# Myocardial Kinetics of TcN-NOET: A Neutral Lipophilic Complex Tracer of Regional Myocardial Blood Flow

Catherine Ghezzi, Daniel Fagret, Charles C. Arvieux, Jean-Paul Mathieu, René Bontron, Roberto Pasqualini, Joel de Leiris and Michel Comet

*Equipe de Physiopathologie Cellulaire Cardiaque et Laboratoire d'Etude des Radiopharmaceutiques, Université Joseph Fourier, Grenoble, France; and CIS Bio International, Gif sur Yvette, France*

[Bis (N-etoxy, N ethyl dithiocarbamato) nitrido] $^{99m}$Tc (V) (TcN-NOET) is a new neutral lipophilic myocardial imaging agent proposed for clinical use for detecting coronary artery disease. We studied the relation between myocardial retention of TcN-NOET and myocardial blood flow (MBF) in a canine model. **Methods:** A wide range of MBF was induced by partial regional coronary occlusion and dipyridamole infusion (protocols 1, 2 and 3). Myocardial activity of TcN-NOET was determined by in vitro tissue counting at 15 or 90 min postinjection. Tracer activity was correlated with radiolabeled microspheres using linear regression analysis. **Results:** There was a linear correlation between myocardial TcN-NOET activity and microspheres in protocol 1 (r = 094, 15 min postinjection, protocol 2 (r = 0.94, 15 min postinjection after dipyridamole) and protocol 3 (r = 0.91, 90 min postinjection after dipyridamole). When arterial occlusion was discontinued (protocol 4), there was no longer a close linear correlation (r = 0.26). The first-pass myocardial extraction action of TcN-NOET was 75.5% ± 4% under basal conditions and 85% ± 2% under hyperemic conditions ($p < 0.01$). **Conclusion:** Up to 90 min after injection, the relationship between TcN-NOET myocardial retention and blood flow is excellent over a wide range of flows. After reflow, TcN-NOET redistributes almost completely within 90 min.

**Key Words:** technetium-99m(N)-NOET; myocardial retention; myocardial blood flow; coronary artery disease

**J Nucl Med 1995; 36:1069–1077**

The suboptimal physical characteristics of $^{201}$Tl (low energy photopeak and long physical half-life) have led to investigations of new radioactive tracers for imaging regional myocardial blood flow (MBF). Several complexes of $^{99m}$Tc have been proposed, and three of these have been assessed clinically (*1,2*): $^{99m}$Tc methoxyisobutyl isonitrile (sestamibi) (*3*), a cationic complex of the hexakis (isonitrile) technetium (I) class; teboroxime (*4*), a neutral boronic acid adduct of technetium dioxime (BATO) complex and tetrofosmin (*5*), a cationic $^{99m}$Tc phosphine compound.

A new neutral lipophilic myocardial imaging agent, bis (N-ethoxy, N-ethyl dithiocarbamato) nitrido $^{99m}$Tc (V) (TcN-NOET) (*6*), shows high myocardial uptake in various animal species (*7*) and has been proposed for clinical use in the detection of coronary artery disease (*8*). The purpose of the present study was to compare the myocardial distribution of TcN-NOET with regional MBF in dogs after permanent and temporary partial coronary occlusion, with and without injection of dipyridamole. Uptake was measured and compared at different times after injection of TcN-NOET to evaluate potential redistribution. Variations in cardiac, pulmonary and hepatic uptake in dogs with normal hearts were determined with a scintillation camera.

## METHODS

### Animal Preparation and Hemodynamic Analysis

Fifteen adult mongrel dogs (mean weight 16.6 ± 2.9 kg) were premedicated with ketamine (10 mg/kg intramuscularly); anesthesia was induced with thiopental (25 mg/kg intravenously) followed by a maintenance dose of 5 mg/kg/hr. After tracheal intubation, controlled ventilation at intermittent positive pressure was initiated at 12 cycles/min with oxygen-enriched air. Esophageal temperature was maintained at 37°–38°C by heating the operating table with an incorporated radiator. A venous catheter was inserted into the femoral vein for perfusion of fluids (NaCl 0.9%) at a flow rate of 4 ml/kg/hr. A size 9F rigid aortic catheter was placed in the left carotid artery and advanced to within 1 cm of the aortic valve to monitor systemic blood pressure. A second rigid catheter was advanced from the right femoral artery to the abdominal aorta to obtain blood samples for determination of arterial pH, $PCO_2$, $PO_2$ and microsphere reference samples. To measure cardiac output, a Swan-Ganz thermodilution catheter was inserted into the jugular vein and passed through the right atrium until its tip rested in the pulmonary artery. The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. A size 9F rigid catheter was placed in the left ventricle through the apex to monitor left ventricular pressure. A size 20G plastic catheter was inserted into the left atrium for the injection of radioactive microspheres. The left anterior descending coronary artery (LAD) was dissected free distally to the origin of the second diagonal branch, and a hydraulic balloon occluder was positioned but not inflated.

Received Jul. 19, 1994; revision accepted Feb. 9, 1995.
For correspondence or reprints contact: Daniel Fagret, MD, Laboratoire d'Etude des Radiopharmaceutiques, URA CNRS 1287, Faculté de Médecine de Grenoble, 38700 La Tronche, France.

A Doppler flow probe was positioned on the coronary artery above the occluder. Two pairs of piezoelectric crystals were inserted into the endocardium perpendicular to the main axis of the heart. One pair was implanted in the area vascularized by the LAD (apex) and the other in the area vascularized by the circumflex artery (base). Aortic and left ventricular pressures, electrocardiographic lead II, coronary flow and variations in the length of apical and basal subendocardial segments were monitored throughout the experiment. During the course of the study appropriate adjustments were made to maintain pH, $PO_2$ and $PCO_2$ within the following ranges: pH, 7.35–7.45; $PCO_2$, 4–5.3 kPa; and arterial $PO_2$ above 13.3 kPa.

### TcN-NOET Preparation

TcN-NOET was prepared through a two-step reaction (*6*). First, [$^{99m}$Tc]pertechnetate was reduced in acidic conditions by a water soluble phosphine in the presence of S-methyl, N methyl dithiocarbazate as the nitrido nitrogen donating agent. This reaction, carried out at 100°C, led to an intermediate bearing the $(Tc \equiv N)^{2+}$ core which, after neutralization, underwent a rapid exchange reaction with the dithiocarbamate ligand giving the neutral complex [bis(N-ethoxy, N-ethyl) dithiocarbamato] nitrido Tc(V). Solutions containing the reagents leading to the final dithiocarbamate complex were prepared as follows: the intermediate reagents were prepared by dissolving 300 mg of trisodium salt of tri(m-sulfophenyl) phosphine and 100 mg of S-methyl N-methyl dithiocarbazate in 100 ml of oxygen-free 0.1 N hydrochloric acid. The solution containing the dithiocarbamate ligand was prepared by dissolving 1 g of sodium salt of N-ethoxy, N-ethyl dithiocarbamate in 100 ml of water for injection, and the pH of the solution was adjusted to 10. We also prepared a 0.2 mole, pH 10 sodium phosphate solution in water for injection. Each solution was sterilized by membrane filtration and 1 ml was dispensed in sterile, pyrogen-free capped vials. The stability of these solutions exceeded 9 mo at 2°–8°C. For TcN-NOET synthesis, 0.5 ml to 3 ml of $^{99m}TcO_4$–eluate (37 MBq to 370 MBq) were added to the vial containing the phosphine and $N^3$–donating ligand. The vial was heated at 100°C for 20 min. After cooling, 1 ml of buffer solution was added followed by 1 ml of dithiocarbamate ligand. The exchange reaction took place almost instantaneously thus forming the final neutral Tc-nitrido dithiocarbamate complex. Due to the lipophilic character of this neutral complex, we observed significant adsorption of radioactivity on the vial walls, plastic syringes and catheters. To avoid any loss of radioactivity, 10 ml of sterile pyrogen-free Tween 80 was added to the final solutions (*9*). Before injection, each TcN-NOET sample was assayed to ensure the purity of the product. TLC was carried out in two systems using silica gel plates developed with $CH_2Cl_2$ and a mixture of EtOH, $CHCl_3$, toluene and 0.5 mole $CH_3$ COO $NH_4$ in the ratio of 6:3:3:1 vol/vol. In the first system, the Rf of TcN-NOET was 0.72 and those of $^{99m}TcO_4$– and $^{99m}$TcN–intermediate species were 0. In the second more polar system, the Rf of TcN-NOET was 0.95; that of $^{99m}TcO_4$– was 0.75; and that of $^{99m}$TcN–intermediate species was 0–0.1 with one spot (≅ 10% total radioactivity) at 0.9 (*6*).

### Comparison of Myocardial TcN-NOET Activity with Regional MBF

These studies were performed according to four different protocols (which are given in Fig. 1) with three dogs in each. Baseline steady-state hemodynamic measurements were recorded in all cases. In protocol 1, two million $^{95}$Nb-labeled microspheres (15 μm) were injected into the left atrium. Microsphere reference blood collection was begun 10 sec before microsphere injection and continued for 2 min postinjection. The same protocol was followed after each microsphere injection. The LAD was then partially occluded by inflation of the hydraulic balloon occluder until 50%–70% reduction in the coronary flow was obtained. The resulting ischemia caused left ventricular dysfunction characterized by systolic bulging and postsystolic contraction. Dysfunction was kept stable on ventricular pressure-length loops appearing on an oscilloscope with image persistence. After 45 min partial occlusion, two million $^{103}$Ru-labeled microspheres were injected into the left atrium. TcN-NOET (185 MBq) was then injected into the femoral vein and the dogs were killed 15 min later. In protocol 2, the LAD was partially occluded and ventricular dysfunction was stabilized for 45 min. The first set of radiolabeled microspheres were injected into the left atrium, and an intravenous infusion of dipyridamole (0. 12 mg/kg/min) was begun and continued over 4 min. Two minutes after discontinuing the dipyridamole infusion, TcN-NOET (185 MBq) was injected into the femoral vein. Simultaneously, a second set of microspheres was injected into the left atrium. The dogs were killed 15 min later. In protocol 3, the dogs underwent partial stenosis of the LAD with dipyridamole infusion as described above, but they were killed 90 min after injection of TcN-NOET and the microspheres were injected just before the dogs were killed. Protocol 4 was the same as 3, except that 10 min after dipyridamole injection, partial occlusion of LAD was interrupted (reflow). Sestamibi was injected instead of TcN-NOET in three dogs and protocol 4 was followed.

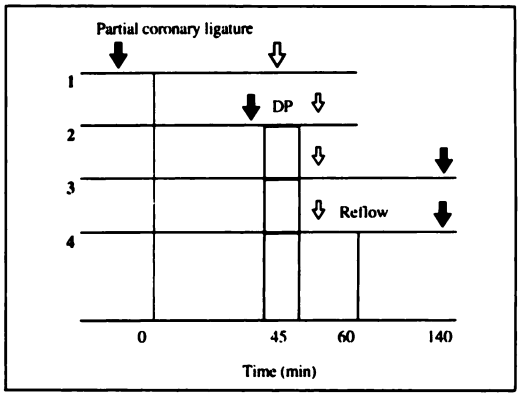


**FIGURE 1.** Diagram of the four different protocols. Open arrows depict simultaneous injection of TcN-NOET and microspheres and solid arrows depict injection of microspheres. DP = dipyridamole.

To monitor the time course of blood $^{99m}$Tc activity, serial 0.5 ml arterial blood samples were taken at 2, 4, 8, 15, 30, 60, 90, 120, 180 and 240 min after TcN-NOET injection (in 3 dogs), and at 2, 4, 8, 15, 30, 60 and 90 min after TcN-NOET (n = 3; protocol 4) and sestamibi (n = 3; protocol 4) injection.

After the dogs were killed, the hearts were removed and the left ventricle was sectioned into five 1-cm thick slices parallel to the base of the left ventricle. Each slice was cut into transmural pieces, which were divided into endocardial and epicardial segments of roughly 1 g each. The total number of myocardial samples for each dog was about 70. Within 5–6 hr of collection, serial blood samples and myocardial tissue samples were counted for $^{99m}$Tc activity in a gamma well-counter. The next day, the microsphere reference blood samples and the same myocardial tissue samples were again counted for $^{103}$Ru activity and $^{95}$Nb activity. The samples were counted for 5 min each using the three appropriate windows: $^{99m}$Tc, 120–180 keV; $^{103}$Ru, 450–550 keV; and $^{95}$Nb, 660–800 keV. A computer was used to perform spill-up and

spill-down corrections. MBF was calculated using the formula R(Cm/Cr) ml/min/g of tissue where R = reference blood flow pump withdrawal rate, Cm = counts per gram in the myocardial samples and Cr = counts in the reference blood sample. Nonischemic and ischemic zones were determined with microspheres injected simultaneously with TcN-NOET or sestamibi. Myocardial segments corresponding to the nonischemic zone (posterior wall) were chosen and the mean activity per gram and its s.d. for this zone were then calculated. Ischemic zones were determined by selecting, within the area irrigated by the LAD, myocardial segments exhibiting activity per gram which was 2.5 s.d. below the mean activity for the nonischemic zone. For each transmural segment, we calculated the flow rate ratio and $^{99m}$Tc tracer activity ratio in the endocardium and epicardium (endo/epi). The mean and s.d. of these ratios were calculated for ischemic and nonischemic zones. The flow rate for each ischemic segment was divided by the mean flow rate of the nonischemic zone. The mean and s.d. of each of these ratios were determined for all dogs in each protocol. The same calculation was performed for $^{99m}$Tc tracers.

To compare TcN-NOET and sestamibi distributions with that of the microspheres injected at the same time, activities per gram were given for each radioisotope as a percentage of the maximal activity determined in a 1-g sample from the nonischemic zones. Myocardial TcN-NOET or sestamibi uptakes, expressed as a percentage of maximal activity in the nonischemic zone, were then compared with microsphere determined blood flow and expressed as a percentage of maximal flow. Furthermore, in protocol 1, in order to compare TcN-NOET distribution with that of microspheres injected at the same time, regional MBF and TcN-NOET activities were expressed as a percentage of the mean value from samples of the nonischemic zone (number of samples for each dog, n = 15 ± 5). Myocardial segments were then divided into 20% flow range and the mean flow in each range, expressed as a percentage of mean flow in the nonischemic zone, was compared to the mean activities of TcN-NOET in the same flow range. Results could not be expressed in a similar way for the other protocols because basal TcN-NOET and microsphere activities would have to be measured in each dog prior to dipyridamole infusion.

### First Pass Extraction Fraction of TcN-NOET

Extraction fractures in nine dogs were determined from arterial (femoral artery)-venous (coronary sinus) $^{99m}$Tc tracer concentration differences relative to the nondiffusible $^{131}$I albumin tracer (*10*). Three catheters were inserted in anesthetized, intubated and ventilated dogs in the left atrium, the femoral artery, and the coronary sinus via the jugular vein. A solution of $^{131}$I albumin and TcN-NOET was quickly injected into the left atrium. Simultaneously, serial blood samples were taken from the coronary sinus and femoral artery by syringe. Sampling time was 4 sec, and a total of six samples were collected over 24 sec in each of the two catheters. Ejection fraction was calculated using the formula 1 − (AvBa/AaBv)] × 100%, where Aa and Ba are the test tracer and $^{131}$I albumin concentrations in the arterial sample and Av and Bv are the corresponding concentrations in the venous sample (*9*). Ejection fraction was measured in four dogs at basal flow and in five dogs at hyperemic flow (after dipyridamole injection) with determination of corresponding MBF with $^{95}$Nb or $^{103}$Ru microspheres.

### Gamma Camera Imaging Technique

After 24 hr of fasting, the dogs were anesthetized with intravenous injection of thiopentane, intubated, and ventilated with oxygen-enriched air. The dogs were injected intravenously with TcN-NOET under the gamma camera (injected dose 148 MBq). A general, all-purpose parallel-hole collimator was used with a 20% energy window encompassing the 140 keV $^{99m}$Tc photopeak. Thoracic images were acquired from the anterior projection. One-minute frames were obtained in a 64 × 64 pixel matrix. Images were recorded for 90 min in two dogs and 30 min in one dog. The regions of interest (ROIs) were manually selected on the heart, lungs and liver. Mean cardiac, pulmonary and hepatic activities per pixel were determined at 5, 15, 30, 45, 60 and 90 min postinjection. Activities were expressed as a percentage of the mean activity per pixel for the organ 5 min postinjection.

### Statistical Analysis

All results are expressed as the mean ± s.d. The significance of differences between means was assessed with Student's t-test. Significance was defined at the level of $p < 0.05$. Correlations between TcN-NOET (or sestamibi) activities and regional myocardial blood flow were determined the linear regression analysis. In protocol 4, to compare relative distribution of TcN-NOET and sestamibi with regard to relative blood flow in each group of dogs (i.e., to compare each group receiving TcN-NOET with the group receiving sestamibi), we performed an analysis of variance with repeated measures using absolute flow (ml/min/100 g) as a covariat. This correction of absolute flow was performed because the group of dogs receiving sestamibi tended to have higher absolute flows after pharmacological stress with dipyridamole than the group of dogs receiving TcN-NOET.

## RESULTS

### Hemodynamics

Partial coronary occlusion, dipyridamole injection and reflow did not significantly modify heart rate or cardiac output (Table 1). Ninety minutes after dipyridamole injection, left ventricular end-diastolic pressure (LVEDP) was significantly lower without reflow than with reflow (4.0 ± 1.6 versus 8.7 ± 3.0 mmHg, $p < 0.05$). Mean aortic pressure was significantly reduced (99.7 ± 20.8 versus 74.3 ± 14.1 mmHg, $p < 0.01$) immediately following dipyridamole injection, but returned to near predipyridamole levels by the end of the experiments in all groups.

### Coronary Blood Flow, TcN-NOET and Sestamibi Myocardial Distribution

*Protocol 1.* The presence of the hydraulic balloon occluder significantly reduced the flow rate in the LAD (108 ± 21 versus 97 ± 21 ml/min 100 g $p < 0.02$). After 45 min of partial occlusion of the LAD, the flow rate in nonischemic zones increased significantly relative to basal levels (126 ± 32 versus 108 ± 21 ml/min/100 g, $p < 0.001$), whereas that of ischemic zones was reduced by 74% (Table 2). The endocardial-to-epicardial blood-flow ratio was higher than 1 in the nonischemic zones (1.19 ± 0.30) and less than 1 in the LAD zone (0.79 ± 14) before partial occlusion. After occlusion the ratio in the ischemic zones dropped significantly (0.63 ± 0.29 versus 0.79 ± 0.14, $p < 0.05$) (Table 3). There was a significant linear correlation

**TABLE 1**
Hemodynamic Data

| | Before occlusion | 45 min occlusion | After DP | 90 min after DP without reflow | 90 min after DP with reflow |
|---|---|---|---|---|---|
| HR bpm | 127 ± 16<br>n = 16 | 132 ± 11<br>n = 16 | 128 ± 11<br>n = 13 | 137 ± 15<br>n = 3 | 141 ± 14<br>n = 6 |
| LVEDP mmHg | 6.8 ± 2.7<br>n = 16 | 8.6 ± 3.6<br>n = 16 | 7.8 ± 3.2<br>n = 13 | 4.0 ± 1.6<br>n = 3 | 8.7 ± 3<br>n = 6 |
| PAo mmHg | 103.8 ± 25.4<br>n = 14 | 99.7 ± 20.8<br>n = 14 | 74.3 ± 14.1<br>n = 11 | 86.4 ± 28.5<br>n = 3 | 89.2 ± 12.4<br>n = 5 |
| CO (l min–1) | 2.83 ± 0.91<br>n = 16 | 2.55 ± 0.66<br>n = 16 | 2.62 ± 0.63<br>n = 13 | 2.1 ± 0.81<br>n = 3 | 2.7 ± 0.99<br>n = 6 |

HR = heart rate; LVEDP = left ventricular end-diastolic pressure; PAo = mean aortic pressure; CO = cardiac output; DP = dipyridamole. All values are mean ± s.d.

between myocardial TcN-NOET activity and regional blood flow ($y = 1.02x + 10.61$, $r = 0.94$, $p < 0.001$) (Fig. 2). If results were expressed as a percentage of the mean activity in the nonischemic zone, it was noted that in segments showing less than 80% nonischemic blood flow, TcN-NOET activity overestimated blood flow. By contrast, TcN-NOET underestimated blood flow in segments showing more than 100% nonischemic blood flow (Table 4). The endocardial-to-epicardial ratio measured with TcN-NOET in nonischemic zones was significantly lower than that determined with microspheres (1.08 ± 0.18 versus, 1.25 ± 0.30, $p < 0.02$) (Table 3); however, there were no significant differences in the ratios measured with TcN-NOET or microspheres in ischemic zones. Hence, in ischemic zones, TcN-NOET clearly revealed a greater reduction of blood flow in the endocardium than in the epicardium 15 min postinjection. The ratio of TcN-NOET activities between ischemic and nonischemic zones was slightly, but significantly, higher than the coronary flow ratio (0.3 ± 0.15 versus 0.24 ± 0.11, $p < 0.05$) (Table 5).

**TABLE 2**
Regional Myocardial Blood Flow (ml/min/100 g)

| Protocol | | n | I | II | III | IV |
|---|---|---|---|---|---|---|
| 1 | N | 46 | | 126 ± 32 | | |
| | I | 40 | 97 ± 21 | 28 ± 14 | | |
| 2 | N | 60 | | 106 ± 25 | 322 ± 164 | |
| | I | 28 | | 35 ± 17 | 29 ± 27 | |
| 3 | N | 68 | | | 301 ± 95 | 94 ± 32 |
| | I | 40 | | | 41 ± 52 | 33 ± 29 |
| 4 | N | 56 | | | 176 ± 61 | 68 ± 19 |
| TcN-NOET | I | 32 | | | 16 ± 10 | 78 ± 26 |
| 4 | N | 60 | | | 249 ± 67 | 132 ± 55 |
| MIBI | I | 32 | | | 21 ± 13 | 133 ± 66 |

n = number of transmural segments; I = before partial coronary ligature; II = after 45 min partial coronary ligature; III = 2 min after dipyridamole injection, IV = 90 min after TcN-NOET (or MIBI) injection with or without reflow; N = nonischemic zone; I = ischemic zone.

*Protocol 2.* After dipyridamole injection, the flow rate was increased by about 300% in nonischemic zones, but there was no significant change in ischemic zones (Table 2); a significant decrease in the endocardial-to-epicardial blood flow ratio was observed in the nonischemic zones (0.95 ± 0.24 versus 1.21 ± 0.29, $p < 0.001$) with no significant change in the ischemic zones (Table 3); and finally, the ratio of flow rates for ischemic versus nonischemic zones dropped sharply (0.09 ± 0.06 versus 0.34 ± 0.18) (Table 5). There was a significant linear correlation between myocardial TcN-NOET activity and normalized regional blood flow ($y = 0.94x + 18.03$, $r = 0.94$, $p < 0.001$) (Fig. 2). Despite this excellent correlation, the ratio of TcN-NOET activities between ischemic and nonischemic zones after dipyridamole injection was much higher than the coronary flow ratio (0.24 ± 0.09 versus 0.06, $p < 0.001$) and did not differ significantly from that determined in protocol 1 without dipyridamole (0.24 ± 0.09 versus 0.30 ± 0.15, ns) (Table 5). This underestimation of the flow deficit may be explained by an overestimation of TcN-NOET activity in the low-flow range and by an underestimation of its activity in the high flow range, as observed in protocol 1. Because TcN-NOET activity was measured 15 min after its injection, these results may reflect a low, early redistribution of TcN-NOET.

*Protocol 3.* Ninety minutes after dipyridamole injection, the flow rate and the endocardial-to-epicardial blood flow ratio were normalized in nonischemic zones, whereas there was no change in the ischemic zones (Table 3). The ratio of flow rates for ischemic versus nonischemic zones was the same as that of protocols 1 and 2 prior to dipyridamole injection (0.28 ± 0.25 versus 0.24 ± 0.11, ns; 0.28 ± 0.25 versus 0.34 ± 0.18, ns) (Table 5). We noted a significant linear correlation between myocardial TcN-NOET activity and normalized regional blood flow ($y = 0.75x + 21.27$ $r = 0.91$, $p < 0.001$) with measurements 90 min subsequent to TcN-NOET and microsphere injection (Fig. 2); however, when these results were compared to those obtained with protocol 2, a reduction in the slope (from 0.94 to 0.75) was

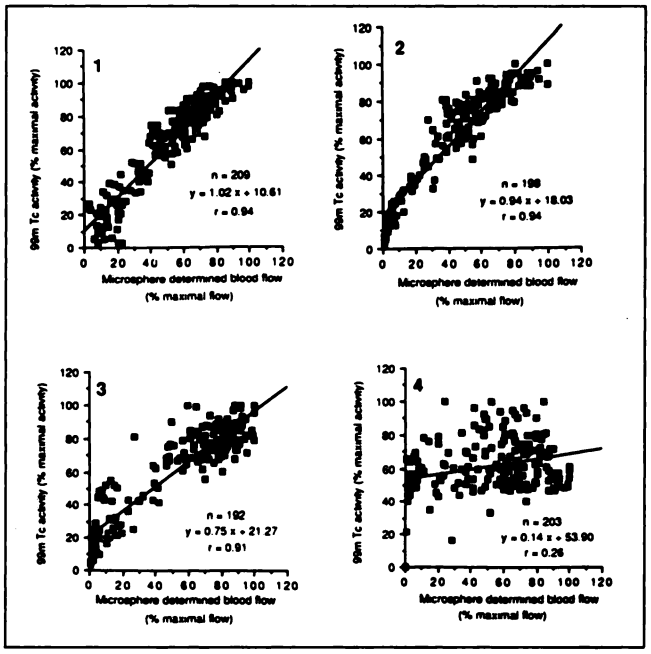


**FIGURE 2.** Plots of relation between TcN-NOET activity (% maximal activity) and microsphere determined blood flow (% maximal flow) in myocardial samples from dogs studied with protocols 1, 2, 3 and 4. Solid lines represent regression lines.

noted, as well a some increase in myocardial TcN-NOET activity relative to microsphere flow in the 0%–20% range of maximal flow, and some decrease of TcN-NOET relative to microsphere flow in the 60%–100% range of maximal flow. This would suggest little redistribution of TcN-NOET; however, there was no significant difference in the ratio of TcN-NOET activities between ischemic and nonischemic zones when measured 90 min postinjection compared with that determined 15 min postinjection in protocol 2 (0.22 ± 0.15 versus 0.24 ± 0.09, ns) (Table 5). We only noted a greater heterogeneity in the ischemic-to-nonischemic ratio 90 min after dipyridamole injection.

*Protocol 4 (TcN-NOET).* Dipyridamole caused a slight increase in the mean flow rate in nonischemic zones compared with the increases noted in protocols 2 and 3 (176 ± 61 versus 301 ± 95 ml/min/100 g, $p < 0.001$). The flow in the ischemic zones was also significantly lower than levels for the same zones in protocols 2 and 3 (16 ± 10 versus 41 ± 52 ml/min/100 g, $p < 0.02$). It is possible that reperfusion caused reperfusion injury with associated membrane injury and intracellular edema. With increasing wet weight, flow measured prior to reperfusion would be underestimated. After 80 min of reflow, the flow of the temporarily ischemic zones was significantly higher than that of the nonischemic zones (78 ± 26 versus 68 ± 19 ml/min/100 g, $p < 0.05$) (Table 2); there was a significant increase ($p < 0.001$) in the endocardial-to-epicardial blood flow ratio in the temporarily ischemic zones (Table 3) and the ratio of flow rate for ischemic versus nonischemic zones was 1.07 ± 0.27 (Table 5). When analysis was carried out after 80 min of reflow, the myocardial distribution of TcN-NOET was no longer closely correlated with normalized blood flow ($y = 0.14 x + 53.90$; $r = 0.26$; $p < 0.02$) (Fig. 2), and there was a significant increase in TcN-NOET activity versus relative microsphere flow at the time of injection. The TcN-NOET endocardial-to-epicardial distribution was homogeneous in the myocardium, and the ratio between TcN-NOET activities in ischemic and nonischemic zones was much higher than that measured without reflow (0.89 ± 0.15 versus 0.22 ± 0.15) indicating redistribution of TcN-NOET (Table 5).

*Protocol 4 (Sestamibi).* Dipyridamole caused a higher flow rate increase in the nonischemic zones than noted in protocol 4 (TcN-NOET) (249 ± 67 versus 176 ± 61 ml/min/100 g, $p < 0.001$), but levels in the ischemic zones were not significantly different. After 80 min of reflow, there were no significant differences in the flow rates of nonischemic zones and temporarily ischemic zones (Table 2). We noted a significant increase ($p < 0.001$) in the endocardial-to-epicardial blood flow ratio after 80 min of reflow in the

**TABLE 3**
Endocardial-to-Epicardial Ratios

| Protocol | | n | I | II | III | IV | $^{99m}$Tc |
|---|---|---|---|---|---|---|---|
| 1 | N | 23 | 1.19 ± 0.30 | 1.25 ± 0.30 | | | 1.08 ± 0,18 |
| | I | 20 | 0.79 ± 0.14 | 0.63 ± 0.29 | | | 0.59 ± 0.28 |
| 2 | N | 30 | | 1.21 ± 0.29 | 0.95 ± 0.24 | | 0.98 ± 0.09 |
| | I | 14 | | 0.53 ± 0.30 | 0.64 ± 0.49 | | 0.61 ± 0.26 |
| 3 | N | 34 | | | 0.96 ± 0.15 | 1.09 ± 0.27 | 0.96 ± 0.11 |
| | I | 20 | | | 0.44 ± 0.21 | 0.43 ± 0.25 | 0.45 ± 0.15 |
| 4 | N | 28 | | | 1.14 ± 0.22 | 1.11 ± 0.20 | 0.87 ± 0.11 |
| TcN-NOET | I | 16 | | | 0.40 ± 0.20 | 0.97 ± 0.28 | 0.86 ± 0.16 |
| 4 | N | 30 | | | 0.99 ± 0.19 | 1.08 ± 0.22 | 1.10 ± 0.09 |
| MIBI | I | 15 | | | 0.60 ± 0.34 | 1.08 ± 0.32 | 0.74 ± 0.09 |

n = number of endocardial-epicardial ratios; I = before partial coronary ligature; II = after 45 min of partial coronary ligature; III = 2 min after dipyridamole injection; IV = 80 min after TcN-NOET (or MIBI) injection with or without reflow; N = nonischemic zone; I = ischemic zone; $^{99m}$Tc = endo/epi ratios measured after TcN-NOET or MIBI injection.

**TABLE 4**
Relation between TcN-NOET Activity (Percent Nonischemic) and Microsphere Determined Blood Flow (Percent Nonischemic) in Myocardial Samples from Dogs Studied Using Protocol 1*

| Flow range | n | Normalized blood flow | Normalized TcN-NOET activity | p | Baseline flow (ml/min/100 g) | Stenosis flow |
|---|---|---|---|---|---|---|
| 0–20 | 17 | 13 ± 4 | 20 ± 11 | 0.04 | 89 ± 21 | 16 ± 7 |
| 20–40 | 26 | 29 ± 5 | 30 ± 14 | NS | 92 ± 25 | 36 ± 11 |
| 40–60 | 17 | 50 ± 6 | 57 ± 14 | 0.01 | 94 ± 24 | 58 ± 14 |
| 60–80 | 43 | 71 ± 6 | 77 ± 11 | 0.002 | 89 ± 17 | 81 ± 17 |
| 80–100 | 52 | 91 ± 5 | 90 ± 10 | NS | 102 ± 18 | 102 ± 23 |
| 100–120 | 36 | 109 ± 6 | 104 ± 10 | 0.001 | 116 ± 16 | 135 ± 29 |
| 120–140 | 15 | 126 ± 5 | 112 ± 5 | 0.001 | 133 ± 20 | 163 ± 31 |

*All values are the mean percent of nonischemic activity ± s.d.; n = number of samples in each flow range; p = normalized blood flow vs. corresponding normalized TcN-NOET activity.

temporarily ischemic zones (Table 3), and the ratio of flow rates for ischemic versus nonischemic zones was 0.96 ± 0.23 (Table 5). The distribution of sestamibi was linearly related to the normalized regional MBF distribution after 80 min of reflow ($y = 0.82x + 20$, $r = 0.89$, $p < 0.001$) (Fig. 3); however, the scatter plot demonstrated a slope of 0.82 and an increase in sestamibi myocardial activity relative to microsphere flow in the flow range of 0%–40% of maximum. This would suggest little redistribution of sestamibi. By contrast, in flows in the 80%–100% range, sestamibi activity did not differ from the flow value. The endocardial-to-epicardial ratio measured with sestamibi after 80 min of reflow did not significantly differ from the ratio determined with microspheres (Table 3), and the ratio between sestamibi uptake in ischemic and nonischemic zones was 0.41 ± 0.12 (Table 5).

*Protocol 4 (Comparison of the Activities of TcN-NOET and Sestamibi Relative to MBF).* We compared normalized TcN-NOET and, sestamibi activities between the two groups of dogs (the group receiving TcN-NOET and the group receiving sestamibi), using an analysis of variance with repeated measures. The sum of squares analysis demonstrated a significant overall group effect for both TcN-NOET and sestamibi ($p < 0.0001$); however, there was a significant difference in the flows between both groups ($p < 0.0001$), which could explain this effect. When flow was taken as a covariat, a significant difference persisted ($p < 0.0002$) between sestamibi activity and TcN-NOET activity, which allows one to conclude that these two tracers behave differently.

### Blood Clearance of TcN-NOET and Sestamibi

Serial blood samples taken over 4 hr in three dogs and over 90 min in three dogs (protocol 4) showed that after 30 min, TcN-NOET activity per milliliter was 20% of that measured 2 min postinjection. This level remained constant until 90 min postinjection (Fig. 4) (18.8% ± 8%). At 240 min, activity was still high (13.6% ± 8.4% the level at 2 min). In contrast, sestamibi blood activity decreased much faster. Sestamibi activity was found to be half that of TcN-NOET 30 min postinjection (10.2% ± 1.5% the level at 2 min) and negligible 90 min postinjection (4.5% ± 0.1% the level at 2 min) (n = 3, protocol 4). In one dog monitored for 60 min after injection of TcN-NOET, no metabolite was detected in the blood (using TLC) at 2 or 60 min.

When blood disappearance of TcN-NOET was analyzed with a biexponential model, an initial half-time of 4.67 ± 0.58 min and a late half-time of 674 ± 241 min were obtained (n = 3, protocol 4). With sestamibi, the blood disappearance was biexponential with an initial half-life of 1.66 ± 0.58 min and a late half-life of 55 ± 3 min (n = 3, protocol 4).

**TABLE 5**
Blood Flow Ratios between Ischemic and Nonischemic Zones

| Protocols | n | I | II | III | IV | $^{99m}$Tc |
|---|---|---|---|---|---|---|
| 1 | 40 | 0.92 ± 0.20 | 0.24 ± 0.11 | | | 0.30 ± 0.15 |
| 2 | 26 | | 0.34 ± 0.18 | 0.09 ± 0.06 | | 0.24 ± 0.09 |
| 3 | 28 | | | 0.06 ± 0.05 | 0.28 ± 0.25 | 0.22 ± 0.15 |
| 4 Tc-NOET | 33 | | | 0.09 ± 0.07 | 1.07 ± 0.27 | 0.89 ± 0.15 |
| 4 MIBI | 30 | | | 0.09 ± 0.06 | 0.96 ± 0.23 | 0.41 ± 0.12 |

n = number of ratios; I = before partial coronary ligature; II = after 45 min of partial coronary ligature; III = 2 min after dipyridamole injection; IV = 90 min after TcN-NOET (or MIBI) injection with or without reflow; $^{99m}$Tc = ratios determined after TcN-NOET or MIBI injection.

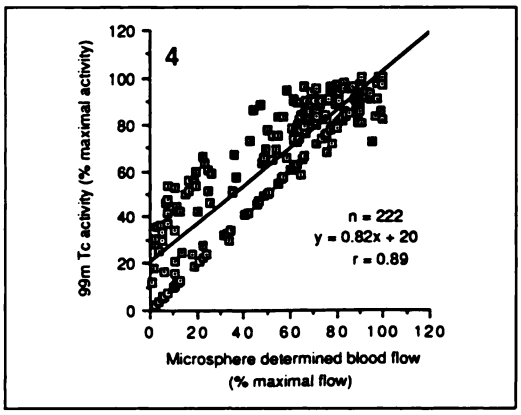


**FIGURE 3.** Plot of relation between sestamibi activity (% maximal activity) and microsphere determined blood flow (% maximal blood flow) in myocardial samples from dogs studied with protocol 4. Solid lines represent regression lines.

### First-Pass Extraction Fraction

The first-pass extraction fraction of TcN-NOET was 75.5 ± 4% under basal conditions (n = 4; mean blood flow 114 ± 16 ml/min/100 g) and 85% ± 2% under hyperemic conditions (n = 5, mean blood flow: 304 ± 129 ml/min/100 g). The difference was significant ($p < 0.01$).

### Imaging

At 60 min postinjection, cardiac uptake had decreased by 43.5% ± 2% relative to that measured at 5 min (Fig. 5). Pulmonary uptake decreased faster than cardiac uptake, and the mean heart-to-lung activity ratio per pixel increased over the time course (1.04 ± 0.13, 5 min postinjection; 1.84 ± 0.33, 60 min postinjection) (Fig. 5). Hepatic uptake remained constant. The heart was distinguishable on scans at 30 min postinjection, but it took 1 hr to obtain high quality images (Fig. 6).

## DISCUSSION

TcN-NOET is a new neutral lipophilic complex of $^{99m}$Tc. This imaging agent was found to have interesting biological characteristics in studies on myocardial cells of

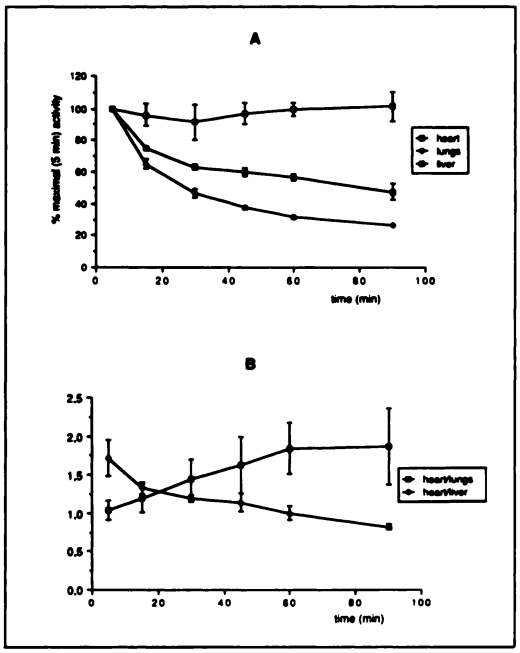


**FIGURE 5.** (A) Variations in mean (± s.d.) TcN-NOET activities per pixel in the heart, lungs and liver. Results are expressed as a percentage of the activity determined at 5 min postinjection. (B) Variations in mean (± s.d.) TcN-NOET activity ratios per pixel (heart-to-lungs and heart-to-liver).

newborn rats (*11*). Its peak cell concentration in percentage total activity is 73% ± 1%, much higher than that of sestamibi (8.9% ± 1.3%) and teboroxime (23% ± 2%), and the half-life of its cellular washout is more than 120 min, whereas that of sestamibi is 29 min and that of teboroxime is 13 min.

The first aim of the present study was to compare myocardial TcN-NOET activity with regional MBF. Partial stenosis of the LAD was established and the investigation was carried out with and without dipyridamole injection so that a wide range of coronary flows could be compared. Blood flow was between 29 ± 27 ml/min/100 g in ischemic zones and 322 ± 164 ml/min/100 g in normal zones. A

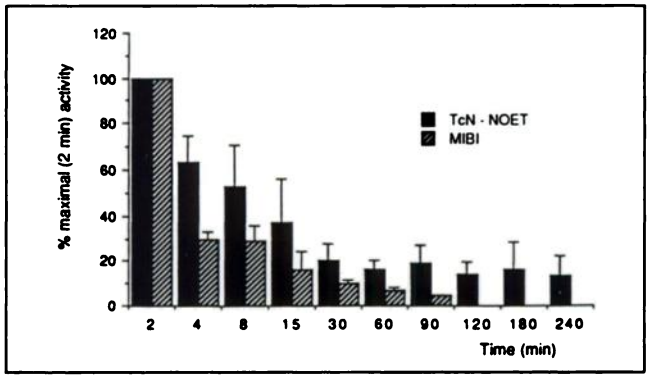


**FIGURE 4.** Variations in blood activity after i.v. injection of TcN-NOET (six dogs) and sestamibi (three dogs). Results (mean ± s.d.) are expressed as a percentage of the activity determined at 2 min postinjection.

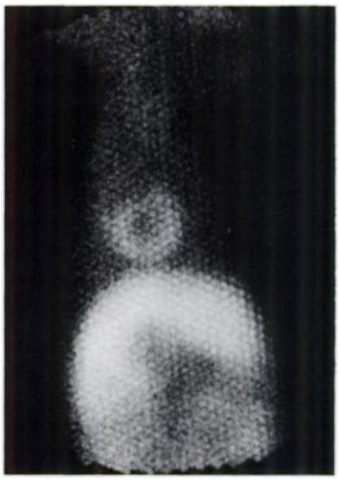

**FIGURE 6.** TcN-NOET myocardial scintigraphy (anterior projection).

highly significant linear correlation between TcN-NOET and normalized blood flow was noted when animals were killed 15 min after TcN-NOET injection. In ischemic zones, the higher blood flow reduction in the endocardium than in the epicardium was clearly traced by TcN-NOET; however, under basal conditions, the results showed that TcN-NOET tended to overestimate flow in the low-flow range and to underestimate flow in the high-flow range when activity was measured 15 min after its injection. With dipyridamole, TcN-NOET also appears to underestimate high flows and overestimate low flows because the ratio between ischemic and nonischemic zones was always higher with TcN-NOET than that determined with blood-flow data. Yet, extraction fraction was 75.5% ± 4% under basal conditions and 85% ± 2% under hyperemic conditions. This increase in extraction fraction under dipyridamole and the underestimation of flow deficit are contradictory, but can be explained by the fact that extraction fraction corresponds to the first pass of the tracer in the heart, whereas its regional myocardial distribution was measured 15 min after injection. This argument reinforces the case for early redistribution of TcN-NOET. The increase in extraction fraction is unlike that of many commonly used perfusion tracers that demonstrate decreased extraction fraction at higher flow because of shorter transit times through the intravascular space; however, a similar result was reported by Stewart et al. (*12*) who used a lipophilic complex, teboroxime. The extraction fraction of this complex was 88% ± 5% under basal conditions and 91% ± 3% under dipyridamole, which is higher on average than that of TcN-NOET. This extraction fraction, which is very high for both TcN-NOET and teboroxime, may be explained by the lipophilic properties of both tracers and, consequently, by a large permeability/surface area product for these tracers in canine myocardium.

The significant linear correlation between TcN-NOET activity and regional MBF persisted 90 min postinjection when partial coronary stenosis was maintained; however, we noted an increase in myocardial TcN-NOET activity relative to microsphere flow in the 0%–20% (of maximum) flow range, and a decrease in the 80%–100% flow range. This result seems to show that TcN-NOET continues to redistribute slowly between 15 and 90 min, despite a good correlation. In ischemic zones, TcN-NOET uptake again revealed the substantial blood flow difference between the endocardium and epicardium; however, when partial stenosis was discontinued 10 min after TcN-NOET injection, there was no longer a significant linear correlation between TcN-NOET activity and MBF at 90 min postinjection. At this point, the distribution of TcN-NOET was uniform, as shown by the activity ratios between temporarily ischemic zones and normal zones (which were close to 1) and by the similar endocardial-to-epicardial ratios for the same zones. The distribution of TcN-NOET activity thus traced blood flow when assessed 15 min postinjection, whereas reflow led to homogenization of its distribution, which was almost complete at 90 min. As reported for $^{201}$Tl (*13*), this redistribution could be a combined result of radioactivity clearance in nonischemic zones and increased radioactivity in temporarily ischemic zones. Increased radioactivity in temporarily ischemic zones could be due to persistently high levels of TcN-NOET in the blood. Indeed, its blood activity (as a percentage of its level at 2 min) was reduced by 80% within 30 min. Activity then remained high and steady; at 90 min postinjection it was 18.8% ± 8% the level at 2 min, and at 240 min it was still 13.6% ± 6.4%. After interruption of occlusion at 10 min postinjection, blood activity of TcN-NOET was 50% of its level recorded at 2 min.

TcN-NOET could thus be used as a blood-flow tracer if scanning is performed before substantial redistribution takes place. The time course of cardiac, hepatic and pulmonary uptake was determined in vivo with a scintillation camera. Relative to uptake at 5 min postinjection, cardiac uptake decreased by 43.5% ± 2% after 60 min. A time-dependent plateau of hepatic activity was noted. Pulmonary uptake decreased more rapidly than cardiac activity with the mean heart-to-lung activity ratio per pixel increasing over time. The ratio was 1.84 ± 0.33 at 60 min postinjection, and high quality myocardial images were obtained. In dogs, interpretable myocardial images can be obtained once the myocardial activity distribution no longer expresses coronary flow in case of reflow.

The results of this study do not indicate whether the distribution of TcN-NOET activity after redistribution could be a marker of viable myocardium since myocardial viability was not assessed. The potential use of neutral lipophilic tracers to evaluate cell viability should be investigated. Accumulation of teboroxime, a neutral lipophilic tracer, actually occurs in cells when the metabolism has been inhibited long enough to produce a nonreversible state (*14*). Iodoacetic acid (IAA) alone (*14*), ouabain alone or ouabain combined with cyanide and IAA (*15*) leads to a significant decrease in cellular uptake of teboroxime. The biological behavior cannot be fully explained by the neutral lipophilic character, because TcN-NOET and teboroxime show very different behaviors in isolated cells (*11*) and in dogs. In addition, myocardial clearance of teboroxime is much more rapid than that of TcN-NOET; two-thirds of the activity washes out within 3.6 min ± 0.6 min (4).

To overcome the problems of comparing results of experimental studies with different protocols, we investigated TcN-NOET and sestamibi with the same protocol. In contrast to TcN-NOET, the distribution of sestamibi matched that of coronary flow even when measured 90 min postinjection with reflow, however, we noted an increase in myocardial sestamibi activity relative to microsphere flow in the flow range 0%–40% of maximums, and the activity ratios between ischemic and nonischemic zones were higher than the ratios calculated with the corresponding coronary flows. These results are in agreement with those Li et al. (*16*) obtained in a model of occlusion-reperfusion, as well as those of Sinusas et al. (*17*), who used a model of occlusion without reflow. Their results demonstrate that

this overestimation of the activity corresponded to a redistribution of sestamibi. Our results could therefore be explained by such a redistribution, but we cannot demonstrate it because sestamibi was studied in one protocol only. This redistribution, however, seem to be far less important than that obtained with TcN-NOET under similar conditions.

## CONCLUSION

TcN-NOET is a good tracer of regional MBF over a wide range of coronary flow except in low-flow zones where the tracer overestimates blood flow, and in high-flow zones where the tracer underestimates blood flow. TcN-NOET showed a slight redistribution 90 min after injection, which was almost complete after 90 min of reflow. Further studies are required to explain this redistribution and to determine if TcN-NOET can be, according to these properties, a tracer of myocardial viability and, thus, a potential technetium compound analog of $^{201}$Tl.


## ACKNOWLEDGEMENTS

The authors thank to A. Fiore for developing the computer software program used to calculate coronary flow with a correction for spillover activity. They also thank C. Arnaud and C. Heyer for administrative assistance and Dupont-de-Nemours Laboratories for generously providing the sestamibi. This work was partly supported by a grant from Cis Biointernational, Oris Industrie SA, Gif sur Yvette, France.


## REFERENCES


1. Leppo JA, De Puey EG, Johnson LL. A review of cardiac imaging with sestamibi and teboroxime. *J Nucl Med* 1991;32:2012–2022.
2. Tamaki N, Takahashi N, Kawamoto M, et al. Myocardial tomography using technetium 99m-tetrofosmin to evaluate coronary artery disease. *J Nucl Med* 1994;35:594–600.
3. Mousa SA, Williams SJ, Sands H. Characterization of in vivo chemistry of cations in the heart. *J Nucl Med* 1987;28:1351–1357.
4. Narra RK, Nunn AD, Kuczynski BL, Feld T, Wedeking P, Eckelman WC. A neutral technetium-99m complex for myocardial imaging. *J Nucl Med* 1989;30:1830–1837.
5. Kelly JD, Forster AM, Higley B, et al. Technetium-99m- tetrofosmin as a new radiopharmaceutical for myocardial perfusion imaging. *J Nucl Med* 1993;34:222–227.
6. Pasqualini R, Duatti A. Synthesis and characterization of the new neutral myocardial imaging agent [$^{99m}$TcN(noet)$_2$](noet = *N*-Ethyl-*N*-ethoxydithiocarbamato). *J. Chem Soc Chem Commun* 1992;18:1354–1355.
7. Pasqualini R, Duatti A, Bellande E, et al. Bis(dithiocarbamato) nitrido technetium-99m radiopharmaceuticals: a class of neutral myocardial imaging agents. *J Nucl Med* 1994;35:334–341.
8. Fagret D, Marie PY Brunotte F, et al. Stress and delayed SPECT myocardial imaging with a new neutral technetium-99m nitrido dithiocarbamate complex (TcN-NOET): comparison with thallium-201 SPECT imaging and coronary angiography [Abstract]. *J Nucl Med* 1994;35:114P.
9. Varma RK, Kaushal R, Junnarkar AY, et al. Polysorbate 80: a pharmacological study. *Arzneim-Forsch/Drug Res* 1985;35:804–808.
10. Weich HF, Strauss HW, Pitt B. The extraction of thallium-201 by the myocardium. *Circulation* 1977;56:188–191.
11. Maublant J, Zhang Z, Ollier M, et al. Uptake and release of bis (*N*-ethoxy, *N*-Ethyldithiocarbamato) nitrido 99m-Tc (v) in cultured myocardial cells: comparison with Tl-201, MIBI and teboroxime [Abstract]. *J Nucl Med* 1992;19:597.
12. Stewart RE, Schwaiger M, Hutchins GD, et al. Myocardial clearance kinetics of technetium-99m. SQ 30217: a marker of regional myocardial blood flow. *J Nucl Med* 1990;31:1183–1190.
13. Beller GA, Watson DD, Ackell P, Pohost GN. Time course of thallium-201 redistribution after transient myocardial ischemia. *Circulation* 1980;61:791–797.
14. Kronauge JF, Chiu ML, Cone JS, et al. Comparison of neutral and cationic myocardial perfusion agents: characteristics of accumulation in cultured cardiac cells. *Nucl Med Biol* 1992;19:141–148.
15. Maublant JC, Moins N, Gachon P. Uptake and release of two new Tc-99m labeled myocardial blood-flow imaging agents in cultured cardiac cells. *Eur J Nucl Med* 1989;15:180–182.
16. Li QS, Solot G, Frank TL, Wagner HN, Becker LC. Myocardial redistribution of technetium 99m-methoxyisobutyl isonitrile (sestamibi). *J Nucl Med* 1990;31:1069–1076.
17. Sinusas AJ, Bergin JD, Edwards NC, et al. Redistribution of $^{99m}$Tc-sestamibi and $^{201}$Tl in the presence of a severe coronary artery stenosis. *Circulation* 1994;89:2332–2341.