Myocardial Uptake and Kinetic Properties of Technetium-99m-Q3 in Dogs

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We postulated that ^{99m}Tc-Q3, a cationic imaging agent, produces myocardial activity related to myocardial blood flow during myocardial ischemia and pharmacologic coronary artery vasodilation, and shows little or no myocardial redistribution over 4 hr after intravenous injection. **Methods:** In six Group 1 dogs, the chest was opened, the left circumflex coronary artery was acutely ligated, and dipyridamole (0.32, 0.56 or 0.84 mg/kg) was infused into the right atrium, followed by 10 mCi of ^{99m}Tc-Q3. Myocardial blood flow was measured by radiolabeled microspheres. The animals were euthanized and 357 myocardial samples were assayed in a well counter for ^{99m}Tc activity. One week later, radiolabeled microsphere activity was counted and myocardial blood flow calculated. In nine Group 2 dogs, a variable occluder was placed around the left circumflex coronary artery and an ischemic level of circumflex blood flow was maintained constant over 4 hr as measured by an ultrasonic flow meter. Dipyridamole (0.56 mg/kg) was then infused into the right atrium followed by 10 mCi of ^{99m}Tc-Q3. Gamma camera images were acquired at 5, 15, 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection. Microsphere blood flow and endocardial biopsies (n = 6 dogs) were performed at 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection. **Results:** In the Group 1 animals, ^{99m}Tc activity (y) was related to myocardial blood flow (x) from 0 to 6.1 ml/min/g by the relationship $y = 0.83X + 0.18$, $r = 0.95$, $p = 0.0001$. The scintigraphic ratio of myocardial perfusion defect zone counts-to-normal myocardial zone counts (0.54 ± 0.05 at 30 min) remained constant over 4 hr, as did technetium counts from direct endocardial sampling. Scintigraphic count ratios allowed discrimination between perfusion defect and normal myocardial regions beginning at 5 min following ^{99m}Tc-Q3 injection. **Conclusions:** Over a range of myocardial blood flows from 0 to 6.1 ml/min/g, ^{99m}Tc-Q3 myocardial activity is related to myocardial flow at the time of tracer injection. Technetium-99m-Q3 shows no evidence of myocardial redistribution over a 4-hr period.

Key Words: myocardial perfusion; radionuclide imaging; technetium-99m

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Technetium-99m-N,N'-ethylenebis(acetylacetoneiminato)bis(tris(3-methoxy-l-propyl)phosphine), also known as ^{99m}Tc-Q3, produces prompt myocardial visualization with rapid hepatic clearance in humans (*1,2*). As a result of the higher physical energy of ^{99m}Tc compared to ^{201}Tl, it is likely that ^{99m}Tc-Q3 could provide myocardial images with less image degradation by soft tissue attenuation compared to ^{201}Tl. If ^{99m}Tc-Q3 is confirmed to have more rapid hepatic clearance in humans compared to the clinically available ^{99m}Tc myocardial tracer, sestamibi, the time from tracer injection to the beginning of imaging could be shortened without significant interference from hepatic activity. This could result in shorter imaging sequences for ^{99m}Tc-Q3 and improved convenience for patients. For ^{99m}Tc-Q3 to provide diagnostically useful myocardial images, tracer activity at the time of imaging must be related to myocardial blood flow over a clinically relevant range of flows.

In addition, design of an optimal clinical imaging sequence requires documentation of the kinetic properties of the tracer after uptake into the myocardium. If the myocardial tracer does not demonstrate detectable washout from the myocardium over time, then the imaging sequence can be selected to minimize patient time while assuring satisfactory myocardial visualization without interference from activity in adjacent organs.

In a recent study (*2*), exercise ^{99m}Tc-Q3 and exercise ^{201}Tl imaging provided comparable detection of coronary artery stenoses documented by coronary angiography. No previous data are available to document the relationship between ^{99m}Tc-Q3 myocardial activity and myocardial blood flow or to characterize the time course of ^{99m}Tc-Q3 distribution in the myocardium. Studies of earlier ^{99m}Tc "Q" complexes (*3*) in animals confirmed that some of them exhibited prominent myocardial activity while chemical studies established that their cationic Tc (III) core is electrochemically inert; thus, these complexes cannot be reduced to their neutral Tc (II) forms in vivo and would be expected to remain fixed in the myocardium. Based upon these observations, in a canine model, we hypothesized that: (1) ^{99m}Tc-Q3 myocardial activity is related to myocardial blood flow during conditions of myocardial ischemia and

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pharmacologic coronary artery vasodilation and (2) that ^{99m}Tc-Q3 shows little or no myocardial redistribution up to 4 hr after intravenous injection.

METHODS

Animal Instrumentation

Animal studies conformed to the guidelines of the American Physiological Society and were approved by the Institutional Animal Care and Use Committee. Seventeen male mongrel dogs weighing 21.0–27.0 kg were instrumented. Two animals developed sustained ventricular fibrillation following coronary artery occlusion and required prolonged resuscitation and support with inotropic drugs. These two animals remained hemodynamically unstable and were therefore excluded from all further consideration. Six dogs (Group 1) were studied by a myocardial perfusion protocol to compare initial ^{99m}Tc-Q3 myocardial distribution to the distribution of myocardial blood flow as assessed by radiolabeled microspheres.

The remaining nine dogs (Group 2) were studied by a myocardial kinetics protocol in which the myocardial distribution of ^{99m}Tc-Q3 in ischemic and nonischemic myocardium was repeatedly measured over 4 hr. The animals were anesthetized with morphine sulfate (3 mg/kg) subcutaneously and either pentobarbital 20 mg/kg (Group 1 dogs) or 1% alpha-chloralose 70 mg/kg (Group 2 dogs) intravenously. Anesthesia was supplemented with either agent as needed. The animals were intubated with a 10-mm internal diameter endotracheal tube, placed on a positive pressure ventilator (Harvard Apparatus Co., South Natick, MA) and ventilation was supplemented with 95% O_2 and 5% CO_2 to maintain an arterial blood pO_2 greater than 100 mmHg. The chest was opened with a left lateral thoracotomy through the fourth intercostal space and the heart was suspended in a pericardial cradle. For the nine Group 2 animals studied over 4 hr, an ultrasonic flow probe (Transonic Systems, Inc., Ithaca, NY) was placed on the proximal portion of the left circumflex coronary artery (Fig. 1). In these animals, a hydraulic occluder was placed distal to the flow probe and a 22-gauge, 2.5-cm plastic catheter was inserted into the distal portion of the left circumflex artery to monitor distal circumflex artery pressure.

In all animals, a 2.7-mm diameter plastic catheter was placed in the left atrium for recording pressure and injecting radiolabeled microspheres. A 2.3-mm diameter Goodale-Lubin catheter was inserted through a femoral artery and advanced into the ascending aorta for measurement of central aortic pressure and withdrawal of microsphere blood samples. A femoral vein was cannulated for administration of intravenous fluids and withdrawal of serial blood samples for measurement of ^{99m}Tc blood radioactivity levels. A separate femoral vein catheter advanced to the right atrium was used for injection of dipyridamole and ^{99m}Tc-Q3. A heated water circulation pad was placed under the dogs to assure normothermia during the studies.

Radiopharmaceutical

The structure of the ^{99m}Tc-Q3 cation is depicted in Figure 2. It was prepared as follows. N,N'-ethylenebis(acetylacetoneimine) (H_2acac_2en, 15–25 mg in 0.1–0.2 ml of ethanol) was combined with KOH (0.03 ml of 1 *M* solution in 50% ethanol) and $Na^{99m}TcO_4$ (1–2 ml saline containing 80–120 mCi) in a 5-ml sterile vial. The solution was deaerated with a vigorous stream of oxygen-free argon for 10–15 min. At the same time, a solution of $SnCl_2$ in ethanol (2–3 mg/ml) was prepared by first deaerating the ethanol, then adding the $SnCl_2$, and finally stoppering the vial

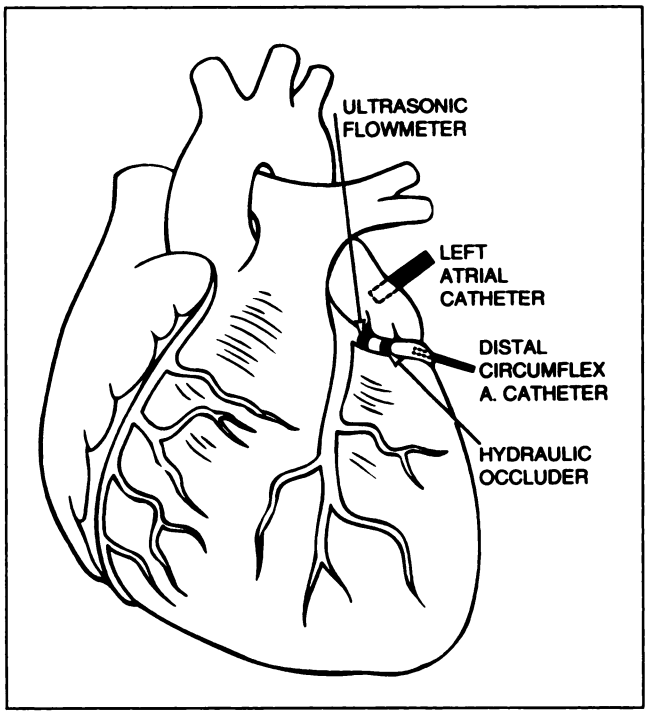

FIGURE 1. Instrumentation in Group 2 animals.

immediately to ensure anaerobic conditions. Under anaerobic conditions, 10–20 μl of the $SnCl_2$ solution were added to the reaction vial, and the solution was heated for 15 min at 70–90°C. The air-sensitive product formed, ($[^{99m}Tc(V)(acac_2en)O]^+$), and was assayed for purity by reversed-phase HPLC. This intermediate was then reduced to the cationic complex, ^{99m}Tc-Q3 ($[^{99m}Tc(III)(acac_2en)(TMPP)_2]^+$), by anaerobic addition of tris(3-methoxy-1-propyl)phosphine (TMPP) hydrochloride (0.1 ml of 50 mg/ml solution in ethanol) and heating at 80–90°C for 10 min.

Purification was necessary to remove excess ligands prior to animal injection. The crude ^{99m}Tc-Q3 preparation was diluted to 20 ml with water and loaded onto a preconditioned Waters C18 Sep-Pak Plus cartridge. The Sep-Pak was rinsed with 20 ml of water and then with 4 ml of 80% ethanol/20% water. The purified radiopharmaceutical was then eluted with 2 ml of 80% ethanol/20% saline, collecting the middle 1-ml fraction, filtered through a 0.2-μm filter and finally diluted with 4 ml of sterile saline.

Quality control was performed by reversed-phase HPLC on a

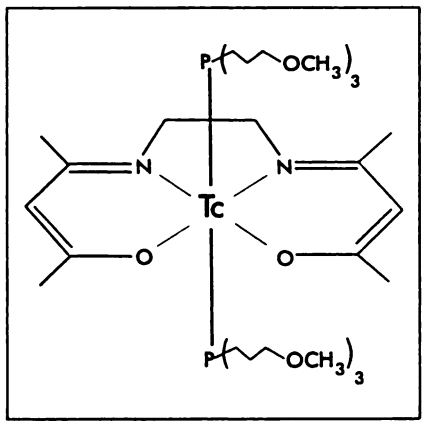

FIGURE 2. Structure of ^{99m}Tc-Q3.

Hamilton 150 mm × 4.1 mm PRP-1 column, using a mobile phase of 90% methanol/10% 0.01 *M* ammonium acetate at flow rate of 1.0 ml/min. The radioactivity was monitored by a Beckman Model 170 radioisotope detector. The chromatograms were recorded and integrated using a Shimadzu Chromatopac C-R3A integrator. A radiochemical purity above 85% was considered acceptable for dog injection. All ^{99m}Tc preparations of Q3 were tested for sterility using both trypticase soy broth and fluid thioglycollate medium; results of both tests (all negative) were available on a post-hoc basis.

For the 14 preparations of ^{99m}Tc-Q3 for 15 animals (2 animals were studied on the same day) the radiochemical purity of the final ^{99m}Tc-Q3 complex was 96.2% ± 3.7% (mean ± s.d.). The radiochemical yield (not corrected for decay) was 40.3% ± 8.5%. Technetium-99m-Q3 preparations were relatively stable. The decomposition rate determined for 10 batches at times from 2 to 24 hr after preparation averaged 2.3%/hr. The only decomposition product observed was ^{99m}Tc-pertechnetate.

Myocardial Perfusion Protocol

The relationship between ^{99m}Tc-Q3 myocardial activity and myocardial blood flow was examined in six dogs over a wide range of flows (Group 1). Blood flow was reduced in the distribution of the left circumflex artery by complete occlusion. Coronary flow through the left anterior descending artery was transiently increased by administration of intravenous (right atrial) dipyridamole.

Baseline pressure measurements were made following instrumentation with fluid-filled catheters. Left atrial, right atrial and systemic blood pressures, as well as the electrocardiogram (lead II) were recorded continuously on a polygraph (Model 7D, Grass Instruments Co., Quincy, MA). The left circumflex coronary artery was ligated and the animal was allowed to stabilize hemodynamically over 15 min. Evidence of myocardial ischemia (i.e., epicardial cyanosis or greater than or equal to 1 mm ST segment depression or elevation by ECG) was present in the anatomic distribution of the left circumflex coronary artery in each animal. Postocclusion hemodynamic measurements were recorded. Dipyridamole was then infused into the right atrium at a constant rate over 4 min with a total dose of 0.32 mg/kg in two dogs, 0.56 mg/kg in three dogs, and 0.84 mg/kg in one dog. Four minutes after completion of the infusion, ^{99m}Tc-Q3, (10 mCi) was injected intravenously (right atrium). Postdipyridamole hemodynamic measurements were made.

Regional myocardial blood flow was determined with radiolabeled microspheres by the method of Heymann et al. (*4*). Blood was withdrawn from the ascending aorta at a constant rate (Harvard Apparatus, South Natick, MA) into a heparinized syringe over 3 min. Blood withdrawal was initiated 15 sec before 1–3 million 15-μ spheres labeled with chromium-51, ruthenium-103, niobium-95 or cerium-141 (E.I. Dupont, New England Nuclear, North Billerica, MA) were rapidly injected into the left atrium. Arterial blood withdrawal continued for 3 min. The number of injected microspheres was estimated to yield 350 to 1000 microspheres per gram of normally perfused myocardium. Microspheres were injected 1 min following ^{99m}Tc-Q3 in each dog.

Heart rate, systemic arterial blood pressure, and cardiac output remained constant over the mean interval of 7 min following ^{99m}Tc-Q3 injection. Each animal was then euthanized with 100 mg/kg of intravenous pentobarbital and the heart was removed from the chest. From the six animals, a total of 357 myocardial samples were taken from the entire left ventricle including the interventricular septum. Approximately 90% of samples were transmural slices with the remaining 10% of samples taken selectively from the subendocardium, subepicardium, or midmyocardium of the left ventricle. The samples were weighed and placed in 10% formalin in counting vials. The myocardial samples weighed 1.39 ± 0.03 g (range 0.4–3.5 g). Within 4 hr after the animal was euthanized, each sample was counted for ^{99m}Tc in a gamma well counter with a multichannel analyzer (Model 1185, Tracor, Elk Grove, IL). The samples were stored for 1 wk to allow ^{99m}Tc activity to decay to background levels, and were then recounted for measurement of radiolabeled microsphere activity.

Myocardial Kinetics Protocol

Technetium-99m-Q3 activity was assessed over 4 hr for evidence of tracer redistribution. Nine dogs were instrumented and baseline hemodynamic measurements were made as described above. The left circumflex artery was occluded sufficiently so that reactive hyperemia was abolished in response to a proximal 10-sec complete occlusion and subsequent release of a ligature on the artery. All animals had a zone of visible epicardial cyanosis distal to the coronary occluder before and/or during subsequent dipyridamole infusion. The animals were allowed to stabilize hemodynamically over 15 min. Dipyridamole (0.56 mg/kg) was infused over 4 min. Four minutes after dipyridamole infusion, 10 mCi of ^{99m}Tc-Q3 was injected into the right atrium.

Blood ^{99m}Tc-Q3 disappearance was measured in four dogs by withdrawing 0.5 cc of blood into a heparinized 1-cc syringe every minute for 6 min, followed by every 2 min for 20 min, and then at 25, 30, 60, 90, 120, 180 and 240 min. Technetium-99m-Q3 blood disappearance curves were constructed by methods reported previously (*5*).

Following ^{99m}Tc-Q3 injection, serial gamma camera images obtained in the right anterior oblique projection on a portable gamma camera with a 10″ field of view and a high-resolution technetium collimator (Siemens LEM, Siemens Corporation, Iselin, NJ) were entered into a dedicated computer (A^3, Medasys, Inc., Ann Arbor, MI). The position of the animal remained constant throughout the entire experiment, as did the position of the gamma camera detector. Gamma camera images were obtained over 5 min at 5, 15, 30, 60, 120, 180 and 240 min after ^{99m}Tc-Q3 injection.

Regional myocardial blood flow was measured by radiolabeled microspheres at four timepoints following ^{99m}Tc-Q3 injection. Microsphere radionuclides (^{141}Ce, ^{51}Cr, ^{103}Ru, ^{95}Nb) were randomly assigned for administration at 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection.

In the last six animals studied, ^{99m}Tc endocardial counts were sampled by direct Cope needle biopsy of the beating heart in the distributions of the patent left anterior descending and partially occluded left circumflex arteries at 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection. The biopsy area was selected between the major coronary artery and a principal branch. The locations of myocardial biopsies in the circumflex artery distribution were selected based on the presence of visible epicardial cyanosis. A purse-string suture was placed around the biopsy site to assure hemostasis. Endocardial tissue samples weighed 0.021 ± 0.001 g (range 0.011–0.032 g, Model 2403 analytical balance, Brinkman Instruments, Westbury, NY) and were placed in a labeled counting vial containing buffered formalin. Tissue samples weighing less than 10 mg were considered inadequate for accurate assessment of ^{99m}Tc-Q3 activity per gram of tissue (only samples ≥ 10 mg were used).

Left and right atrial blood pressures, left circumflex coronary artery blood flow, distal circumflex artery blood pressure (in 7 of 9 dogs), systemic blood pressure, and cardiac rhythm were monitored constantly over 4 hr in all animals. Hemodynamic changes, other than those attributable to dipyridamole infusion, were corrected with fluids or adjustment of the level of anesthesia.

On completion of the 4-hr protocol, all animals were euthanized with 100 mg/kg of intravenous pentobarbital. The heart was excised from the chest and each coronary artery perfused for 10 min at 100 mmHg pressure with a different color dye (acid magenta, acid orange or methyl green certified biological stains, Sigma Chemicals, St. Louis, MO) for visualization of respective perfusion zones. The heart was then opened. Duplicate transmural myocardial tissue samples were taken from central areas in the distribution of the left anterior descending and left circumflex coronary arteries as guided by dye coloration. Additional adjacent slices were divided into subendocardial, midmyocardial and subepicardial samples. Tissue samples weighed 1.45 ± 0.08 g. Samples were placed in appropriately labeled counting vials containing 10% formalin. Direct in vivo biopsy (Cope needle samples) and postmortem tissue samples were counted in a gamma well counter (Model 1185, Tracor, Elk Grove, IL) for ^{99m}Tc activity within 4 hr following conclusion of the experiment. One week later, when ^{99m}Tc activity was fully decayed to background levels, the samples were recounted for microsphere radioactivity. A computer program corrected for spillover of radioactive counts into the counting window of other microspheres. In a separate validation study carried out in our laboratory, myocardial ^{99m}Tc activity was serially counted in a dose calibrator before and after dye coloration in two dog hearts. The net change in myocardial ^{99m}Tc activity in response to intracoronary dye infusion was ≤1% of total myocardial ^{99m}Tc activity.

Myocardial blood flow (MBF) measurements in ml/min/g were calculated from the ratio of myocardial microsphere counts per gram of myocardium (M_c) times the rate of arterial blood withdrawal into a syringe (SBF) in ml/min divided by total microsphere counts in the reference blood sample (B_c):

$$MBF = \frac{M_c \times SBF}{B_c}.$$

Gamma camera images were viewed on the nuclear medicine computer screen and analyzed by a single observer (Fig. 3). On the 30-min image from the kinetic protocol, a 5 × 5 pixel defect region of interest (DROI) was located centrally in the perfusion defect. A second 5 × 5 pixel region of interest (NROI) was located centrally in the normally perfused anterior wall. A 5 × 5 pixel background ROI was placed approximately 5 pixels external to the anterior wall overlying the lung. Background ROI activity was subtracted from both myocardial regions, and the ratio of corrected DROI counts divided by corrected NROI zone counts was calculated. The procedure was repeated on the 5-, 15-, 60-, 120-, 180- and 240-min images for each animal studied by the kinetics protocol.

Data Analysis

Data are expressed as the mean ± one standard error. All hemodynamic data were assessed for changes over time by a two-way repeated measures analysis of variance, followed by the Scheffe F-test. In order to combine data from all Group 1 (myocardial perfusion protocol) dogs, ^{99m}Tc counts/g were normalized to total counts/total weight of the entire left ventricular myocardium in each dog and microsphere myocardial blood flows were normalized to the mean myocardial blood in each dog (*6*). Normalized ^{99m}Tc counts were plotted against normalized microsphere myocardial blood flow for the group of six animals and linear regression analysis was performed on the group data.

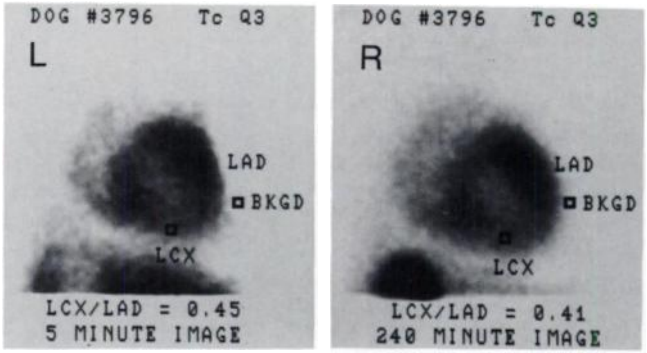

FIGURE 3. Right anterior oblique gamma camera images from a supine anesthetized dog, that were obtained 5 and 240 min after injection of 10 mCi of ^{99m}Tc-Q3. Regions of interest (5 × 5 pixels) are located over the posterior wall in the distribution of the stenotic left circumflex artery (left), over the anterior wall in the distribution of the unobstructed left anterior descending artery (center) and over a background region (right). Following background subtraction, the ratio of defect-to-nondefect zone total counts was 0.45 at 5 min after ^{99m}Tc-Q3 injection. The animal and camera were not moved and the same regions of interest were evaluated at 15, 30, 60, 120, 180 and 240 min after ^{99m}Tc-Q3 injection. The ratio of background-corrected defect-to-nondefect zone total counts was 0.41 at 240 min after ^{99m}Tc-Q3 injection.

For the Group 2 animals, myocardial blood flow and radionuclide image count ratios were assessed for changes over time and postmortem tissue ^{99m}Tc counts per gram were assessed by myocardial layer using a two-way repeated measures analysis of variance followed by a Scheffe F-test. Cope needle biopsy samples of adequate tissue weight (≥10 mg) were not available for all sample times on all animals. Samples of left anterior descending and left circumflex Cope biopsy ^{99m}Tc tissue counts were analyzed at each point in time by a two-tailed paired t-test. A p value less than 0.05 was considered to be statistically significant.

RESULTS

Relationship of Technetium-99m-Q3 Distribution to Myocardial Perfusion

With complete occlusion of the left circumflex artery, systemic blood pressures fell (from 133/97 ± 9/6 mmHg to 117/94 ± 8/7 mmHg) while heart rate and left atrial pressure rose (from 102 ± 9 beats/min to 119 ± 7 beats/min and from 9 ± 1 mmHg to 17 ± 3 mmHg) but none of the changes was statistically significant. Further hemodynamic changes with dipyridamole infusion were also not statistically significant.

For six Group 1 animals, normalized ^{99m}Tc-Q3 myocardial counts from 357 postmortem tissue samples are compared to normalized microsphere myocardial blood flow in Figure 4. Normalized ^{99m}Tc counts (y) and normalized microsphere myocardial blood flow (x) were related over a range of myocardial flows from 0 to 6.1 ml/min/g by the equation $y = 0.83X + 0.18$ ($r = 0.95$, $p < 0.0001$).

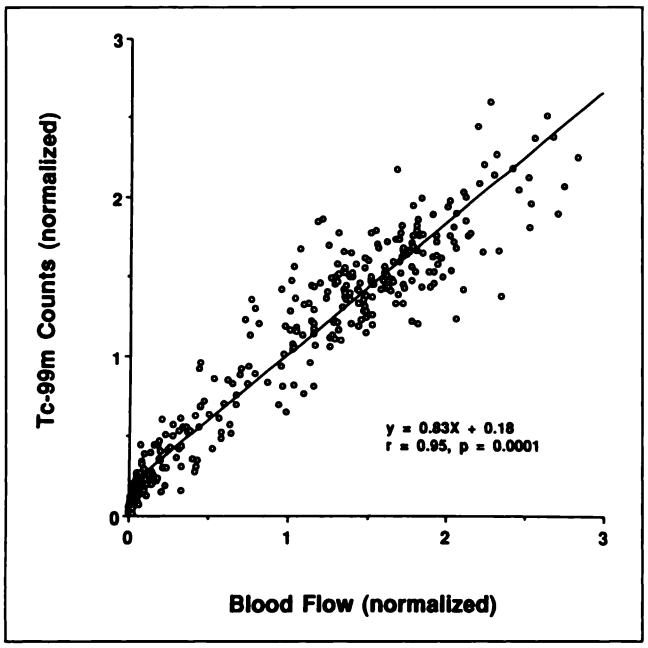

FIGURE 4. Plot of normalized myocardial blood flow determined from radiolabeled microspheres versus normalized ^{99m}Tc-Q3 counts per gram of myocardium. Data from six animals have been combined by normalizing ^{99m}Tc counts to the number of ^{99m}Tc counts per gram of myocardium in the entire left ventricular myocardium for each dog. The linear fit incorporates data from flows of 0 to 6.1 ml/min/g.

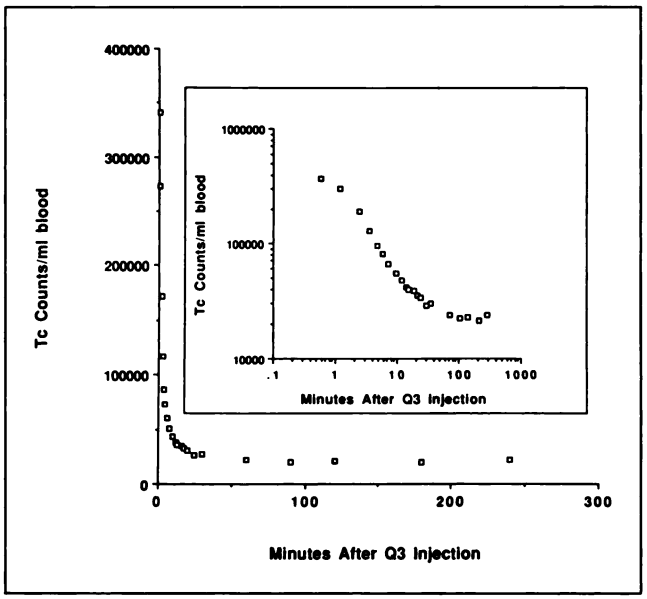

FIGURE 5. Representative ^{99m}Tc-Q3 blood clearance curve following intravenous injection in an anesthetized dog. Inset, plot of logarithmic transformation of the blood clearance curve.

Blood Clearance of Technetium-99m-Q3

A representative blood disappearance curve for ^{99m}Tc-Q3 over time is shown in Figure 5. Blood disappearance of ^{99m}Tc-Q3 in all four dogs was biexponential with an initial half-time of 1.7 ± 0.3 min and a late half-time of 195 ± 26 min. Calculated blood clearance of ^{99m}Tc-Q3 activity was 0.25 ± 0.07 ml/min/kg. The biexponential fit is characterized by the equation: $C = Ae^{-\alpha t} + Be^{-\beta t}$ where $A = 0.897 \pm 0.012$, $B = 0.102 \pm 0.012$, $a = 28.46 \pm 3.82\ s^{-1}$ and $\beta = 0.213 \pm 0.028\ s^{-1}$.

Relationship of Technetium-99m-Q3 Myocardial Kinetics to Myocardial Blood Flow Over Four Hours

Hemodynamics. Table 1 summarizes the hemodynamic data from the myocardial kinetics protocol. Distal left circumflex artery pressure and blood flow in the left circumflex coronary artery were reduced significantly by design with inflation of the circumflex artery occluder.

During dissipation of the dipyridamole effect, myocardial blood flow in the left anterior descending coronary artery distribution as measured serially by radiolabeled microspheres (Fig. 6) decreased significantly from 1.31 ± 0.18 ml/min/g at 30 min following ^{99m}Tc-Q3 injection to 0.62 ± 0.12 ml/min/g at 240 min after ^{99m}Tc-Q3 injection ($p < 0.05$). Myocardial blood flow in the distribution of the left circumflex coronary artery remained unchanged from 30 to 240 min after ^{99m}Tc-Q3 injection.

Scintigraphy. The transmural scintigraphic count ratios of defect-zone-to-nondefect-zone ^{99m}Tc ROI counts at 5, 15, 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection are shown by solid bars in Figure 7. The ratios (0.51 ± 0.05 at 5 min, 0.49 ± 0.05 at 15 min, 0.54 ± 0.05 at 30 min, 0.52 ± 0.05 at 60 min, 0.46 ± 0.04 at 120 min, 0.50 ± 0.04 at 180 min and 0.49 ± 0.04 at 240 min) did not change over 4 hr despite decreasing myocardial blood flow in the area of the left anterior descending coronary artery.

Direct Myocardial Tissue Counting. Technetium-99m counts from left ventricular subendocardial biopsies were obtained by serial Cope needle biopsies in six animals (Fig. 8). Over 4 hr following ^{99m}Tc-Q3 injection counts per gram in the left anterior descending coronary artery distribution were 143576 ± 22639 at 30 min, 161123 ± 22050 at 60 min, 170630 ± 35880 at 120 min and 149951 ± 31068 at 240 min (no significant change over time). In the left circumflex coronary artery distribution, subendocardial ^{99m}Tc-Q3 counts per gram by Cope needle biopsy were 54953 ± 14048 at 30 min, 56364 ± 10963 at 60 min, 75425 ± 22128 at 120 min and 75679 ± 22844 at 240 min (no significant change over time).

The relative distribution by the myocardial layer of ^{99m}Tc-Q3 4 hr after its administration was evaluated in the subendocardium, midmyocardium and subepicardium by dividing postmortem transmural sections from the left anterior descending and circumflex artery territories each into three slices. The ratio of subendocardial left circumflex counts per gram of myocardium-to-subendocardial left anterior descending counts per gram from myocardial slices obtained 240 min following ^{99m}Tc-Q3 injection was 0.31 which is similar to the corresponding subendocardial ratio of 0.38 present at 30 min after tracer injection as acquired from Cope needle biopsies of the beating heart. The distribution of ^{99m}Tc-Q3 in the left anterior descending

TABLE 1
Myocardial Kinetics Protocol: Hemodynamics*

	Baseline	Postocclusion	Postdipyridamole	30 min	60 min	120 min	240 min
Heart rate (bpm)	109 ± 12	131 ± 7	132 ± 10	130 ± 6	126 ± 6	111 ± 5	119 ± 5
Systolic blood pressure (mmHg)	129 ± 5	116 ± 4	95 ± 8†	92 ± 7†	105 ± 7	119 ± 6‡§	116 ± 6‡§
Diastolic blood pressure (mmHg)	88 ± 6	84 ± 4	58 ± 5†	64 ± 6†	79 ± 6‡	89 ± 4‡§	81 ± 6‡
Right atrial pressure (mmHg)	10 ± 1	9 ± 1	8 ± 1	9 ± 1	8 ± 1	9 ± 2	8 ± 2
Left atrial pressure (mmHg)	13 ± 1	16 ± 2	13 ± 1	13 ± 1	15 ± 1	16 ± 2	16 ± 2
Distal circumflex systolic pressure (mmHg)	118 ± 10¶	57 ± 7	46 ± 5	46 ± 5	56 ± 9	69 ± 10‡§	59 ± 7
Circumflex coronary blood flow (ml/min)	21 ± 3¶	7 ± 2	4 ± 0.3	9 ± 3	9 ± 3	8 ± 2	7 ± 3

*n = 9 dogs.
†$p < 0.05$ vs. postocclusion.
‡$p < 0.05$ vs. postdipyridamole infusion.
§$p < 0.05$ vs. 30′ postinjection.
¶$p < 0.05$ vs. all postocclusion measurements.
All other comparisons p = NS (preocclusion baseline is compared to postocclusion but not to later times).

region was uniform across the myocardial wall (Fig. 9, top). Technetium-99m-Q3 activity in the distribution of the stenotic left circumflex artery was reduced in all myocardial layers relative to the anterior descending territory ($p < 0.05$), with a significantly greater reduction in circumflex subendocardial compared to subepicardial activity ($p < 0.05$). Corresponding myocardial blood flow measurements in the left anterior descending and left circumflex artery distributions are shown at the bottom of Figure 9.

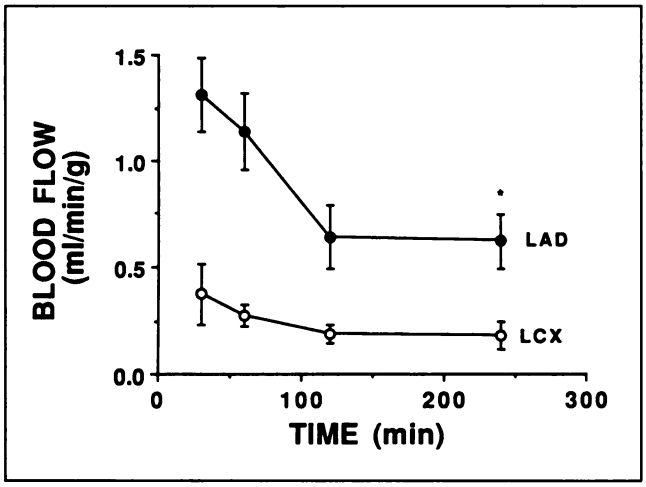

FIGURE 6. Plot of transmural myocardial blood flow measured by radiolabeled microspheres in nine dogs of Group 2 from 30 to 240 min following injection of ^{99m}Tc-Q3. Myocardial blood flow in the left anterior descending territory decreased significantly from 30 to 240 min following injection of ^{99m}Tc-Q3 as dipyridamole-induced vasodilation dissipated. Myocardial flow in the distribution of the left circumflex coronary artery flow where the hydraulic stenosis was applied remained unchanged from 30 to 240 min after ^{99m}Tc-Q3 injection.

DISCUSSION

Technetium-99m-Q3 has many positive attributes as a myocardial-imaging agent in man. Rossetti et al. (*1*) carried out the first clinical study of ^{99m}Tc-Q3 in six healthy volunteers and in eight patients with coronary artery disease documented by angiography. Effective visualization of infarcted and ischemic myocardium was reported, along with rapid clearance through the hepatobiliary system. These results have been confirmed and extended in a report by

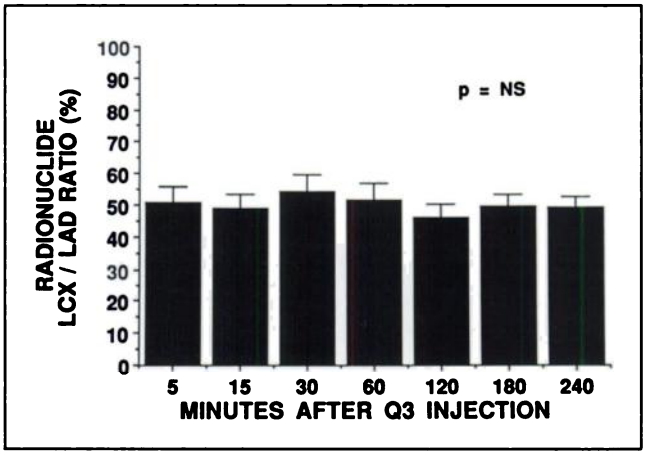

FIGURE 7. Scintigraphic transmural defect zone ^{99m}Tc-Q3 activity as a percentage of nondefect zone activity from 5 to 240 min following tracer injection in nine dogs. The ratio was unchanged over time, consistent with absence of tracer redistribution.

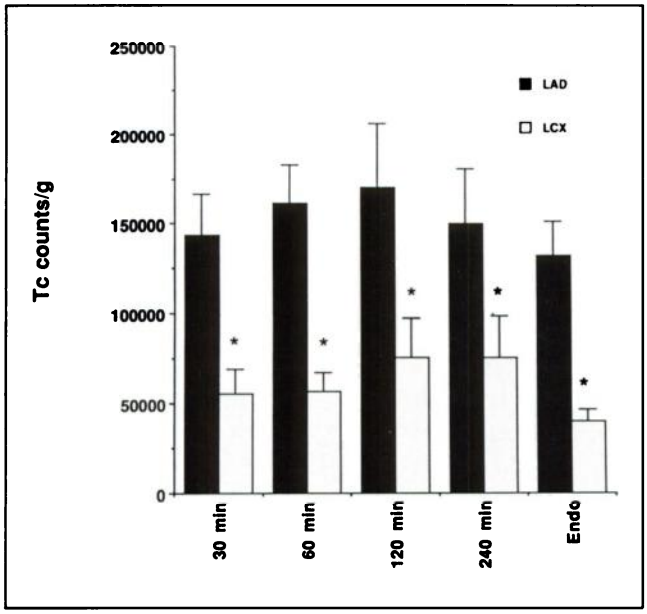

FIGURE 8. Technetium-99m counts per gram of myocardium from Cope needle biopsies taken from the left anterior descending (LAD) and left circumflex (LCX) coronary artery distributions at 30, 60, 120 and 240 min following ^{99m}Tc-Q3 injection. Also shown are the subendocardial (Endo) ^{99m}Tc-Q3 counts/gram acquired from tissue slices following euthanasia at 240 min. Technetium-99m counts per gram were significantly lower in the left circumflex artery distribution compared to the left anterior descending distribution at each point in time (*$p < 0.05$). There was no change in ^{99m}Tc counts per gram over time in either coronary artery distribution for the six dogs studied with subendocardial biopsies.

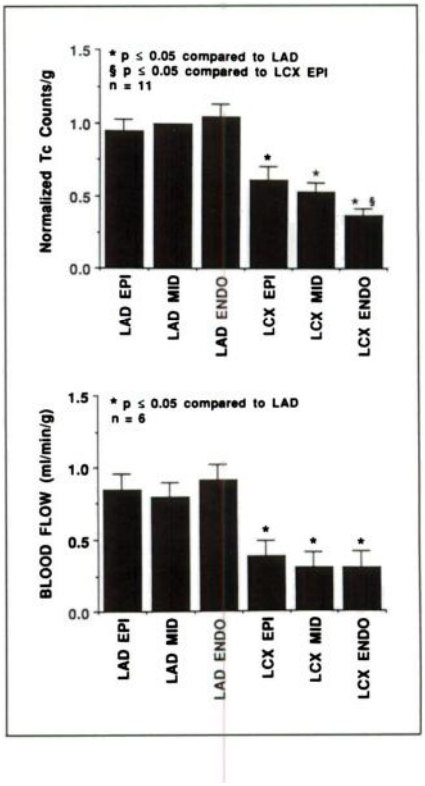

FIGURE 9. (Top) Technetium-99m-Q3 activity in subepicardial (EPI), midmyocardial (MID), and subendocardial (ENDO) segments obtained from post-mortem slices in nine Group 2 dogs. Subendocardial, midmyocardial and subepicardial tracer activities in the distribution of the stenotic left circumflex artery (LCX) were significantly reduced compared to tracer activities associated with the nonstenotic left anterior descending artery (LAD) ($p < 0.05$). Technetium-99m normalized counts per gram were significantly lower in the subendocardial compared to the subepicardial circumflex artery distribution. (Bottom) Myocardial blood flow values in the samples shown above.

Gerson et al. (*2*), who found comparable sensitivity and normalcy rates for detection of coronary artery disease with postexercise tomographic imaging of ^{99m}Tc-Q3 and ^{201}Tl in 19 patients with angiographic coronary disease and in eight normal study participants. Technetium-99m Q3 imaging uses to advantage the favorable physical properties of ^{99m}Tc by yielding higher counting statistics and resultant improved image quality compared to ^{201}Tl.

The relationship of ^{99m}Tc-Q3 myocardial uptake and subsequent kinetics to actual myocardial blood flow has not been previously evaluated and is the focus of the present report. In the present study, ^{99m}Tc-Q3 myocardial activity was related to radiolabeled microsphere measurements of myocardial blood flow over a range of flows from approximately 0 to 6.1 ml/min/g. Previous studies have examined the relationships of other myocardial perfusion tracers to myocardial blood flow. Thallium-201 myocardial uptake closely approximates microsphere assessments of myocardial blood flow under conditions of normal resting myocardial flow and under conditions of myocardial ischemia or infarction (*7–9*).

In response to treadmill exercise, myocardial blood flow and myocardial oxygen demand increase in a parallel fashion and a close relationship between myocardial thallium distribution and measurements of myocardial blood flow is maintained (*10*). When coronary blood flow increases out of proportion to myocardial oxygen requirements, as occurs with pharmacologic coronary artery vasodilation with dipyridamole infusion, ^{201}Tl myocardial uptake substantially underestimates high myocardial blood flows (*7,8,11*). Technetium-99m-teboroxime, a boronic acid adduct, shows myocardial uptake parallel to actual myocardial blood flow in a manner similar to ^{201}Tl for normal resting flows and for augmented flow following dipyridamole infusion (*12–14*).

Myocardial uptake of ^{99m}Tc-sestamibi, a cationic isonitrile, is related linearly to myocardial blood flow determined by radiolabeled microspheres in a flow range from 0.3 to 2.0 ml/min/g (*15,16*). Technetium-99m-sestamibi uptake overestimates myocardial blood flow for flows <0.3 ml/min/g (*15,17*), and substantially underestimates myocardial blood flow for flows >2.0 ml/min/g (*15,17,18*). Similarly, the diphosphine tracer, ^{99m}Tc-tetrofosmin, has been reported to have myocardial uptake in excess of myocardial blood flow at low flows and to underestimate myocardial blood flow at high flows induced by pharmacologic coronary vasodilation (*19*). Technetium-99m-Q12 or furifosmin, a mixed ligand cation with structure similar to ^{99m}Tc-Q3, shows myocardial uptake increased out of proportion to myocardial blood flow at low flows and a good relation of uptake to myocardial blood flow for flows up to 2.0 ml/min/g (*20*). Thus, myocardial distribution character-

istics of ^{99m}Tc-Q3 in dogs compare favorably to sestamibi, tetrofosmin and ^{99m}Tc-Q12.

Clearance of ^{99m}Tc-Q3 from the central circulation is rapid with an initial phase $T_{1/2}$ of 1.7 ± 0.3 min. This is similar to other myocardial perfusion imaging agents in current use. This property facilitates early myocardial imaging following tracer injection.

The present study also demonstrates that once ^{99m}Tc-Q3 is taken up into the left ventricular myocardium, the relative myocardial distribution of tracer remains constant over time. This observation was confirmed by external myocardial imaging and by direct counting of myocardial samples in a well counter. In the ischemic distribution of the left circumflex artery and in the transiently hyperemic left anterior descending arterial distribution, myocardial ^{99m}Tc-Q3 activity remained constant over 4 hr. The relative distribution of tracer activity in the left ventricular myocardium was also confirmed at the end of the experiments from larger left ventricular slices counted for ^{99m}Tc activity in a well counter. No differential washout of ^{99m}Tc-Q3 from ischemic compared to nonischemic myocardial zones or other evidence of tracer redistribution was detected over 4 hr of study. The lack of myocardial redistribution over 4 hr observed with ^{99m}Tc-Q3 is similar to the kinetic properties previously reported for ^{99m}Tc-sestamibi (*15,21*) and for ^{99m}Tc-Q12 (furifosmin) (*20*). The kinetic properties of ^{99m}Tc-Q3 differ from those of ^{201}Tl (*22–24*) which shows clinically important myocardial redistribution and ^{99m}Tc-teboroxime (*25–27*) which shows evidence of myocardial washout.

Possible limitations of this study include the blind nature of the serial endocardial biopsies used to measure ^{99m}Tc-Q3 activity over 4 hr. The endocardium is not visualized during the Cope needle biopsies of the beating heart and, therefore, the possibility exists that all putative endocardial muscle samples may not be accurately located in the central distribution of either the left anterior descending or left circumflex coronary artery but could be located in an area with overlapping flow from both arteries. Other investigators have implanted miniature cadmium telluride radiation detectors on the endocardial and epicardial walls for counting regional myocardial tracer activity (*15,21,22*). This approach assures a relatively constant location of serial radioactivity detection but samples a transmural section of tissue that may have a heterogenous flow and ^{99m}Tc distribution by myocardial layer (as illustrated in Fig. 9). In the present study, location of the Cope needle endocardial biopsy sites was verified at postmortem examination of the heart. Additionally, in this study, the lack of change in ^{99m}Tc-Q3 activity over time was verified independently by external gamma camera quantitation of a ratio of defect zone-to-nondefect zone activity in all animals.

A second potential technical limitation of this study relates to the level of augmentation of left anterior descending coronary blood flow at the time of the first endocardial biopsies 30 min following dipyridamole administration. Hintze and Vatner (*28*) showed that coronary artery cross-sectional area increased and diastolic coronary vascular resistance decreased significantly within 5 min following dipyridamole infusion, but had largely returned to baseline levels by 30 min. Afonso (*29*) produced marked increases in coronary blood flow by administering 5 mg of dipyridamole into the right atrium in mongrel dogs. By 15–20 min later, coronary blood flow had returned nearly to baseline levels. In the present study, in the Group 1 animals, microsphere measurements of coronary blood flow at 5 min following dipyridamole infusion demonstrated coronary blood flows up to 6.1 ml/min/g (6–9 times normal basal flow). In the nine Group 2 animals studied by the 4-hr myocardial kinetics protocol, maximal myocardial blood flow by microsphere techniques at 30 min following dipyridamole infusion was 2.03 ml/min/g (group mean 1.31 ± 0.18 ml/min/g). If earlier samples of ^{99m}Tc-Q3 tissue activity and corresponding microsphere blood flow measurements had been taken at 10–15 min following dipyridamole infusion, a larger augmentation and subsequent decline in blood flow over 4 hr might have been recorded. This was not, however, part of the original study design, at which time it was not known how much time following tracer injection was required to permit complete myocardial extraction and blood pool clearance of ^{99m}Tc-Q3. Although the measured increase in myocardial blood flow in the left anterior descending coronary artery distribution at 30 min following ^{99m}Tc-Q3 injection was limited in this open-chest anesthetized model, the change in myocardial blood flow from 30 min to 4 hr following tracer injection was statistically significant.

A third potential limitation of this and all other studies of myocardial tracer distribution versus myocardial blood flow is the unavailability of a universally accepted method for combining myocardial tracer versus blood flow data from multiple animals. In a preliminary report (*30*) of ^{99m}Tc-Q3 myocardial tracer distribution versus myocardial blood flow by microsphere injection, we normalized the data from each individual dog by assigning a value of 1 to the number of ^{99m}Tc-Q3 counts per gram of myocardium at a myocardial blood flow of 1 ml/min/g. Data were then combined from three dogs. With this approach to data normalization, the combined data from three animals suggested a relationship of tracer activity versus myocardial blood flow at flows above 2.0 ml/min/g. Subsequently, an additional three animals were studied and the data from all six animals were reanalyzed by the more widely reported methods of Bassingthwaighte et al. (*6*). This method normalizes the data from each animal by dividing the number of ^{99m}Tc counts per gram of myocardial sample by the number of ^{99m}Tc counts per gram in the entire ventricular myocardium and also normalizes each measurement of myocardial blood flow for an animal to the mean myocardial blood flow for that animal (the method used in the present report).

Finally, we cannot exclude the possibility that myocardial washout of ^{99m}Tc-Q3 may have occurred during the first 5 min following tracer injection. Very early washout of

tracer has been observed with the neutral tracer ^{99m}Tc-teboroxime. Examination of very early tracer myocardial kinetics following injection is potentially confounded by scattered counts from adjacent blood pool activity and by overlap of myocardium and blood pool regions. Very early substantial myocardial tracer washout has not been documented with other cationic ^{99m}Tc myocardial imaging agents and is not suspected to be a major component of ^{99m}Tc-Q3 kinetics.

Caution is required in attempting to extrapolate from the results of these canine studies to the likely perfusion and kinetic properties of ^{99m}Tc-Q3 in man. Nevertheless, Rossetti and associates (*31*) have reported that ^{99m}Tc-Q3 showed no evidence of myocardial washout over 5 hr in six normal volunteers. Technetium-99m-Q3 appears to hold substantial promise as a clinical myocardial perfusion imaging agent. Preliminary reports of rapid hepatic clearance of ^{99m}Tc-Q3 in humans (*31*), with a high myocardial-to-liver activity ratio, suggest a potential advantage in terms of convenience for clinical imaging of ^{99m}Tc-Q3.

We conclude that ^{99m}Tc-Q3 activity in the myocardium is related to actual myocardial blood flow over a clinically relevant range of flows, the tracer is rapidly cleared from the blood, and once extracted, ^{99m}Tc-Q3 remains relatively fixed in the myocardium for at least 4 hr. The correlates of these findings in humans require further clinical study.

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