SUMMARY. The respective importance of flow and cellular viability in determining initial myocardial thallium uptake was studied in reperfused and nonreperfused experimental myocardial infarction. Open-chest dogs were subjected to permanent coronary artery occlusion of 70-minute duration (n = 3) or 5-hour duration (n = 5), or to a 3-hour temporary occlusion followed by reflow (n = 14). Thallium uptake 10 minutes after intravenous injection was compared directly with radioactive microspheres in myocardial samples from excised hearts. Triphenyl tetrazolium chloride staining was used to differentiate necrotic and viable samples with confirmation by electron microscopy. In nonreperfused infarcts, thallium uptake occurred despite necrosis, and a close correlation was found between thallium uptake and regional myocardial blood flow. In reperfused infarcts, thallium uptake again occurred, but was reduced relative to flow in necrotic myocardium and, to a lesser extent, in reperfused viable areas. However, because of the high levels of reflow, actual thallium uptake was often more than 50% of normal in reperfused necrotic regions. This study demonstrates that the presence of thallium uptake is an unreliable indicator of myocardial injury and that reperfused necrotic tissue may have remarkably high levels of thallium uptake.

THE initial distribution of thallium in the myocardium is dependent on regional perfusion (Strauss et al., 1975; Zaret et al., 1976; DiCola et al., 1977; Pohost et al., 1981; Nishiyama et al., 1982; Chu et al., 1982), but structural and functional integrity of the sarcolemma may also determine myocardial thallium uptake. Studies of fetal mouse hearts in tissue culture have shown reduced thallium uptake when the hearts are injured by hypoxia (Goldhaber et al., 1983). The idea that thallium uptake may be a useful marker of myocardial viability has been utilized in two recent studies of thrombolytic reperfusion in patients with acute myocardial infarction (Maddahi et al., 1981; Markis et al., 1981). A reduction in the size of scintigraphic perfusion defects following reperfusion was interpreted as evidence for myocardial salvage. However, before this conclusion can be accepted, further clarification is required of the respective roles of flow and cellular integrity on thallium uptake in the intact animal. In this study, regional myocardial thallium uptake and myocardial blood flow were compared in viable and necrotic myocardium from dogs with reperfused and nonreperfused myocardial infarcts.

Methods

Experimental Preparation

Twenty-two adult mongrel dogs were anesthetized with sodium pentobarbital, intubated, and ventilated with room air. The heart was exposed through a left thoracotomy, and polyethylene catheters were inserted into the left atrium, jugular vein, and carotid artery. A snare occluder was placed around the left circumflex coronary artery just below the first marginal branch. Microspheres (7.10 μm in diameter) labeled with ⁹⁵Nb or ⁶⁵Sc (New England Nuclear) were used to determine regional myocardial blood flow, as described in detail elsewhere (Jugdutt et al., 1979).

Experimental Protocols

Two different protocols were used. Group I consisted of eight dogs with permanent coronary artery occlusion, and group II, of 14 dogs with temporary coronary occlusion followed by reflow. In group I, the circumflex artery was occluded for 70 minutes in three dogs and 5 hours in five dogs to produce a broad range of ischemic damage. At the end of the occlusion period, microspheres were injected into the left atrium, and, immediately after the withdrawal of the reference blood sample, 500 μCi of thallium-201 were injected intravenously. Ten minutes
later, monastral blue dye (Dupont Co.), 1 ml/kg, was infused into the left atrium over 30 seconds to define the myocardial region of hypoperfusion, and the hearts were arrested by injection of KCl.

In group II, all 14 dogs had a 3-hour coronary occlusion followed by reperfusion, accomplished by abrupt removal of the snare. Three hours of occlusion was chosen instead of the 5 hours used in group I to provide sufficient amounts of nonnecrotic myocardial tissue within the ischemic region for analysis. Myocardial blood flow was determined initially 30 minutes after occlusion. In nine animals, after 20 minutes of reperfusion a second myocardial blood flow measurement was made, and immediately thereafter, 500 μCi of thallium were administered. These dogs were killed 10 minutes later, as in group I, except that the circumflex artery was reoccluded terminally for the monastral blue injection. In the other five dogs of group II, in order to reduce flow to levels comparable with nonreperfused infarcts, the coronary artery was reoccluded 15 minutes after reflow. Five minutes later, microspheres and thallium were given. These animals were killed 10 minutes later after monastral blue injection, as in group I.

Postmortem Tissue Preparation

After sacrifice, the hearts were excised and the ventricles were sectioned parallel to the ativoventricular groove, forming five slices 1–1.5 cm thick, which were then placed in a solution of triphenyl tetrazolium chloride (TTC) at 37°C for 30 minutes. Sampling for regional myocardial blood flow and thallium activity was made in the center of the infarct (TTC−), in ischemic but noninfarcted tissue (area unstained by monastral blue but TTC+), and in the nonischemic anterior papillary muscle. Only samples with homogeneous, confluent areas of necrosis were considered for analysis of the infarct region. Similarly, for the ischemic but noninfarcted region, samples were cut in order to avoid contamination by the nonischemic tissue and by the necrotic area. All surfaces of each sample were examined to assess the homogeneity of staining and suitability for inclusion. Samples were weighed (0.41–1.6 g) and counted in a scintillation counter at appropriate energy windows. A standard computer program was used to correct for activity overlap, and thallium and microsphere activities were determined per gram of myocardium in each sample. Activities in ischemic and infarcted samples were normalized by dividing these values by the nonischemic zone activity in the corresponding left ventricular ring.

In eight dogs of group II, immediately after sacrifice and transverse sectioning of the heart, approximately 20-μg tissue specimens were excised with a scalpel from the center of the non-blue region, lateral portion of the non-blue region, and the nonischemic region. One endocardial sample and one epicardial sample were taken from each of the three regions, making a total of six samples per heart. After TTC staining of the left ventricular rings, sampling sites were identified as being in the region of nonstaining with TTC, in the area stained with TTC but inside the hypoperfused region, or in the nonischemic region. The specimens were prepared for electron microscopy, and 30–40 electron micrographs per heart (six per tissue sample) were obtained and reviewed for ischemic ultrastructural changes. Each specimen was classified as showing cell death or cell injury without definite evidence of cell death. Injury was graded on a 1–4+ scale based on the severity and extent of the ultrastructural abnormalities. The criteria for injured cells were subsarcolemmal blebs, separation and disruption of sarcomeres, and mitochondrial swelling. Criteria for cell death included amorphous matrix densities in mitochondria, disruption of the mitochondrial DNA, and marked clumping of nuclear chromatin along the nuclear membrane.

In eight dogs of group II, the myocardial samples (n = 58) were desiccated after scintillation counting in an oven at 105°C for 12 hours. Water content was calculated from weights before and after drying (accuracy: 0.01 g). Tissue edema was expressed as the percent increase in water content of ischemic samples over nonischemic samples in the same ring.

Statistical Analysis

Student's t-tests for paired and unpaired data were utilized for statistical comparisons between groups. Linear regression analysis was done by the least-squares fit method. All results are presented as mean ± SEM.

Results

Hemodynamic Data

At the time of paired thallium and microsphere injection, there were no significant differences in heart rate, left atrial pressure, or arterial pressure between group I (permanent occlusion) and group II (reflow). Mean heart rates were 172 ± 11 and 181 ± 8 beats/min, mean left atrial pressures were 8 ± 1 and 7 ± 2 mm Hg, and mean arterial pressures were 96 ± 8 and 104 ± 10 mm Hg, respectively. In group II, 30 minutes after the coronary occlusion, regional flow in the infarcted area was reduced below 20% of the corresponding nonischemic flow in all samples.

Relationship of Thallium Uptake to Microsphere-Determined Regional Myocardial Blood Flow

Figure 1 shows the relationship between normalized thallium activity and normalized myocardial blood flow for all samples taken from the infarcted zone in group I dogs. Normalized flows ranged from 0.2 to 23.7% and normalized thallium activities from 2 to 25%. There was a significant linear relation between thallium activity and microsphere flow after both 70 minutes and 5 hours of coronary occlusion. The slopes were close to the line of identity for both time periods. The vertical axis intercepts were similar (3.5 and 2.2%, respectively) and indicated a slight overestimation of flow by thallium in the very low flow ranges (<5% of nonischemic flow).

Figure 2 shows the relationship between normalized thallium activity and normalized microsphere flow in samples from the infarcted zone (TTC−) in group II dogs after reflow. The data include infarcted samples from the nine dogs with simple reperfusion, where normalized flow ranged from 24 to 124%,
and the five dogs with reperfusion followed by coronary reocclusion, where flow ranged from 2.0 to 23.2%, comparable to the level in nonreperfused infarcts. As in Figure 1, there again was a linear relationship between thallium activity and microsphere flow, but in contrast to group I, thallium activity in those reperfused group II samples underestimated microsphere flow (regression line below the line of identity), except at very low flows. However, because blood flow in the reperfused infarcted samples was generally >75% of nonischemic flow (in the absence of reocclusion of the artery), the actual thallium content was often more than 50% of normal.

Table 1 compares mean normalized thallium and microsphere activities at comparable low levels of flow (0–25%) in samples from groups I and II. Whereas thallium activity was slightly but significantly greater than microsphere activity in the 70-minute and 5-hour permanent occlusion subgroups, thallium activity was significantly less than microsphere activity in the reperfusion group.

Group II was analyzed further by comparing thallium and microsphere activities in infarcted (TTC−) and ischemic but noninfarcted (TTC+) samples (Table 2). Samples were grouped according to their normalized microsphere activity (25–49%, 50–74%, 75–99%, 100–124%). Mean thallium activity was only about half of mean microsphere activity in TTC− samples in each flow range. In TTC+ samples, mean thallium activity was also reduced, but to a lesser extent, averaging 9–33% less than microsphere activity in the different flow ranges.

The extent of underestimation of flow by thallium was correlated with the amount of edema in reperfused samples (Fig. 3). On average, TTC− samples had a 4.1% increase in water content, relative to nonischemic myocardium, and a 44.3% underestimation of flow by thallium. In contrast, samples within the ischemic region but TTC+ had a 3.3% increase in water content and a 22.8% underestimation of flow. Taking all samples together, underestimation of flow was linearly related to tissue edema, although considerable scatter in the data was evident.

Electron Microscopy of Reperfused Samples

Electron microscopy was performed on specimens from group II hearts from TTC− regions (96 electron micrographs, 16 tissue samples, eight dogs) and ischemic but TTC+ regions (84 electron micrographs, 14 tissue samples, eight dogs). Fourteen of

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Normalized Thallium and Microsphere Activity in Samples with Comparable Low Levels of Flow</td>
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<tr>
<td></td>
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<tr>
<td>No. of samples</td>
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<tr>
<td>Microsphere activity (%)</td>
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<tr>
<td>Thallium activity (%)</td>
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TABLE 2

Normalized Thallium and Microsphere Activity in Tissue Reperfused after Coronary Occlusion

<table>
<thead>
<tr>
<th>Range of normalized microsphere activity</th>
<th>TTC−</th>
<th>TTC+</th>
<th>TTC−</th>
<th>TTC+</th>
<th>TTC−</th>
<th>TTC+</th>
<th>TTC−</th>
<th>TTC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>25−49%</td>
<td>42 ± 3</td>
<td>49 ± 3</td>
<td>64 ± 2</td>
<td>89 ± 2</td>
<td>111 ± 2</td>
<td>109 ± 3</td>
<td></td>
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</tr>
<tr>
<td>50−74%</td>
<td>27 ± 1</td>
<td>64 ± 2</td>
<td>85 ± 4</td>
<td>109 ± 3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>75−99%</td>
<td>18 ± 2</td>
<td>63 ± 9</td>
<td>55 ± 3</td>
<td>81 ± 4*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100−124%</td>
<td>11 ± 2</td>
<td>64 ± 2</td>
<td>85 ± 4</td>
<td>109 ± 3</td>
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</table>

No. of samples 4 11 18 10

Microsphere activity (%) 42 ± 3 27 ± 1 64 ± 3 64 ± 2 89 ± 2 85 ± 4 111 ± 2 109 ± 3

Thallium activity (%) 21 ± 3 18 ± 2 33 ± 3 58 ± 4* 46 ± 2 63 ± 9 55 ± 3 81 ± 4*

P 0.02 0.04 <0.001 0.06 <0.001 0.01 <0.001 0.004

* P < 0.001 vs. TTC− (unpaired t-test).

Discussion

These experiments demonstrate that, in infarcted myocardium without reperfusion, the regional distribution of thallium 10 minutes after injection closely approximates myocardial blood flow measured by radioactive microspheres. In contrast, in reperfused infarcts, thallium underestimates myocardial blood flow in necrotic areas and, to a lesser extent, in viable areas. However, because blood flow to reperfused infarcted myocardium is so high, actual thallium content may sometimes approach normal levels. As pointed out by Schaper (1979), the level of reflow after a long period of ischemia does not correlate with tissue viability or the intensity of the prior ischemic insult.

The close correlation between thallium uptake and regional myocardial blood flow in nonreperfused animals is similar to previous studies using Rb-86 and K-43 as well as thallium (Becker et al., 1974; Prokop et al., 1974; Strauss et al., 1975; Zaret et al., 1976; DiCola et al., 1977; Schwartz et al., 1978; Selwyn et al., 1978; Wharton et al., 1980; Pohost et al., 1981; Chu et al., 1982). In agreement with DiCola (1977) and Chu (1982), we also observed a relative excess of thallium in regions with low microsphere flow (<5% of flow in the nonischemic zone). This may be due to more efficient tissue extraction of monovalent cations with more prolonged circulatory exposure (Love and Burch, 1959; Moir, 1966). Alternatively, plasma skimming may occur at low flow rates, causing a dissociation between red cell and plasma flow; microspheres, being particulate, may accurately measure erythrocyte flow, while the diffusible thallium may accurately reflect plasma flow (Yipintsoi et al., 1973).

The relative importance of perfusion and metabolic factors in the myocardial uptake of thallium has been the subject of considerable investigation. Studies from Gehring (1967), Zimmer (1979), and Ku (1975, 1976) suggested that the sodium-potassium pump was responsible for thallium uptake. The effect of ischemic-like injury on cellular extraction of thallium independent of blood flow was studied in the fetal mouse-heart organ-culture model (Goldhaber et al., 1983). The data suggested that extraction of thallium from the medium was depressed only when irreversible cell damage existed, although these results may pertain more to late thallium uptake during redistribution than to initial uptake. In the intact animal, our study indicates good agreement between thallium uptake and flow in myocardium made necrotic by 70 minutes and 5 hours of ischemic injury. DiCola (1977) and Chu (1982) also observed that the reduction in regional thallium uptake correlated well with microsphere estimates of blood flow in 24- to 96-hour canine infarct models, and Buja et al. (1976) noticed thallium uptake in areas of nearly homogeneous necrosis. The initial trapping of thallium by the myocardium thus appears to be proportional to flow.
even in infarcted myocardium, despite the lack of a normally active sodium potassium ATPase. In further support of this, Krivokapich and Shine (1981) showed that, in the isolated perfused rabbit septum, the influx of thallium was not dependent on the sodium-potassium pump. Their data suggested that thallium is transported passively across the cell membrane along an electropotential gradient.

In our study, the close agreement that existed between thallium uptake and flow in nonreperfused infarcts was not seen after reperfusion. Except for flows <5% of the nonischemic value, disproportionately reduced thallium uptake was found for the broad range of flow rates obtained, including low flows comparable to nonreperfused infarcts. Fukuyama (1978) found that 86Rb uptake by reperfused infarcts was also reduced disproportionately to myocardial blood flow. Several possible explanations should be considered for this phenomenon. We cannot determine from our data whether extraction fraction was reduced in the reperfused infarct, or whether cellular trapping was reduced, with increased back diffusion. Myocardial thallium content 10 minutes after injection, as measured in this study, reflects a combination of extraction and cellular trapping, and the two components cannot be separated without further information.

Extraction fraction could have been reduced in the reperfused necrotic as well as viable areas merely as a consequence of higher flow rates, but thallium uptake was also reduced in reperfused infarcts with low flows (Table 1). Extraction fraction might also have been reduced because of microvascular damage occurring during reflow, but it is difficult to believe that vascular injury in reperfused viable regions was greater than that occurring in nonreperfused necrotic areas subjected to 5 hours of ischemia.

It would appear more likely that reduced cellular trapping and/or increased back diffusion was responsible for the disproportionate decrease of thallium uptake in reperfused infarcts. Reduced trapping could have been related to the greater fall in intracellular potassium concentration that occurs in reperfused infarcts (Whalen et al., 1974). Alternatively, more severe sarcolemmal damage after reperfusion could have led to a decrease in intracellular transport of thallium, or to an accelerated loss after initial uptake. Also, interstitial edema could have limited the diffusion of thallium from the capillaries to the sarcolemma, increasing the possibility of back diffusion before cellular trapping. Whereas increased water content was found in reperfused necrotic areas, and to a lesser extent in viable regions, it has not been observed in acute nonreperfused infarcts (Whalen et al., 1974).

Despite an underestimation of flow by thallium in reperfused necrotic samples, the actual thallium uptake was often quite high, frequently 50% or more of the nonischemic value, because of the high levels of reflow. Since this level of thallium uptake is easily detectable scintigraphically, our findings have important implications for studies in patients with acute myocardial infarction treated with early nonsurgical reperfusion. In the studies of both Markis et al. (1981) and Maddahi et al. (1981), thallium scintigrams were obtained hours after reperfusion by intracoronary thrombolysis and compared with scintigrams performed pretreatment. A reduction in defect size was found and interpreted as evidence of myocardial salvage. Animal experiments were reported by Maddahi (1981) to support the conclusion that improved thallium uptake signified tissue viability, but the imaging techniques employed by these authors may have been insufficiently sensitive and quantitative to detect uptake by nonviable myocardium. Our study clearly indicates that necrotic tissue reperfused after 3 hours may have surprisingly high levels of both blood flow and thallium uptake. The presence of thallium uptake in this setting is therefore an unreliable indicator of myocardial cell viability.

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J A Melin, L C Becker and B H Bulkley

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