Progression of Myocardial Necrosis During Reperfusion of Ischemic Myocardium

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Background—The occurrence of myocyte necrosis during reperfusion of ischemic myocardium is controversial. This study measured myocardial 2-deoxyglucose uptake, correlated with histology, to determine whether loss of viability occurred during reperfusion of ischemic myocardium.

Methods and Results—In 12 anesthetized dogs, the left anterior descending coronary artery was occluded for 90 minutes before 4 hours reperfusion. Myocardial blood flow was measured by microspheres and the tracers 14C-2-deoxyglucose and 18F-2-deoxyglucose were injected intravenously after 5 and 180 minutes of reperfusion, respectively. After 240 minutes, the heart was stained with thioflavin-S (size of no-reflow zone) and triphenyl-tetrazolium chloride (TTC, extent of necrosis). Samples from normal, salvaged, and necrotic myocardium were counted for 14C- and 18F-2-deoxyglucose and microspheres. With the use of a three-compartment model of 2-deoxyglucose uptake, the rate constant k3 for phosphorylation of 14C- and 18F-2-deoxyglucose was calculated for each sample. Viability was defined as k3 ≥ 0.125 min⁻¹ (predictive accuracy 88% versus electron microscopy and 97% versus TTC). Among 58 samples from no-reflow regions, 97% were nonviable after 5 minutes of reperfusion (k3 = 0.096 ± 0.027 min⁻¹). Among 164 samples from salvaged myocardium, 95% were viable after both 5 and 180 minutes of reperfusion (k3 = 0.170 ± 0.056 min⁻¹ P < .01 versus no-reflow). Among 179 samples from infarcted myocardium, mean k3 after 5 minutes of reperfusion was 0.184 ± 0.070 min⁻¹ and 65% of samples were viable, but after 180 minutes of reperfusion mean k3 had decreased to 0.077 ± 0.032 min⁻¹ (P < .0001) and 98% of samples were nonviable.

Conclusions—A large proportion of samples from infarcted myocardium are viable at the end of the ischemic period but lose viability during the first hours of reperfusion. (Circulation. 1998;97:795–804.)

Key Words: metabolism ■ myocardial infarction ■ ischemia ■ reperfusion ■ radioisotopes

Coronary reperfusion improves ventricular function and survival after infarction, but concern persists that damaged but otherwise viable myocytes may undergo necrosis during reperfusion. Although interventional studies with scavengers of oxygen radicals, inhibition or removal of neutrophil leukocytes, and administration of adenosine suggest that myocardial necrosis does occur during reperfusion, direct evidence has been lacking. Sequential measurements of viability, using a marker of basic cellular metabolism, are required to address this question. Radionuclide-labeled 2-deoxyglucose is used as a tracer of glucose uptake and phosphorylation in brain and heart. Although rapidly phosphorylated by hexokinase, 2-deoxyglucose is not a substrate for further glycolytic metabolism and is trapped in the cell. The rate constant for phosphorylation of 18F-2-deoxyglucose by hexokinase (k3) is correlated with glucose metabolism in the reperfused myocardium. We used 2-deoxyglucose for sequential measurements of viability in reperfused myocardium, with injection of 14C-2-deoxyglucose immediately after reperfusion and 18F-2-deoxyglucose 3 hours later. Uptake of 2-deoxyglucose was compared with histochemical and ultrastructural evidence of reversible and irreversible myocardial injury and correlated with collateral blood flow during ischemia to differentiate lethal injury occurring during ischemia from that occurring during reperfusion.

Methods

Thirty-three mongrel dogs of either sex (weight, 20 to 27 kg) were studied after an overnight fast. The dogs were anesthetized with sodium thiopental (12.5 mg/kg IV) followed by chloralose (14 mg/kg IM) in urethane (136 mg/kg). Polyvinyl catheters were placed in the right femoral artery and vein for reference sampling of microspheres and administration of intravenous fluids, respectively. After left thoracotomy, a catheter was placed in the left atrium for microsphere injections and a catheter-tip pressure transducer (5F, Millar Instruments) was advanced to the left ventricle from the left atrium. An inflatable occluder was placed around the proximal LAD. Hemodynamics were continuously recorded on chart paper (Gould Instruments).

Myocardial Distribution and Retention of 2-Deoxyglucose

Because 14C-2-deoxyglucose and 18F-2-deoxyglucose are not structurally identical, the distribution of the two tracers in reperfused myocardium was compared with histochemical and ultrastructural evidence of reversible and irreversible myocardial injury and correlated with collateral blood flow during ischemia to differentiate lethal injury occurring during ischemia from that occurring during reperfusion.
Myocardial reperfusion necrosis

Myocardial reperfusion was studied in three dogs. After 90 minutes of LAD occlusion, the dogs received simultaneous intravenous \(^{14}\text{C}-2\text{-deoxyglucose}\) (25 \(\mu\text{Ci}\); specific activity, 59 mCi/mmol; Sigma) and \(^{18}\text{F}-2\text{-deoxyglucose}\) (0.25 to 1.5 mCi; Division of Nuclear Medicine, Johns Hopkins Medical Institutions). Tracers were injected after 5 minutes of reperfusion in one dog, after 15 minutes in the second, and after 3 hours in the third. At 60 minutes after tracer injection, the LAD was reoccluded and monoeval blue dye injected into the left atrium to define the ischemic region. Multiple (n = 30 to 40) biopsies (30 to 85 mg wet wt) were obtained from reperfused myocardium for counting of \(^{14}\text{C}\) and \(^{18}\text{F}\) activities.

Retention of \(^{14}\text{C}-2\text{-deoxyglucose}\) in reperfused myocardium was studied in three other dogs. After 90 minutes of LAD occlusion and 5 minutes of reperfusion, \(^{14}\text{C}-2\text{-deoxyglucose}\) was given intravenously. Transmural biopsies (15 to 30 mg wet wt) were taken in triplicate from LAD and circumflex artery territories by biopsy drill at 1, 2, and 3 hours after tracer injection. The heart was harvested after 6 hours and further biopsies taken. The biopsies were weighed and counted for \(^{14}\text{C}\) activity. In two other dogs, myocardial retention of \(^{18}\text{F}-2\text{-deoxyglucose}\) was compared with myocardial blood flow during ischemia and reperfusion. The LAD was occluded for 90 minutes and blood flow measured by radionuclide-labeled 15-\(\mu\text{m}\) microspheres (\(^{111}\text{In}, ^{113}\text{Sn}, ^{46}\text{Sc}; \text{Du Pont Co}) injected into the left atrium, with reference sampling from the right femoral artery, at 10 minutes before reperfusion. After 10 minutes reperfusion, \(^{18}\text{F}-2\text{-deoxyglucose}\) was injected intravenously. A biopsy was excised by scalpel from reperfused myocardium 35 minutes later and divided into endocardial and epicardial halves. After 3 hours of reperfusion, blood flow was measured with microspheres before repeat biopsy of reperfused myocardium and the normal circumflex artery territory. Samples were weighed and counted for \(^{18}\text{F}\) activity and microspheres.

Comparison of \(^{18}\text{F}-2\text{-Deoxyglucose Uptake With Histology}\)

In seven dogs the LAD was occluded for 90 minutes and myocardial blood flow measured by microspheres at 10 minutes before reperfusion. After 5 minutes of reperfusion, \(^{18}\text{F}-2\text{-deoxyglucose}\) was injected intravenously, followed by a second microsphere injection. After 35 minutes of reperfusion, the heart was arrested by intravenous potassium chloride, excised, and sectioned into short-axis slices. Multiple transmural sections from normal and reperfused regions were divided into fifths from endocardium to epicardium (30 to 100 mg wet wt per sample). Samples were randomly selected from the control and reperfused myocardium in each dog for electron microscopy. A small section of each sample was immersed in cold (4°C) 3% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, and kept in fixative at 4°C for 24 hours before rinsing in 0.1 mol/L cacodylate buffer (with saccharose added, pH 7.4) and storage at 4°C before examination. The remainder of the sample was counted for \(^{18}\text{F}\) activity and microspheres.

Operators studied all samples by light and electron microscopy, without knowledge of sample location or regional blood flow. Samples were embedded in epon with the use of a Wakuta automatic tissue processor, after fixation in 2% osmic acid anhydride, dehydration in an ethanol series, and substitution by propylene oxide. Semithin \((1 \text{ to } 2 \mu\text{m})\) sections were stained with toluidine blue. Artifact-free areas were selected for preparation of thin sections (50 to 60 nm), which were attached to uncoated copper grids, stained with uranyl acetate and lead citrate, and viewed in a Phillips EM 300-electron microscope. For each myocardial sample, 30 to 40 micrographs were examined according to previously established criteria. Reversible injury was identified by absence of contraction bands, an intact sarcolemma, absence of mitochondrial amorphous densities, and absence of nuclear clearing and shrinkage. Irreversible injury was identified by the presence of amorphous densities, matrix clearing and/or cristae breakage in the mitochondria, clearing and shrinkage of nuclei, and/or disruption of the sarcolemma. A sample was deemed to have suffered irreversible injury if >50% of the micrographs from that sample showed evidence of irreversible injury.

Sequential Measurements of 2-Deoxyglucose Uptake in Reperfused Myocardium

Myocardial uptake of 2-deoxyglucose after 5 minutes and 3 hours of reperfusion was studied in 18 dogs in which the LAD was occluded for 90 minutes before free reperfusion was allowed. Myocardial blood flow was measured by microspheres 10 minutes before reperfusion and 10 minutes and 3 hours after reperfusion. After 5 minutes of reperfusion, \(^{14}\text{C}-2\text{-deoxyglucose}\) (25 \(\mu\text{Ci}\)) was injected intravenously and after 3 hours of reperfusion, \(^{18}\text{F}-2\text{-deoxyglucose}\) (\(\approx 1\) mCi; range, 0.25 to 2 mCi) was injected intravenously. The \(^{18}\text{F}-2\text{-deoxyglucose}\) was used as the second tracer because of the short half-life of \(^{18}\text{F}\). One hour after injection of \(^{18}\text{F}-2\text{-deoxyglucose}\), the fluorescent dye thioflavine-S (2% solution) was injected into the left atrium to define no-reflow zones. Two minutes later the LAD was reoccluded and monoeval blue dye (20 mL) was injected into the left atrium to define the ischemic risk region. The heart was then arrested with potassium chloride and excised for tissue sampling and measurement of infarct size.

The left ventricle was sectioned and sectioned into five transverse slices (8 to 10 mm thick), which were weighed and examined under ultraviolet light to define no-reflow zones (absent thioflavin-S fluorescence). Endocardial and epicardial surfaces of each myocardial slice and borders of the ischemic region and the no-reflow zones were traced on acetate sheets. Transmural sections were excised at multiple sites in the LAD and circumflex territories of each heart and divided into fifths (70 to 100 mg wet wt per sample). A total of 60 to 70 samples were obtained from each heart, and sample sites were recorded on the acetate sheets. The myocardial slices were incubated in 2,3,5-TTC solution at 37°C for 30 minutes to differentiate infarcted myocardium (absent or negative TTC staining) from salvaged myocardium (brick-red or positive TTC staining). The borders of infarct and noninfarct regions were traced on the corresponding acetate sheets and planimetered to measure the ischemic risk region, infarct region, and no-reflow region.

Radionuclide Measurements

Myocardial samples were weighed and counted with flow reference samples and radionuclide standards in a NaTl crystal well counter (Packard 5986) set for photopeaks of \(^{111}\text{In}, ^{113}\text{Sn}, ^{46}\text{Sc}\), and \(^{141}\text{Ce}\). Decay-corrected counts were corrected for crossover between radionuclides and blood flow calculated according to standard methods. Samples were then solubilized (Protosol, Du Pont) and incubated at 50°C for 48 hours, before addition of 10 mL scintillation cocktail (EconoFluor, Du Pont) and liquid scintillation counting (Packard Tri-Carb 2660) for \(^{14}\text{C}\) activity. To eliminate any error in \(^{14}\text{C}\) measurement due to the presence of gamma emitters in the samples, calibration curves were determined for beta activity observed in the presence of \(^{113}\text{Sn}\) and \(^{141}\text{Ce}\). Sc. Activity counts were corrected for crossover between radionuclides and blood flow calculated according to standard methods. Samples were then solubilized (Protosol, Du Pont) and incubated at 50°C for 48 hours, before addition of 10 mL scintillation cocktail (EconoFluor, Du Pont) and liquid scintillation counting (Packard Tri-Carb 2660) for \(^{18}\text{F}\) activity. To eliminate any error in \(^{18}\text{F}\) measurement due to the presence of gamma emitters in the samples, calibration curves were determined for beta activity observed in the presence of \(^{113}\text{Sn}\) and \(^{141}\text{Ce}\). Sc. Activity counts were corrected for crossover between radionuclides and blood flow calculated according to standard methods.
the overall reaction rate in the sample of interest $R_i$ can be described as:

$$R_i = (BG/LC) \times \frac{(k_i^0 \times k_i^1)}{(k_i^0 + k_i^1)}$$

where $BG$=blood glucose level; $k_i^0$=rate of transport of 2-deoxyglucose into myocyte from plasma; $k_i^1$=rate of reverse transport of 2-deoxyglucose from myocyte to plasma; and $k_i^2$=rate of phosphorylation of 2-deoxyglucose by hexokinase. Because the lumped constant (LC) and arterial input function are the same for all samples in each heart, the ratio of reaction rates in two samples can be calculated as the ratio of tracer activities:

$$\frac{C_{iT}/C_{rT}}{\left[\frac{(k_i^0 \times k_i^1)}{(k_i^0 + k_i^1)}\right]}$$

where $C_{iT}$=tracer content in sample of interest; and $C_{rT}$=tracer content in reference sample.

The rate constants of 2-deoxyglucose uptake and phosphorylation in reperfused canine myocardium have been previously described, allowing calculation of $k_i$ for the infarct region. The previous data show that $k_i$ increases in normal myocardium after an infarct, but $k_i$ in salvaged postischemic myocardium remains comparable to that of the control state. Therefore, the salvaged (TTC-positive) myocardium was used as the reference region. For all samples from reperfused myocardium the rate constants used were $K_i^0=0.61 \text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, $k_i^1=0.87 \text{ min}^{-1}$. For samples from normal myocardium, the rate constants used were $K_i^0=0.83 \text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ and $k_i^1=1.44 \text{ min}^{-1}$. The same rate constants were used for calculation of $k_i$ for $^{14}$C- and $^{18}$F-2-deoxyglucose.

### Statistics

The proportion of samples in reperfused myocardium that were viable with $^{14}$C-2-deoxyglucose (early reflow) was compared with the proportion that were viable with $^{18}$F-2-deoxyglucose (late reflow) by $\chi^2$ analysis. Hemodynamic and regional myocardial blood flow measurements during ischemia and reperfusion were compared by ANOVA. Myocardial 2-deoxyglucose contents were compared between control and postischemic regions in each dog by ANOVA. The proportions of samples that appeared to undergo necrosis during the ischemic period, or necrosis during reperfusion, or remained viable were compared with collateral blood flow by regression analysis. Results are reported as mean±SD, and a value of $P<.05$ is described as significant.

### Results

#### Uptake and Distribution of 2-Deoxyglucose

The distribution of $^{14}$C-2-deoxyglucose and $^{18}$F-2-deoxyglucose in reperfused myocardium, after simultaneous injection of both tracers, is shown in Fig 1. The slope (0.93) reflects slightly lower $^{18}$F-2-deoxyglucose uptake after 5 minutes of reperfusion, but neither the group regression nor individual regressions differed from the line of identity, indicating that tissue distributions and retention of the tracers were equivalent. Retention of $^{14}$C-2-deoxyglucose in normal and reperfused myocardium is shown for another three dogs in Fig 1. One hour after injection, mean $^{14}$C-2-deoxyglucose content in reperfused myocardium was half of that in normal myocardium ($P<.01$). Myocardial count activities were similar to those in subsequent experiments. During 3 hours after injection, there was no significant change in mean $^{14}$C activity in normal or reperfused myocardium. Biopsies obtained 4 hours after reperfusion had $^{14}$C activity similar to that of biopsies taken after 1 hour. In the other two dogs, which had both flow and $^{18}$F-2-deoxyglucose uptake measurements, samples were grouped according to myocardial blood flow. Samples with severe ischemia (collateral flow <10% of control) and impaired reperfusion (flow <40% of control), which are likely to have the most severe necrosis, had similar $^{18}$F-2-deoxyglucose content after 35 minutes (40% of control) and after 3 hours (41% of control) reperfusion. These data show that radiolabeled 2-deoxyglucose, injected during early reperfusion, is retained over 4 hours in reperfused myocardium.

#### Comparison of 2-Deoxyglucose Uptake With Histopathology

The uptake of $^{18}$F-2-deoxyglucose in normal and reperfused myocardium was compared with electron microscopy findings in seven dogs. Blood flow to control (1.45±0.54 mL/min per gram) and reperfused (1.54±0.34 mL/min per gram) myocardium was similar, but $^{18}$F-2-deoxyglucose content was less in reperfused myocardium (61 465±30 328 counts/min per gram) than in the control region (106 760±71 013 counts/min per gram, $P<.05$). Samples were randomly selected from control (n=6) and reperfused (n=28) regions. All control samples had the ultrastructural features of viable myocardium.
Among samples from reperfused myocardium, 8 manifested reversible ischemic injury (Fig 2B) and 20 had irreversible injury (Fig 2C). The mean $k_3$ for these samples were $0.320 \pm 0.152$ min$^{-1}$ for control, $0.194 \pm 0.070$ min$^{-1}$ for reversible injury, and $0.098 \pm 0.055$ min$^{-1}$ for irreversible injury ($P < .01$ versus reversible injury). The individual $k_3$ values were compared with the electron microscopy findings to determine which value of $k_3$ was the best discriminator between reversible and irreversible injury (Fig 3). A value of $k_3 = 0.125$ min$^{-1}$ appeared to be the best indicator of viability (sensitivity = 93%, specificity = 85%, predictive accuracy = 88%). If a value of $k_3 = 0.100$ min$^{-1}$ was used, specificity decreased to 60%, and if a value of $k_3 = 0.150$ min$^{-1}$ was used, sensitivity decreased to 86%. For the subsequent serial studies of 2-deoxyglucose uptake, samples with $k_3 < 0.125$ min$^{-1}$ were considered non-viable and those with $k_3 \geq 0.125$ min$^{-1}$ were considered viable.

**Serial Studies of 2-Deoxyglucose Uptake in Reperfused Myocardium**

Among 18 dogs included in the group, two had ventricular fibrillation shortly after reperfusion and were not resuscitated. Four dogs with collateral blood flows > 30% control flows and no evidence of infarction on TTC staining were excluded from analysis. Data are reported for 12 dogs that completed 90 minutes of ischemia and 4 hours of reperfusion with TTC evidence of infarction. Heart rate did not change from before ischemia (136 ± 19 bpm) to 3 hours of reperfusion (135 ± 28 bpm), but mean arterial pressure was lower during ischemia (92 ± 22 mm Hg) than before ischemia (102 ± 24 mmHg) or after 3 hours of reperfusion (102 ± 20 mm Hg, $P < .05$ versus ischemia). Blood flow in the circumflex territory was 1.07 ± 0.57 mL/min per gram after 5 minutes of reperfusion and 1.03 ± 0.55 mL/min per gram after 3 hours. Collateral flow during LAD occlusion was 0.07 ± 0.04 mL/min per gram, increasing to 1.19 ± 0.34 mL/min per gram during early reperfusion. After 3 hours, flow in the LAD myocardium was 0.68 ± 0.23 mL/min per gram ($P < .05$ versus early reperfusion). Collateral flow to the TTC-positive region was greater than flow to the TTC-negative region ($P < .01$) (Table 1). Blood flow was reduced in the no-reflow zones, but there was no other difference in flow between TTC-negative and TTC-positive regions during reperfusion.

A total of 850 myocardial samples were examined (340 from the control circumflex territory and 510 from the reperfused LAD territory). Among samples from reperfused myocardium, 237 were from TTC-negative regions, including 58 from the no-reflow zone, and 164 were from TTC-positive regions. There were 109 samples from borders of TTC-negative and TTC-positive myocardium, which were not included in the data analysis. Among the 401 samples from reperfused myocardium, the $^{18}$F-2-deoxyglucose $k_3$ threshold of 0.125 min$^{-1}$ identified 235 of the 237 TTC-negative samples as nonviable and 155 of the 164 TTC-positive samples as viable (sensitivity for identifying viable myocardium, 93%; specificity, 99%; predictive accuracy, 97%).

Contrasting examples of 2-deoxyglucose uptake during early and late reperfusion are shown in Figs 4 and 5. Data from one heart are shown in Fig 4. Samples are grouped by origin from TTC-positive, TTC-negative, or no-reflow regions.
This heart had reduced 14C-2-deoxyglucose uptake in TTC-negative myocardium after 5 minutes of reperfusion (Fig 4, A and B). In TTC-negative samples the $k_3$ for 14C-2-deoxyglucose was 0.082±0.019 min⁻¹, and all but one of these samples had $k_3<0.125$ min⁻¹, indicating necrosis by 5 minutes after reperfusion. The same TTC-negative samples all had $k_3<0.125$ min⁻¹ for 18F-2-deoxyglucose (Fig 4, C and D), with $k_3=0.067±0.015$ min⁻¹ (NS versus 14C-2-deoxyglucose). Eight samples from no-reflow regions all had $k_3<0.125$ min⁻¹ at both early and late reperfusion. The data in this heart are consistent with irreversible injury occurring during ischemia.

Data for a different heart are shown in Fig 5. There was avid uptake of 14C-2-deoxyglucose during early reperfusion in both TTC-positive and TTC-negative regions (Fig 5A). The calculated $k_3$ for 14C-2-deoxyglucose in the TTC-negative region was 0.228±0.077 min⁻¹. All samples in the TTC-negative region had $k_3>0.125$ min⁻¹, indicating viability at 5 minutes of reperfusion (Fig 5B). After 3 hours of reperfusion the $k_3$ for 18F-2-deoxyglucose in the same TTC-negative samples was 0.057±0.016 min⁻¹ ($P<.001$ versus 14C-2-deoxyglucose), and all TTC-negative samples had $k_3<0.125$ min⁻¹, indicating irreversible injury by the time of 18F-2-deoxyglucose injection (Fig 5, C and D). The data in this heart are consistent with the occurrence of necrosis during the reperfusion period.

The 2-deoxyglucose $k_3$ values in TTC-positive and TTC-negative myocardium are summarized for the group in Table 2. In the no-reflow region, $k_3$ after 5 minutes of reperfusion was 0.096±0.027 min⁻¹ and after 3 hours 0.060±0.023 min⁻¹ (NS). All but 2 of these samples were classified as nonviable after 5 minutes and all were nonviable after 3 hours of reperfusion. In the TTC-negative region, $k_3$ decreased from 0.184±0.070 min⁻¹ after 5 minutes of reperfusion to 0.077±0.032 min⁻¹ ($P<.0001$) after 3 hours. After 5 minutes, 117 of these 179 samples were viable according to the $k_3$ threshold, but after 3 hours only 4 were viable ($P<.0001$). In the TTC-positive region, mean $k_3$ after 5 minutes (0.170±0.087 min⁻¹) and after 3 hours (0.170±0.056 min⁻¹) were similar, and 155 of these 164 samples were viable. Among 237 samples from no-reflow and TTC-negative infarct regions, 119 (50.2%) were viable at the time of 18F-2-deoxyglucose injection, but only 4 (1.7%) were viable at the time of 14C-2-deoxyglucose injection, consistent with the development of irreversible injury during reperfusion. The proportion of samples classified as viable in the no-reflow, TTC-negative, and TTC-positive regions, according to different values of $k_3$ are shown in Fig 6. For $k_3$ between 0.100 and 0.200 min⁻¹, almost no samples in the no-reflow zones were classified as viable by 14C-2-deoxyglucose or 18F-2-deoxyglucose. In TTC-negative myocardium, the proportions of samples classified as viable by 14C-2-deoxyglucose and 18F-2-deoxyglucose were similar, whichever value of $k_3$ was used. In TTC-negative myocardium, there was a marked difference between the proportions of samples classified as viable by 14C-2-deoxyglucose and those classified as viable by 18F-2-deoxyglucose.

**Ischemic Necrosis, Reperfusion Necrosis, and Infarct Size**

The ischemic risk region occupied 30.0±4.0% of the left ventricle. The mean infarct size was 37.6±21.0% of the risk region (range, 5.7% to 68.3%), and the mean size of the no-reflow region was 8.3±7.7% of the risk region. Total

<table>
<thead>
<tr>
<th>Infarct region</th>
<th>Occlusion</th>
<th>10 Minutes</th>
<th>3 Hours</th>
</tr>
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<tbody>
<tr>
<td>TTC-positive</td>
<td>0.14±0.08</td>
<td>1.21±0.47*</td>
<td>0.74±0.25†</td>
</tr>
<tr>
<td>% Normal region</td>
<td>13±8</td>
<td>113±44*</td>
<td>72±24†</td>
</tr>
<tr>
<td>TTC-negative</td>
<td>0.03±0.02‡</td>
<td>1.29±0.57*</td>
<td>0.86±0.42†</td>
</tr>
<tr>
<td>% Normal region</td>
<td>3±2‡</td>
<td>121±53*</td>
<td>85±41†</td>
</tr>
<tr>
<td>No-reflow region</td>
<td>TFL-negative</td>
<td>0.02±0.02‡</td>
<td>0.57±0.29†‡‡</td>
</tr>
<tr>
<td>% Normal region</td>
<td>2±2‡</td>
<td>53±27*‡‡</td>
<td>23±7*‡‡</td>
</tr>
</tbody>
</table>

TTC indicates triphenyl-tetrazolium chloride stain; TFL, thioflavine stain. Values are mean±SD. Data are shown for mean blood flows in each region in the 12 dogs.

*P<.01 vs occlusion; †P<.01 vs early reperfusion; ‡P<.01 vs TTC-positive; §P<.01 vs TTC-negative.
infarct size was inversely related to collateral blood flow during ischemia ($r = -0.87$), and the extent of the no-reflow zone was also inversely related to collateral blood flow ($r = -0.64$).

The proportion of anatomic infarct size due to necrosis during ischemia or reperfusion in each dog was calculated from the number of infarct samples that were nonviable by both $^{14}$C-2-deoxyglucose and $^{18}$F-2-deoxyglucose (ischemic necrosis) or viable by $^{14}$C-2-deoxyglucose but nonviable by $^{18}$F-2-deoxyglucose (reperfusion necrosis) for that particular dog. The proportions of infarct size due to ischemic or reperfusion necrosis varied according to the level of collateral blood flow (Fig 7). The four dogs with the lowest collateral blood flow (2.8±1.1% of control flow) had the largest infarcts (54±7% of risk region) and in these dogs 86±7% of the infarct samples were irreversibly injured by 5 minutes of reperfusion. The four dogs with intermediate collateral flows (4.8±0.9% of control) had an infarct size of 46±15% of risk region, but only 30±23% of infarct samples were irreversibly injured by 5 minutes of reperfusion. In contrast, the four dogs with the highest collateral flows (12.7±3.1% of control) had small infarcts (13±10% of risk region) and only 7±7% of infarct samples were nonviable by 5 minutes of reperfusion. As collateral blood flow increased, the proportion of infarction due to irreversible injury during reperfusion increased.

**Discussion**

This study examined changes in viability of reperfused myocardium. In salvaged myocardium, uptakes of $^{14}$C-2-deoxyglucose during early reperfusion and $^{18}$F-2-deoxyglucose 3 hours later were similar and above a threshold of viability. In samples from severely injured no-reflow regions, both $^{14}$C-2-deoxyglucose and $^{18}$F-2-deoxyglucose uptakes were below the viability threshold. In most samples from the infarct region, $^{14}$C-2-deoxyglucose uptake during early reperfusion was above the viability threshold, but 3 hours later uptake of $^{18}$F-2-deoxyglucose in the same samples was reduced to levels associated with irreversible injury, consistent with the occurrence of myocyte necrosis during reperfusion. Regions with low collateral flows had nearly complete loss of viability by the end of ischemia, but regions with higher collateral flows appeared to undergo necrosis during reperfusion.

**Myocardial Viability and 2-Deoxyglucose**

The tracer $^{18}$F-2-deoxyglucose is widely used as a viability marker in clinical studies with PET. The phosphorylated tracer accumulates within myocytes, reaching steady state by 60 minutes, because dephosphorylation is slow and alternate metabolic pathways are limited. Myocardial uptake of 2-deoxyglucose depends on tracer delivery and kinetics of 2-deoxyglucose transport and phosphorylation. We calculated $k_3$, the rate constant for 2-deoxyglucose phosphorylation, in individual myocardial samples, using parameters derived from PET studies of reperfused infarcts in canine hearts. The values of $k_3$ calculated for normal, salvaged, and infarcted myocardium in this study are similar to the previous PET data. To
define an appropriate value of $k_3$ as a marker of viability, we compared tissue $k_3$ with ultrastructural appearance and histochemical staining of reperfused myocardium. Myocardial samples with an ultrastructural pattern of irreversible injury, after 1 hour of reperfusion, had $k_3 < 0.125 \text{ min}^{-1}$, but samples showing reversible injury had $k_3 \geq 0.125 \text{ min}^{-1}$. Similarly, samples that were necrotic by TTC stain after 4 hours of reperfusion had $k_3 < 0.125 \text{ min}^{-1}$, but samples that were viable by TTC stain had $k_3 \geq 0.125 \text{ min}^{-1}$. The lowest $k_3$ values were found in the no-reflow regions with the most severe ischemic injury. Small differences in predictive accuracy of $k_3$ for detection of viability compared with electron microscopy or TTC stain reflect different numbers of samples in each comparison. It should be noted that this threshold may not be universally applicable, particularly for clinical PET studies when patients are given a glucose load.

The $k_3$ in the reference region and the value of the viability threshold might change during reperfusion, but our pathology comparisons indicate the same viability threshold at 35 minutes and at 4 hours after reperfusion. There was no systematic change in $k_3$ in the normal myocardium in this study and the previous PET study found no change in $k_3$ in salvaged myocardium. The proportion of samples deemed viable by $^{14}$C-2-deoxyglucose and $^{18}$F-2-deoxyglucose in TTC-positive myocardium were the same across a wide range of $k_3$ values. To avoid any bias related to selection of the threshold value of $k_3$, we determined the number of samples that were viable in the infarct region according to a wide range of threshold values of $k_3$. Irrespective of the $k_3$ threshold used, many samples from

### TABLE 2. Regional 2-Deoxyglucose Uptake in Control and Reperfused Myocardium

<table>
<thead>
<tr>
<th>Region</th>
<th>10-Minute Reparfusion, $^{14}$C-2-Deoxyglucose</th>
<th>3-Hour Reparfusion, $^{18}$F-2-Deoxyglucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-reflow</td>
<td>Counts $\cdot \min^{-1} \cdot \text{g}^{-1}$</td>
<td>$k_3 \min^{-1}$</td>
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<tr>
<td></td>
<td>12.455$\pm$0.5557*‡§</td>
<td>0.096$\pm$0.027*‡§</td>
</tr>
<tr>
<td>TCC-negative</td>
<td>Counts $\cdot \min^{-1} \cdot \text{g}^{-1}$</td>
<td>$k_3 \min^{-1}$</td>
</tr>
<tr>
<td></td>
<td>2.071$\pm$0.5486*‡</td>
<td>0.184$\pm$0.070*‡</td>
</tr>
<tr>
<td>TCC-positive</td>
<td>Counts $\cdot \min^{-1} \cdot \text{g}^{-1}$</td>
<td>$k_3 \min^{-1}$</td>
</tr>
<tr>
<td></td>
<td>20.125$\pm$0.5005*‡</td>
<td>0.170$\pm$0.087*‡</td>
</tr>
<tr>
<td>Normal</td>
<td>Counts $\cdot \min^{-1} \cdot \text{g}^{-1}$</td>
<td>$k_3 \min^{-1}$</td>
</tr>
<tr>
<td></td>
<td>36.939$\pm$15600</td>
<td>0.463$\pm$0.278</td>
</tr>
</tbody>
</table>

Values are mean$\pm$SD. Data are shown for mean count activities and $k_3$ for each of the 12 dogs.

*P<.01 vs control region, †P<.05 vs TCC-positive region, ‡P<.01 vs 10 minutes-reperfusion, §P<.01 vs TTC-negative region.
the TTC-negative infarct region, which were viable at the time of $^{14}$C-2-deoxyglucose injection after 5 minutes of reperfusion, were nonviable by the time of $^{18}$F-2-deoxyglucose injection 3 hours later.

**Interpretation of Findings**

Approximately half of myocardial samples from the TTC-negative infarct region were apparently viable during early reperfusion, but during the next 3 hours exhibited a decrease in 2-deoxyglucose phosphorylation to the levels found in necrotic myocardium from the no-reflow zone. This observation is consistent with the occurrence of myocardial necrosis during reperfusion, but several other possible interpretations should be examined. Loss of $^{18}$F-2-deoxyglucose during late reperfusion is unlikely to account for the observed differences in myocardial 2-deoxyglucose content between 5 minutes and 3 hours of reperfusion. First, our initial experiments showed that myocardial content of 2-deoxyglucose was largely unchanged during 4 hours of reperfusion. Second, if $^{18}$F-2-deoxyglucose were lost from necrotic myocytes, then $^{14}$C-2-deoxyglucose would also be lost. Third, increased metabolism of $^{18}$F-2-deoxyglucose-phosphate is unlikely, as the rate constant for dephosphorylation remains an order of magnitude below $k_3$ during reperfusion.14

Impaired delivery of $^{18}$F-2-deoxyglucose to the infarct region after 3 hours of reperfusion is also unlikely to account for our findings. Blood flow to the infarct zone was mildly reduced after 3 hours, but mean flows in the TTC-negative and TTC-positive regions were similar, and myocyte uptake of $^{18}$F-2-deoxyglucose at steady state is independent of blood flow. Reperfusion of infarcted myocardium is associated with myocyte swelling and interstitial edema.21 The true uptake of $^{14}$C-2-deoxyglucose during early reperfusion might be underestimated when the sample is weighed after 4 hours. This error would underestimate the calculated $k_3$ for early reperfusion but could not explain the differences in $k_3$ found in this study. Correction for tissue edema in the infarcted myocardium was used in this study, but even in the absence of any such correction, 60% of samples from TTC-negative myocardium were viable after 5 minutes of reperfusion, with $k_3 > 0.125$ min$^{-1}$ for $^{14}$C-2-deoxyglucose.

Reperfusion of irreversibly injured myocytes is associated with contraction bands, cell swelling, and sarcolemmal disruption.27,28 Such cells would be unlikely to accumulate $^{14}$C-2-deoxyglucose, consistent with our finding in samples from the no-reflow zones. An increase in sarcolemmal permeability29 leads to loss of enzymes such as creatine kinase. The reduced $^{18}$F-2-deoxyglucose content seen during later reperfusion might reflect washout of hexokinase from necrotic myocytes, but $^{14}$C-2-deoxyglucose would also be lost from the same myocytes. Our observations cannot be explained as an artifact of tissue edema or tracer washout. The most likely explanation is that myocytes, which were viable during early reperfusion, subsequently lost viability during the next 3 hours of reperfusion.

**Irreversible Myocardial Injury During Reperfusion**

Many samples from infarcted myocardium appear to have undergone necrosis after restoration of coronary blood flow.
Myocytes can undergo necrosis in the presence of apparently adequate coronary perfusion, as with catecholamine stress, loss of calcium homeostasis, or reoxygenation after anoxia. It is also now known that myocytes may be programmed to die through the process of apoptosis. Although normally a mechanism for removal of senescent cells, it is possible that apoptosis may be responsible for large scale cell loss under pathological conditions.

The small residual uptake of 2-deoxyglucose observed in infarcted myocardium may represent a few surviving myocytes, or uptake in endothelial cells or fibroblasts, but the volume of these elements is small compared with myocyte volume. Neutrophil leukocytes accumulating during reperfusion are a potential site of 2-deoxyglucose uptake, but leukocyte uptake of 18F-2-deoxyglucose in reperfused myocardium is small compared with overall myocyte uptake. Furthermore, any such error would result in an increase in tissue 18F-2-deoxyglucose uptake, which is opposite to our observations.

Two other studies have reported data consistent with the occurrence of irreversible myocardial injury during reperfusion. One study in rabbits, using sequential tissue staining with horseradish peroxidase and TTC, found an apparent increase in infarct size during 3 hours of reperfusion. A canine study, using radionuclide-labeled antimyosin antibodies, found a progressive increase in antibody binding in reperfused myocardium, which suggests lethal injury, although these observations could also be explained by increasing sarcolemmal permeability in infarcted myocytes.

This study does not define the mechanism of lethal myocardial injury occurring during reperfusion, although the concordance between the time course of reperfusion injury and neutrophil infiltration is compelling. Intervention studies have implicated neutrophil leukocytes in the pathogenesis of irreversible injury during reperfusion. Although it is possible that myocytes are “programmed” for inevitable necrosis during reperfusion, as a result of an irreversible ischemic insult, our data show that a significant proportion of samples from the infarct region are viable at the time of reperfusion and many interventional studies argue that reperfusion necrosis is not inevitable.

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References


Progression of Myocardial Necrosis During Reperfusion of Ischemic Myocardium
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