<tr>
<th>Heart no.</th>
<th>No. of samples</th>
<th>Average no. of microspheres/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>22</td>
<td>8.0</td>
</tr>
<tr>
<td>37</td>
<td>8</td>
<td>6.4</td>
</tr>
<tr>
<td>54</td>
<td>8</td>
<td>2.9</td>
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<tr>
<td>55</td>
<td>8</td>
<td>5.1</td>
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<tr>
<td>73*</td>
<td>10</td>
<td>9.3</td>
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<tr>
<td>148</td>
<td>20</td>
<td>2.8</td>
</tr>
<tr>
<td>156*</td>
<td>32</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Mean ± SE: 6.0 ± 1.0, 2.6 ± 0.8, 0.5 ± 0.3

* P < 0.02, † P < 0.05

* Sections in these dogs were 16 μm in thickness compared to 8 μm in the five others.
† 20 x 10^6 microspheres were given preocclusion in this dog compared to 5 x 10^6 in others.
‡ P < 0.001 vs. normal; by analysis of variance the microsphere count was significantly different in the three regions (F = 20.23, P < 0.006).
mass and decrease in the number of RM per gram tissue. We measured the average thicknesses (to the nearest mm) of ischemic LC and nonischemic LAD samples included in calculations of RM content in 67 hearts from group 1, but we found no significant difference between LC and LAD thicknesses in hearts with infarcts (14.5 ± 0.3 vs. 14.3 ± 0.3 mm, n = 40, P < 0.1) or no infarcts (13.4 ± 0.6 vs. 13.2 ± 0.6 mm, n = 27, NS), suggesting that edema was not great.

To investigate further the importance of edema, we dried samples from ischemic LC and nonischemic LAD regions and re-computed I:NI ratios of the preocclusion RM in terms of dry tissue weight for 11 hearts in group 3 (Table 3). There was greater weight loss from the necrotic LC than from normal LAD samples (77 ± 74%, P < 0.001). Although this weight loss was associated with a significant increase in I:NI ratios in all dogs (0.75–0.85, P < 0.001), there was not a complete restoration to unity (0.85 vs. 1.0, P < 0.02, n = 11) for the group. In four dogs (nos. 3, 6, 7, 8) whose dry I:NI ratios were close to unity, the amount of weight loss did not correlate with the abnormality of the wet weight I:NI ratio. The explanation for these observations may be that the degree of correcion of I:NI ratios by drying and the water content may depend on relative amounts of normal tissue, CBN, and CN in the samples and their water contents. Although it may be reasonable to expect more tissue water in zones of CBN than in CN, and less in normal myocardium, the water content in these regions has not been quantified previously. We, therefore, measured the water content by dehydration (100°C for 12 hours) in the core and marginal regions of infarcts in seven dogs (group 4) in which histology was done for the type and distribution of necrosis in 2-mm thick samples encompassing the entire infarct and its borders from the mid LV ring. We found water content to be as follows: 79.4 ± 0.4% in the infarct core which had 93% CN and 7% CBN; 78.8 ± 0.4% in the infarct margin which had 26% CN and 70% CBN; 77.6 ± 0.4% in the normal-looking border region which had 0% CN, 17% CBN, and 83% normal tissue; and 75.8 ± 0.7% in the totally normal LAD tissue. The small differences in water content between infarct core and margin were not statistically significant in the seven hearts. A further explanation of the variable degrees of correction of I:NI ratios on drying could have been the relative amounts of hemorrhage and inflammatory infiltrate. In the 2-day infarcts in this study, hemorrhage was not significant, and small areas of inflammatory infiltrate were not quantifiable accurately.

We considered the possibility that the processing of the hearts could have contributed to the low I:NI ratios. Thus, the formalin could have dehydrated normal tissue more than infarcted tissue, or the barium injection could have increased the mass of the necrotic region more than the normal tissue. However, in six fresh hearts, with 48-hour infarcts, which were sectioned immediately after killing the dog without barium injections or formalin fixation, and dehydrated after counting for radioactivity, weight loss was 80.1 ± 0.8% from the necrotic LC region vs. 78.2 ± 0.3% in the normal LAD region (P < 0.05). Both of those values were higher than the corresponding values for formalin-fixed hearts (Table 3), but the relationship between necrotic and normal tissue remained similar.

In 22 dogs, we attempted to locate the lost myocardial RM. We examined the preocclusion RM content in the regional lymph nodes, lungs, and other tissues and found slightly more (P ≤ 0.05) of this RM injection (as percent of RM content in nonischemic LAD tissue) in the lungs than in other tissues in 16 dogs with necrosis, but unexpectedly few RM in the nodes (Table 4). However, the amount of preocclusion RM in the lungs was greater in dogs with necrosis than in those without necrosis (550 vs. 293, P < 0.05). Furthermore, we compared the preocclusion RM content in the tissues with the

---

**Table 3 Drying Experiment (Group 3)**

<table>
<thead>
<tr>
<th>Heart no.</th>
<th>Ischemic LC tissue with necrosis</th>
<th>Normal LAD tissue</th>
<th>(I:NI) RM ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue wt (g)</td>
<td>Tissue wt (g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wet</td>
<td>Dry</td>
<td>Wet</td>
</tr>
<tr>
<td>1</td>
<td>1.26</td>
<td>0.21</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>4.68</td>
<td>0.99</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>40.11</td>
<td>7.56</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>63.17</td>
<td>15.80</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>18.67</td>
<td>5.34</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>18.95</td>
<td>3.62</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>21.00</td>
<td>4.53</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>27.16</td>
<td>6.74</td>
<td>75</td>
</tr>
<tr>
<td>9</td>
<td>14.94</td>
<td>3.56</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>25.74</td>
<td>6.49</td>
<td>75</td>
</tr>
<tr>
<td>11</td>
<td>20.23</td>
<td>4.30</td>
<td>79</td>
</tr>
</tbody>
</table>

Mean ± SE 23.26 ± 5.09 5.49 ± 1.24* 77 ± 1 11.24 ± 1.17 2.95 ± 0.36* 74 ± 2* 0.75 ± 0.04 0.85 ± 0.05* †

* P < 0.001, dry vs. wet; †P < 0.001 vs. corresponding value for ischemic LC tissue; \( \bar{P} < 0.02 \) vs. 1.0.
content of a second RM injected just prior to killing in 11 dogs (Table 4). In dogs with necrosis, excess preocclusion RM (as percent of RM content in LAD tissue) over the prekilling RM were found in the lung (550 vs. 222, \( P < 0.005 \)) and less in the liver (110 vs. 58, \( P < 0.01 \)) but not in regional lymph nodes. The kidney had slightly fewer preocclusion RM than prekilling RM (\( P < 0.1 \)), although the reason for this finding is unknown. Since preocclusion RM were distributed to all systemic organs, the excess RM in the lung may have come from organs other than the heart, and the excess RM in the liver suggests that over the 2 days, some RM may have been lost from the gut via the portal circulation to the liver. However, a similar excess of preocclusion RM in the lungs was not found in dogs without infarcts, suggesting that some of these RM must have come from the occluded LC bed.

To document further the physical loss of myocardial RM, we compared the tissue content of an RM injected directly into the LC bed just prior to occlusion (Table 5). We found more of the RM that were injected into the LC bed in the lung and neck nodes in dogs with necrosis than in those without necrosis.

**Table 4** Summary of RM Content in Some Tissues in Dogs with and without Infarcts (Group 5)

<table>
<thead>
<tr>
<th>Subgroup n</th>
<th>Tissues</th>
<th>Nodes</th>
<th>(I:NI) RM ratio</th>
<th>Necrosis (% LV)</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Neck</th>
<th>Bronchial</th>
<th>Aortic</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 9</td>
<td>With necrosis</td>
<td>0.83 ± 0.03</td>
<td>1.06 ± 0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b 2</td>
<td>Without necrosis</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Discussion

The radioactive microsphere technique is now widely used in studies of coronary blood flow and infarction in animals. It is well known that after a left atrial injection of RM, the RM distribute to the coronary beds in proportion to blood flow (Schaper and Pasyk, 1976) and that the average RM ratio per gram of tissue in the LC and LAD perfusion beds in a series of dogs is close to unity (Rudolph and Heymann, 1967; Domenech et al., 1969; Heymann et al., 1977). It recently has been questioned whether these relationships are also true for the chronic infarct model (Capurro et al., 1977, 1979; Jugdutt and Becker, 1977). In this study, we found that the I:NI preocclusion RM ratio did not deviate significantly from unity in dogs without necrosis but was significantly less than one in dogs with necrosis, a finding that has been noted by others (White et al., 1978a). Several alternative explanations for the decrease in I:NI RM ratio must be considered. First, a loss of radioactive label from RM in necrotic areas without physical loss of the RM themselves could theoretically cause a decrease in the ratio. Although we cannot exclude the pos-

**Table 5** Summary of Tissue Content of Microspheres after Intracoronary Injection in Dogs with and without Necrosis (Group 6)

<table>
<thead>
<tr>
<th>Subgroup n</th>
<th>Tissues</th>
<th>Nodes</th>
<th>(I:NI) RM ratio</th>
<th>Necrosis (% LV)</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Neck</th>
<th>Bronchial</th>
<th>Aortic</th>
</tr>
</thead>
<tbody>
<tr>
<td>a 16</td>
<td>With necrosis</td>
<td>0.88</td>
<td>14.0 ± 0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b 9</td>
<td>Without necrosis</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a = \) Preocclusion RM, \( b = \) Prekilling RM.

\( \* \) RM/g as percent of RM per g in LAD region.

\( \ddagger \) RM/g as percent of RM per g in the normal border in the LC region.

\( \dagger \) \( P < 0.1 \), \( \ddagger \) \( P < 0.05 \), \( \dagger \) \( P < 0.005 \) vs. corresponding value in group with necrosis (unpaired \( t \)-test).
sibility that the radioactive label left the microspheres in vivo, we believe it is unlikely to have been a major factor. We found some evidence of radioactivity loss from microspheres in vitro from samples kept in formalin in the case of the iodine label but not with Ce, Sr, Nb, or Sc. Iodine had been used as a preocclusion RM label in 19 of the 34 dogs given two consecutive RM injections preocclusion, and the two determinations of RM content were identical except for three dogs given $^{125}$I-labeled RM.

Second, a decrease in the I:NI ratio could be due to factors which increase the mass of the infarcted tissue, such as edema, hemorrhage, and/or inflammatory cell infiltration. In our drying experiments, we found that the I:NI ratio calculated on the basis of desiccated tissue samples was closer to, but still significantly less than, unity (0.75–0.85). Thus, tissue edema accounted for approximately 40% of the depression in the I:NI ratio. We did not attempt to correct for hemorrhage or inflammatory infiltrate because we felt that these factors could not be quantified accurately, although we did not find significant hemorrhage in infarcts after permanent coronary occlusion in our study.

Third, a decrease in the I:NI ratio could represent a true physical loss of RM from necrotic myocardium. We believe that RM are in fact lost, based on the following evidence: (1) lack of correction of the I:NI ratio to unity in desiccated samples, (2) decreased RM content in infarcted tissue by microscopy, especially in zones of CN, and (3) finding of excess RM in the lungs and neck nodes after selective intracoronary RM injections in dogs developing myocardial necrosis, but not in dogs without necrosis. The microscopic evidence appears to be particularly important: an even distribution of preocclusion RM was expected throughout the ischemic area, but a marked maldistribution occurred. Noninfarcted zones contained 6.0 ± 1.0 spheres/cm$^2$ (mean ± SEM) and zones of CBN contained 2.6 ± 0.8 spheres/cm$^2$ and zones of CN, only 0.5 ± 0.3 sphere/cm$^2$. Based on the amount of increased tissue water that we measured in infarcted tissue, we can explain a decrease in RM content only to 5.3 spheres/cm$^2$ in the necrotic zones. The remaining discrepancy is explained best by true loss of RM from necrotic tissue.

**Microsphere Loss and Necrosis**

We found a close linear relationship between RM loss, calculated as (1 − I:NI) × 100%, and the extent of necrosis (Fig. 2). This relationship probably is due in part to increased edema associated with larger infarcts and also to increased physical loss of RM. Importantly, Figure 2 shows that no sphere loss is detected until about 40% of the ischemic zone is infarcted (horizontal axis intercept), suggesting that loss may occur from only a portion of the infarct, for example, the most necrotic core. There is support for this view in the microscopic studies, which showed much greater RM loss from areas of CN than CBN. In addition, it is important to realize that RM loss in Figure 2 is determined relative to the RM content in nonischemic myocardium. If spheres were to be lost from nonischemic areas as well, then the net true RM loss from the infarct region would in fact have been underestimated. Consigny et al. (1979) have recently found a 5–10% sphere loss from nonischemic myocardium over 2 hours in anesthetized dogs, whereas other studies in conscious dogs suggest that additional loss may continue to occur from normal myocardium over a period of several weeks (White et al., 1978a).

Although necrosis was more extensive in endocardial layers than epicardial layers within the infarct zone in our study, we found no reduction in the IN:OUT preocclusion RM ratio (Fig. 3). This suggests that RM loss is greater for epicardial layers, per gram of infarct. More extensive loss of RM in this region may be due to higher levels of collateral flow than are found in endocardial regions, resulting in greater "washout."

**Mechanisms for Microsphere Loss: Whither the Microspheres?**

It is known that myocardial necrosis causes a disruption of muscle and capillaries (Barry and Patten, 1968). It is possible that embolized RM are released and become engulfed by macrophages in the inflammatory infiltrate during the healing process. The microspheres eventually may be carried away via the lymphatic channels, present in a network in close association with venules and veins (Barry and Patten, 1968), to regional nodes, and thence to the systemic circulation via the thoracic duct, and finally embolize in the fine pulmonary capillaries. Hales et al. (1974) has emphasized that, after left ventricular injections of 15 μm of RM, there is some initial dislodgement of RM from capillaries and movement via microvascular arteriovenous connections and final entrapment in the lung, even in the no-infarction setting. Early dislodgement also has been documented by others from normal myocardium (Archie et al., 1973; Crystal et al., 1979) or other organs (Tripp et al., 1977a). In rabbit ear capillaries viewed through a Perspex window by phase contrast microscopy, RM were seen to go outside capillaries, presumably as the result of necrosis of the wall, and become engulfed by macrophages (Fim, "Microspheres," 3M Company).

In this study, we found excess preocclusion RM in the lungs in dogs with myocardial necrosis but not in those without necrosis, after both systemic (left atrial) and selective intracoronary injections. A slight excess of RM was found also in the neck nodes in dogs with necrosis, although we had anticipated finding more RM in the regional nodes draining the heart. The explanation may be that RM
pass through the lymph nodes. Ludwig (1971, 1972) has found that RM ≤ 10 µg in size were present in lymph nodes 4 hours after injection into the lymphatics but had a more rapid transit through the node than larger RM. Nearly 70% of 10-µm RM found in the marginal sinus of the node at 4 hours had moved on by 2 days.

Alternatively, RM may leave the heart and pass into the systemic circulation by direct loss into the left ventricular cavity or via microcirculatory arteriovenous connections and thence to the venous side.

Implications of Microsphere Loss for Flow Measurements

The radioactive microsphere technique is used widely for the measurement of regional myocardial blood flow (Fortuin et al., 1971; Cobb et al., 1974; Marcus et al., 1975; Schaper and Paayk, 1976; Rivas et al., 1976; Bishop et al., 1976) and assumes that microspheres do not shift their positions over time (Rudolph and Heymann, 1967). Since flow is derived from the RM content of tissue samples, an underestimation of actual flow would result if embolized RM were physically lost with time. An early postocclusion coronary flow measurement would appear low compared to one made shortly before killing the animal. The effect of interventions, which might increase collateral blood flow and/or lymphatic drainage from the heart, on microsphere loss has not been determined. It is possible that increased flow might lead to increased washout, accentuating the underestimation of flow in necrotic areas. Alternatively, increased flow might also lead to more edema, causing an apparent rather than real decrease in microsphere content.

It should be pointed out that in chronic experiments on myocardial infarction lasting over several weeks, infarcted tissue may be reabsorbed, leading to an apparent "gain" in preocclusion RM per gram in the infarct relative to nonischemic areas (White et al., 1978b). If, in addition, any physical loss of microspheres should occur from nonischemic tissue during this time, the apparent "gain" in RM content would be accentuated.

Possibilities for Correction of Microsphere Loss

In dogs given two preocclusion RM injections, the close agreement between the values of I:NI RM content indicates that the apparent loss of preocclusion RM was similar. One might anticipate that the apparent loss of postocclusion RM might also be similar, at least for some limited period of time following occlusion. If this were the case, then directional changes in flow would be valid, even if absolute values were underestimated.

In our studies, the amount of apparent RM loss from necrotic areas averaged 19%. Approximately 40% of this apparent loss was actually due to edema within the infarct. These values would be expected to be closely dependent on certain experimental conditions. For example, apparent RM loss in tissue samples taken from the infarct zone should depend on the amount of noninfarcted myocardium included in the sample. The method of postmortem processing of the hearts (formalin fixation vs. analysis of fresh tissue, perfusion with saline vs. perfusion with barium-gelatin mixtures) might be expected to influence the amount of apparent loss related to increased water content in the infarct. Also, the amount of infarct edema probably would depend on whether the coronary occlusion was permanent or whether reflow was used.

Correction for edema should be possible by using desiccated tissue samples, although the contributions of hemorrhage and inflammation to apparent RM loss still would not be taken into account. Statistical problems due to low sphere numbers in necrotic areas may be overcome partially by injecting more microspheres for flow measurements (Buckberg et al., 1971; Tripp et al., 1977b). If the amount of reduction in I:NI ratio for the preocclusion RM reflects the combined effects of increased mass in the infarcted area and true physical RM loss, it should be possible to use this ratio to correct the underestimation of postocclusion flows in necrotic tissue. Although theoretically attractive, this approach requires experimental validation in future studies.

At best, the accuracy of absolute values for flow in areas of infarcted myocardium remains uncertain by current methods. However, in other studies we have done, changes in flow in noninfarcted tissue adjacent to the infarct, but located within the perfusion bed of the occluded coronary artery, have been similar to changes in flow in infarcted tissue (Jugdutt et al., 1979a). This has been true both of natural changes over time in untreated animals and of changes following therapeutic interventions (Jugdutt et al., 1978). Since RM loss does not appear to occur from noninfarcted tissue, values for collateral flow in these areas should be valid and reflect the true flow changes throughout the occluded coronary bed.

In conclusion, the loss of microspheres is a limitation that must be borne in mind when the RM technique is used to measure collateral blood flow in studies of myocardial infarction conducted over several days.

Acknowledgments

We are indebted to Patricia Shaw, Anthony DiPaula, and Alexander Wright for technical assistance and to Joanna Walton for typing the manuscript. We thank Dr. Myron L. Weisfeldt for helpful suggestions.

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Circ Res. 1979;45:746-756
doi: 10.1161/01.RES.45.6.746

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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