Development of Nonreducible Technetium-99m(III) Cations as Myocardial Perfusion Imaging Agents: Initial Experience in Humans

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A series of 15 nonreducible technetium-99m(III) complexes of formula *tr*-[^{99m}TcL(Y)$_2$]$^+$ has been prepared by a general synthetic route based on reductive addition of Y to the technetium-99m (^{99m}Tc) intermediate [^{99m}TcL(O)]$^+$. In these complexes, selected for potential use as myocardial imaging agents, L represents one of the two tetradentate Schiff base ligands N,N′-ethylenebis(acetylacetone iminato), (en), or N,N′-propylene-1,2-bis(acetylacetone iminato), (pn), while Y represents a monodentate phosphine, phosphite or isonitrile ligand as exemplified by $P(CH_3)_3$, $P(OCH_3)_3$ and $CN\text{-}C(CH_3)_3$. Of these 15 complexes, several with octanol/saline partition coefficients in the range 0.04–20 exhibit significant myocardial uptake in rats and dogs. Of these, none exhibit detectable myocardial washout, providing strong support for the hypothesis that myocardial washout occurs only for those ^{99m}Tc(III) cations that undergo in vivo reduction to the neutral ^{99m}Tc(II) form. Evaluation of the prototypical complex *tr*-[^{99m}Tc(en)(P(CH$_3$)$_3$)$_2$]$^+$ in seven normal volunteers and patients establishes that it is only a mediocre myocardial imaging agent in man.

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The first technetium-99m (^{99m}Tc) cationic agent to be evaluated as a myocardial perfusion imaging agent in humans was the Tc(III) complex *tr*-[^{99m}Tc(DMPE)$_2$Cl$_2$]$^+$ where DMPE represents 1,2-bis(dimethylphosphino)ethane (*1,2*). This agent proved unsatisfactory because of intense hepatic uptake that interfered with clinical imaging of the cardiac apex. A subsequent study of the comparative biodistributions of *tr*-[^{99m}Tc(DMPE)$_2$Cl$_2$]$^+$ and *tr*-[^{186}Re(DMPE)$_2$Cl$_2$]$^+$ in animals demonstrated that at least one of the factors responsible for the unsatisfactory results obtained with *tr*-[^{99m}Tc(DMPE)$_2$Cl$_2$]$^+$ is that this agent suffers in vivo reduction to the neutral Tc(II) form *tr*-[^{99m}Tc(DMPE)$_2$Cl$_2$]^0 (*3*). This in vivo reduction leads to myocardial washout and enhanced liver uptake of the more lipophilic Tc(II) analog. Consistent with this analysis is the clinical observation that the Tc(I) complexes [Tc(DMPE)$_3$]$^+$ and [Tc(TBIN)$_6$]$^+$ (where TBIN represents *t*-butylisonitrile), which cannot undergo in vivo reduction (*4,5*), do not exhibit detectable myocardial washout even several hours after injection (*6,7*).

In order to obviate the detrimental effects of in vivo reduction, new classes of cationic technetium(III) complexes that would be electrochemically inert in the redox range accessible to biologic systems were sought for evaluation as potential myocardial perfusion imaging agents. Such a class of complexes had been previously prepared and characterized in our laboratory using macroscopic amounts of technetium-99m (*8,9*), but had not been prepared utilizing "no carrier added" technetium-99m. These complexes, designated *tr*-[TcLY$_2$]$^+$, are "mixed ligand" complexes containing both a tetradentate Schiff base ligand (L) and monodentate phosphine, phosphite or isonitrile ligands (Y). The properties of these complexes can be varied over wide ranges by altering the nature of the equatorial Schiff base ligand and by varying the nature of the monodentate ligand and its functional groups. The synthetic route to these complexes involves first converting TcO_4^- to the Tc(V) species [Tc^{V}L(O)]$^+$ and then subsequently converting this intermediate to the final Tc(III) product *tr*-[Tc^{III}L(Y)$_2$]$^+$, and thus a single L readily gives rise to a series of analogous *tr*-[Tc^{III}L(Y)$_2$]$^+$

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complexes. While a large number of tetradentate Schiff base ligands are known (*10,11*), this research has focused on the two shown in Figure 1 [N,N′-ethylenebis(acetylacetone imine) abbreviated (en), and N,N′-propylene-1,2-bis(acetylacetone imine) also called (pn)] since these ligands are readily available and are known to form *tr*-$[^{99m}TcL(Y)_2]^+$ complexes with lipophilicities appropriate for myocardial uptake (*9*). In this paper we describe the preparation of $[^{99m}Tc^{V}L(O)]^+$ and *tr*-$[^{99m}Tc^{III}L(Y)_2]^+$ complexes from "no carrier added" $^{99m}TcO_4^-$, the evaluation of several *tr*-$[^{99m}Tc^{III}L(Y)_2]$ cations as myocardial perfusion imaging agents in animals, and the results obtained with one of these agents, *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$, in human volunteers.

NOMENCLATURE AND ACRONYMS

In this paper the class of tetradentate Schiff base ligands shown in Figure 1 is designated by "L", while the two specific examples given in Figure 1 are designated (en) and (pn). The Tc(V) complexes, of proper formula $[TcL(O)]^+$, are designated TcL^+. The Tc(III) complexes, of proper formula *tr*-$[TcL(Y)_2]^+$, where Y is a monodentate ligand (*9*), are designated $[TcL\text{-}Y]^+$ to indicate the identities of L and Y. Thus, $[^{99m}Tc(en)\text{-}PMe_3]^+$ designates the *trans* octahedral complex in which the tetradentate Schiff base ligand (en) occupies four equatorial positions while two monodentate trimethylphosphine ligands occupy *trans* positions. Within the Y ligands, the following abbreviations and acronyms are used: Me = CH_3; MeOH = CH_2OH; Et = CH_2CH_3; Ph = C_6H_5; TMP = $P(OCH_3)_3$; TBIN = $CN\text{-}C(CH_3)_3$.

MATERIALS AND METHODS

General

Unless otherwise specified, all materials were of reagent grade. Solvents used in high performance liquid chromatography (HPLC) were specified as being of HPLC purity. The tetradentate Schiff base ligands (en) and (pn) were prepared by a general literature procedure (*8,9,12*) then purified by two recrystallizations from dichloromethane/heptane (*13*). The monodentate phosphine ligands* and t-butylisonitrile (TBIN)† were obtained commercially and used without further purification. Trimethylphosphite (TMP) was obtained commercially† and distilled before use. All the phosphine ligands were manipulated under strictly anaerobic conditions. The reducing power of phosphine stock solutions was determined using a previously described procedure (*14*).

High performance liquid chromatographic analyses were performed on either a C-8 reversed phase (250 mm × 4.2 mm) column‡ or a PRP-1 (150 mm × 4.1 mm) column§ using methanol/water mobile phases that contained 0.01*M* sodium heptanesulfonate in the aqueous fraction. The high performance liquid chromatography (HPLC) radiometric detection system was centered at the 140 keV emission of ^{99m}Tc. Detection of macroscopic amounts of ^{99}Tc complexes was accom-

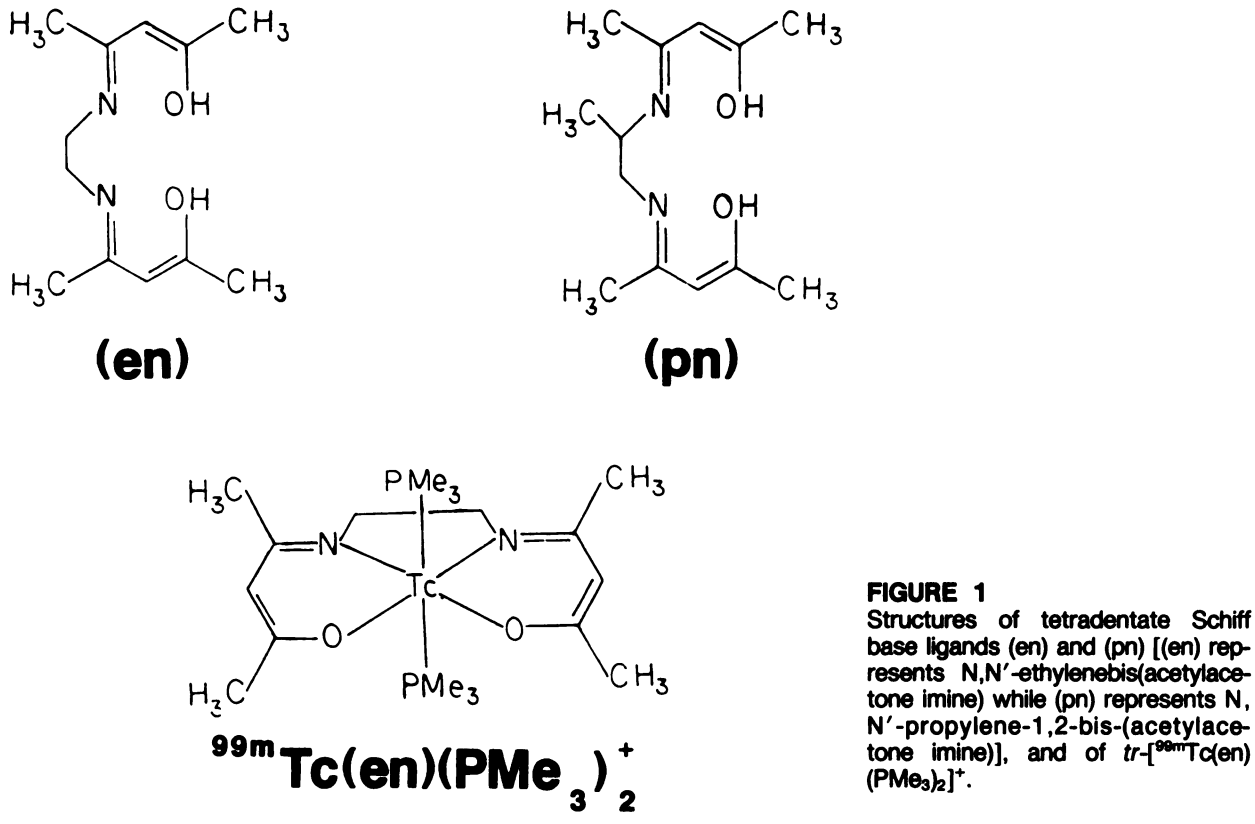

FIGURE 1
Structures of tetradentate Schiff base ligands (en) and (pn) [(en) represents N,N′-ethylenebis(acetylacetone imine) while (pn) represents N,N′-propylene-1,2-bis-(acetylacetone imine)], and of *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$.

plished with either a uv detector set at 254 nm or with a diode array rapid scanning spectrophotometric detector[¶] that recorded the visible-uv spectrum of the eluting complex.

Octanol/saline partition coefficients were determined by standard back extraction techniques. Replicate experiments indicated that the resulting D_o values are reproducible to ±6%.

Radiopharmaceutical Preparation

All labeling procedures were conducted under anaerobic conditions using "no carrier added" $^{99m}TcO_4^-$ obtained by eluting a $^{99}Mo/^{99m}Tc$ generator.[**,††] Solutions of the Tc(V) Schiff base complexes $[^{99m}TcL(O)]^+$ in dichloromethane were prepared as follows. A saline solution of $^{99m}TcO_4^-$ was diluted to 1.8 ml with normal saline in a 5-ml borosilicate vial. To this vial were successively added 0.30 ml of a 0.20*M* solution of Schiff base ligand in absolute ethanol, 0.02 ml of a 0.02*M* solution of stannous chloride (either anhydrous or dihydrate) in absolute ethanol and then 0.02 ml of 1.0*M* NaOH in water. This solution was sealed with a Teflon lined cap and heated at 95 ± 2°C for 15 min. After cooling the preparation to ambient temperature, 0.10 ml of 0.5*M* LiF_3CSO_3 (pH 10.5) in water, 3.0 ml HPLC grade dichloromethane, and 0.02 ml of 0.02*M* stannous chloride in ethanol were added. The vial was capped and the contents were well mixed by vortexing. The layers were then separated by centrifugation and the dichloromethane layer was removed. HPLC analysis shows that at this point the dichloromethane layer contains $[^{99m}TcL(O)]^+$, usually in >95% radiochemical purity. The overall yield of $[^{99m}TcL(O)]^+$ at this point is usually 50–70% (*13*).

The mixed ligand Tc(III) complexes *tr*-$[^{99m}TcL(Y)_2]^+$ were prepared from this dichloromethane solution of $[^{99m}TcL(O)]^+$ by the following procedures which differ in small details because of the different properties of the monodentate Y ligands. If the procedure calls for evaporation of the dichloromethane solution, this was accomplished under vacuum at room temperature after first passing the solution through a short column of $MgSO_4$ to remove traces of water. Unless otherwise noted, the radiochemical purity of the final radiopharmaceutical is >95%.

Y = $P(CH_3)_3$, $P(CH_3)_2(CH_2OH)$, $P(CH_3)(CH_2OH)_2$. The reduction of Tc(V) to Tc(III) is accomplished simply by adding excess phosphine to the $[^{99m}TcL(O)]^+$ solution. Trimethylphosphine, PMe_3, is added as a stream of gas, while $PMe_2(MeOH)$ and $PMe(MeOH)_2$ are added as 5% solutions in ethanol (0.1 ml). After standing at room temperature for 10 min, the dichloromethane solution was evaporated and then the residue was taken up in 2 ml of water. This solution was added to a Sephadex SP C-25[‡‡] cation exchange column and the column was rinsed with 2 ml water and then 2 ml 5% ethanol/water. The desired *tr*-$[^{99m}TcL(Y)_2]^+$ product was eluted with 2 ml normal saline (>70% yield for $PMe_2(MeOH)$ and $PMe(MeOH)_2$, and >85% yield for PMe_3) and filtered through a 0.22 micron-filter.

Y = $P(CH_2CH_3)_3$. The Tc(III) complex was prepared as above, but purification was accomplished on a silica SEP-PAK cartridge[§§] that had been prepared by rinsing with 95% ethanol and then dichloromethane. After loading the reaction mixture, the cartridge was rinsed with 5 ml dichloromethane, dried by suction, washed with three successive 3-ml portions of water, and then washed with 3 ml 95% ethanol. The desired radiopharmaceutical was eluted in ~75% yield with 2.5 ml of a 50% ethanol/saline mixture. This was diluted tenfold with saline and then filtered through a 0.22-micron filter.

Y = $P(CH_2CH_3)_2(C_6H_5)$, $P(CH_2CH_3)(C_6H_5)_2$. The dichloromethane solution of the Tc(V) complex was converted to an ethanol solution either by evaporation of the CH_2Cl_2 and redissolution in ethanol, or by use of a reversed phase Sep-Pak cartridge.[§§] Reaction with the phosphine in ethanol takes place at 95°C in 20 min. Purification is readily accomplished by adding an equal volume of saline to the preparation, and then passing the resulting solution through a Gelman LC-13[¶¶] 0.2-micron filter. The radioactivity that remains on the filter is then eluted with absolute ethanol.

Y = $P(OCH_3)_3$, *TMP*. The dichloromethane solution of the Tc(V) complex was converted to a methanol solution (0.5 ml) as described above, and 0.1 ml 10% TMP in methanol was added. The reaction mixture was heated at 50°C for 45 min and HPLC analysis at this point confirms that the product is formed in >95% radiochemical purity. Purification of *tr*-$[^{99m}TcL(TMP)_2]^+$ is complicated by the relative instability of this complex in aqueous media; all subsequent steps are performed as quickly as possible to minimize decomposition. The reaction mixture was diluted with 3 ml water and then loaded onto a C-18 SEP-PAK cartridge[§§] that had been prepared as described above. The cartridge was rinsed with 4 ml of 20% ethanol/saline and then the desired product was eluted in ~50% yield with 60% ethanol/saline. The rapid decomposition of *tr*-$[^{99m}TcL(TMP)_2]^+$ in aqueous media does not permit a satisfactory estimate of its radiochemical purity.

Y = $CN\text{-}C(CH_3)_3$, *TBIN*. Since TBIN is not a good reductant, the conversion of Tc(V) to Tc(III) was accomplished with stannous ion. To the dichloromethane solution of the Tc(V) complex were added 0.25 ml 95% ethanol, 0.10 ml of 10% TBIN in ethanol, and 0.10 ml of 0.02*M* stannous chloride in ethanol. This mixture was heated at 50°C for 40 min, allowing the CH_2Cl_2 vapors to be vented. The cooled reaction mixture was loaded onto a C-18 SEP-PAK cartridge,[§§] which was then washed with 4 ml of 20% ethanol/saline. The product was eluted in 80% yield with 2 ml of 60% ethanol/saline. HPLC analysis at this point shows ~5–10% $[^{99m}Tc(TBIN)_6]^+$ (*5,7*). To remove this impurity the preparation was diluted five-fold with water and loaded onto a Sephadex SP C-25 cation exchange column.[‡‡] After washing the column with water, the desired product was eluted with saline and filtered through a 0.22-micron filter. The radiochemical purity was ~90% and there was no detectable $[^{99m}Tc(TBIN)_6]^+$.

Animal Studies

In vivo scintigraphic studies were performed using anesthetized (Nembutal) rats and dogs. The radiopharmaceuticals were injected into the jugular vein for the rats, or into the saphenous vein for the dogs. Biodistribution in a dog was monitored for 1 hr after injection by means of a gamma camera in line with a dedicated computer; data were recorded in a 64 × 64 matrix at 60 sec per frame (*15*). Blood clearance data were also obtained in this dog, with samples being taken up to 1 hr after injection.

Samples of heart, kidney and urine from rats were analyzed by HPLC for the presence of metabolites. The urine was directly analyzed after filtration through a 0.45 micron filter

(ACRO LC-13)[¶]; the heart and kidney samples were ground in 3 ml acetonitrile then filtered (ACRO LC-13)[¶] before analysis.

Tissue distribution studies were conducted in anesthetized (Metofane), rats of 200 g average weight. Radiopharmaceuticals were administered through jugular vein injection. At each of 10, 45, and 90 min postinjection, groups of five rats were sacrificed (by cervical dislocation for the 10 min point and by CO_2 asphyxiation for the 45 and 90 min points). Samples of blood, heart, lung, liver, spleen, kidney, stomach, and intestines were collected, weighed and assayed for ^{99m}Tc activity versus appropriate standards and blanks for calculation of percent uptake per gram of tissue.

The potential toxicities of (en), PMe_3, $O{=}PMe_3$ and ^{99}Tc(en)-PMe_3 were briefly investigated by intravenously injecting large amounts (from 100 to 1,000 times the anticipated human dose, on a per weight basis) of these chemicals into rats and dogs. No adverse reactions or toxicological effects were observed in any of these studies.

Human Studies

All subjects signed the informed consent statement used in Ospedale San Raffaele where the human studies were conducted.

Five asymptomatic male volunteers were injected with 8–12 mCi ^{99m}Tc(en)-PMe_3 in an antecubital vein (four at rest, one after stress). Blood samples were obtained at 2, 4, 6, 15, 30, 60, 120, 180, and 240 min after injection through an intravenous catheter inserted in the contralateral arm. Blood samples were weighed and then assayed for ^{99m}Tc activity. The plasma fraction was separated by centrifugation at 1,500 *g* for 10 min, and then it was also weighed and counted. Aliquots of the original radiopharmaceutical were also counted as internal standards. The activity in the blood (and in the plasma) was then corrected for decay and expressed as a percentage of the injected dose per mg of blood (or plasma). Values for the half-lives ($t_{½}$) governing the first portion of the blood clearance were obtained from time/activity curves plotted on a semilog scale. The ratios of activity per mg of plasma to activity per mg of red blood cells (RBC) were also plotted as a function of time.

In the stress study, exercise was performed upright on a cycloergometer and the exercise protocol was characterized by a progressive stepwise load increase of 25 W every 150 sec until physical exhaustion. The tracer was injected 1 min before the end of the stress test.

Anterior images of supine subjects were obtained with a gamma camera, equipped with a high resolution collimator, that was centered over the thorax. Data were continuously recorded for the first hour in a 64 × 64 matrix (10 sec per frame) using a dedicated computer that allowed processing and visualization of the data. One-minute static chest images were collected at 30-min intervals from 1 hr to 6 hr after injection. Magnified myocardium images (200*M* = 2,500,000 counts) were obtained in the anterior and 40° left anterior oblique projections at selected times. Regions of interest (ROI) were defined in the dynamic images over the left anterolateral heart wall, cardiac blood pool, lung, and liver. The resulting counts were normalized to the area of the ROI. The ratios of counts in the left ventricle to counts in the cardiac blood pool, lung, and liver were calculated at each imaging time.

RESULTS

Chemistry

The identities of $[^{99m}Tc(en)(O)]^+$, $[^{99m}Tc(pn)(O)]^+$, *tr*-$[^{99m}Tc(en)(PEt_3)_2]^+$, *tr*-$[^{99m}Tc(en)(PEt_2Ph)_2]^+$ and *tr*-$[^{99m}Tc(en)(PEtPh_2)_2]^+$ are established by HPLC experiments utilizing authentic samples of the ^{99}Tc congeners (*8,9*) and a rapid scanning diode array spectophotometric detector[¶]. These experiments demonstrate that (a) radiopharmaceuticals prepared with "carrier" ^{99}Tc are >95% pure, (b) the eluted ^{99}Tc complexes exhibit the same visible-uv spectra as do the authentic samples, and thus are the same chemical species, and (c) when ^{99}Tc and ^{99m}Tc samples are co-injected, the uv trace (that monitors ^{99}Tc) and the radiometric trace (that monitors ^{99m}Tc) exhibit co-eluting peaks, and thus the ^{99}Tc and the ^{99m}Tc samples contain the same chemical species. The identities of the other technetium-99m(III) complexes prepared in this study are inferred from the procedures used for their preparations, their HPLC characteristics (vide infra) and their properties, all of which are analogous to those of the three more rigorously characterized *tr*-$[^{99m}Tc(en)(Y)_2]^+$ complexes (Y = PEt_3, PEt_2Ph, $PEtPh_2$).

In general, conversion of "no carrier added" $^{99m}TcO_4^-$ to a pharmaceutically acceptable form of *tr*-$[^{99m}TcL(Y)_2]^+$ requires 1–2 hr, with a large fraction of this time being dedicated to HPLC quality control. HPLC quality control chromatograms demonstrate >95% purity for the $[^{99m}Tc(en)(O)]^+$ and *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ used in the human studies. For $[^{99m}Tc(en)(PMe_3)_2]^+$, no difficulty has been encountered in conducting the synthesis using from 1 to 120 mCi $^{99m}TcO_4^-$. In all experiments to measure recovered yields, 100 ± 5% of the injected radioactivity was recovered from the HPLC columns.

Table 1 gives the HPLC capacity factors $k' = [t_x - t_o]/t_o$ for the Tc(III) complexes prepared in this study, along with the octanol/saline partition coefficients, D_o, measured for selected complexes. As expected for reversed phase chromatography, there is a general correlation between k′ and D_o, the more lipophilic complexes being more strongly retained on the reversed phase matrix. Interestingly, the complex which deviates most from this correlation is *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ the cation which, of all those evaluated in animal models, appears to exhibit the most promise as a myocardial perfusion imaging agent (vide infra).

In Vivo Studies

Tables 2–5 give the time dependent biodistributions in rats of the four related Tc(III) complexes $[^{99m}Tc(en)$-$PMe_3]^+$, $[^{99m}Tc(pn)$-$PMe_3]^+$, $[^{99m}Tc(en)$-$PMe_2MeOH]^+$ and $[^{99m}Tc(en)$-$PEt_3]^+$. Target to nontarget organ ratios are also given in these tables. Figure 2 shows the relative blood clearance rates in dogs for three analogous (en)

TABLE 1
HPLC Capacity Factors*, k′, and Octanol/Saline Coefficients, D_o, for ^{99m}Tc Complexes

Complex†	k′	D_o
$^{99m}TcO_4^-$	0	0.01
$[^{99m}Tc(en)\text{-}PMe_3]^+$	3.58	0.56
$[^{99m}Tc(en)\text{-}P(Me)_2MeOH]^+$	0.79	0.05
$[^{99m}Tc(en)\text{-}P(MeOH)_2Me]^+$	0.79	0.04
$[^{99m}Tc(en)\text{-}PEt_3]^+$	6.87	20.5
$[^{99m}Tc(en)\text{-}TMP]^+$	2.10	—†
$[^{99m}Tc(en)\text{-}TBIN]^+$	2.72	—
$[^{99m}Tc(en)\text{-}PEt_2Ph]^+$	6.14	—
$[^{99m}Tc(en)\text{-}PEtPh_2]^+$	10.1	—
$[^{99m}Tc(pn)\text{-}PMe_3]^+$	3.20	1.45
$[^{99m}Tc(pn)\text{-}P(Me)_2MeOH]^+$	1.16	0.25
$[^{99m}Tc(pn)\text{-}P(MeOH)_2Me]^+$	0.78	—
$[^{99m}Tc(pn)\text{-}PEt_3]^+$	6.48	22
$[^{99m}Tc(pn)\text{-}TMP]^+$	2.11	—†
$[^{99m}Tc(pn)\text{-}TBIN]^+$	2.73	5.5
$[^{99m}Tc(pn)\text{-}PEt_2Ph]^+$	6.26	—

*Values of k′ referred to $[^{99m}Tc(DMPE)_3]^+$ (k′ = 3.87) as an internal standard; DMPE = 1,2-bis(dimethylphosphine)ethane, $(CH_3)_2PCH_2CH_2P(CH_3)_2$.

†Value cannot be measured because of decomposition of the complex.

complexes (n = 1). Figure 3 shows comparative scintiphotographs of dogs imaged with three related *tr*-$[^{99m}Tc(en)\text{-}Y]^+$ complexes.

HPLC analysis of heart tissue from rats injected with $[^{99m}Tc(en)\text{-}PMe_3]^+$ shows that >95% of the activity in the heart is still in this chemical form 4 hr after injection. A small amount (~10%) of metabolite is detected in kidney tissue but no detectable metabolites are present in the urine. HPLC analyses of urine from rats injected with $[^{99m}Tc(en)\text{-}PMe_2MeOH]^+$, $[^{99m}Tc(pn)\text{-}PMe_2MeOH]^+$ and $[^{99m}Tc(en)\text{-}PMe(MeOH)_2]^+$ show that these complexes undergo considerable conversion to more hydrophilic species during their passage through the renal system.

The blood clearance of $[^{99m}Tc(en)\text{-}PMe_3]^+$ in normal volunteers, plotted as percent injected dose per gram of blood, is shown in Figure 4. The equivalent per gram data obtained for a dog are essentially superimposable over the time period monitored (1 hr). Thus, on a per organ basis the percent dose in human blood is about six times that in dog blood at all times monitored. Biexponential analysis of the curve in Figure 4 shows the half-life of the faster clearing component to be 5 min (correlation coefficient 0.99). The slower clearing component removes <0.5% of the originally injected activity. The value of the plasma/red blood cell ratio remains constant in the dog while it rapidly decreases

TABLE 3
Tissue Distribution of $[^{99m}Tc(pn)\text{-}PMe_3]^+$ in Rats*

	10 min	45 min	90 min
Blood	0.23 ± 0.01	0.16 ± 0.01	0.14 ± 0.01
Heart	3.7 ± 0.5	3.2 ± 0.5	3.0 ± 0.5
Lung	1.4 ± 0.7	1.1 ± 0.3	0.9 ± 0.2
Liver	1.4 ± 0.4	0.41 ± 0.08	0.31 ± 0.09
Spleen	1.7 ± 0.3	1.4 ± 0.3	1.1 ± 0.2
Kidney	9.1 ± 1.0	5.3 ± 0.9	3.3 ± 0.4
Stomach	2.3 ± 0.8	1.5 ± 0.3	1.07 ± 0.07
Intestines	3 ± 1.5	1.3 ± 0.6†	0.9 ± 0.2†
Heart/blood ratio	16	20	22
Heart/lung ratio	2.6	2.9	3.4
Heart/liver ratio	2.5	7.6	9.5

*Table entries are average % injected dose per gram of organ at the listed time of assay. The quoted error is ± 1 s.d. (n = 5). The radiopharmaceutical was injected in 0.4 ml of a 0.5% ethanol/normal saline solution.

†n = 4.

TABLE 2
Tissue Distribution of $[^{99m}Tc(en)\text{-}PMe_3]^+$ in Rats*

	10 min	45 min	90 min
Blood	0.171 ± 0.004	0.115 ± 0.004	0.093 ± 0.011
Heart	2.95 ± 0.24	2.79 ± 0.27	2.89 ± 0.45
Lung	0.94 ± 0.10	0.74 ± 0.07	0.81 ± 0.33
Liver	1.34 ± 0.31	0.39 ± 0.06	0.30 ± 0.08
Spleen	1.18 ± 0.19	0.86 ± 0.20	0.68 ± 0.1
Kidney	8.6 ± 1.1	4.7 ± 0.5	2.9 ± 0.4
Stomach	1.2 ± 0.4	1.2 ± 0.6	1.0 ± 0.3
Intestines	1.5 ± 0.4†	3 ± 1	0.7 ± 0.1†
Heart/blood ratio	17	24	31
Heart/lung ratio	3.1	3.8	3.4
Heart/liver ratio	2.2	7.1	9.5

*Table entries are average % injected dose per gram of organ at the listed time of assay. The quoted error is ± 1 s.d. (n = 5). The radiopharmaceutical was injected in 0.4 ml of a 0.5% ethanol/normal saline solution.

†n = 4.

TABLE 4
Tissue Distribution of $[^{99m}Tc(en)\text{-}P(Me)_2MeOH]^+$ in Rats*

	10 min	45 min	90 min
Blood	0.134 ± 0.015	0.058 ± 0.005	0.043 ± 0.014
Heart	0.22 ± 0.02	0.18 ± 0.02	0.19 ± 0.02
Lung	0.18 ± 0.02	0.12 ± 0.03	0.105 ± 0.007
Liver	2.1 ± 0.4	0.54 ± 0.10	0.35 ± 0.06
Spleen	0.14 ± 0.03	0.094 ± 0.011	0.088 ± 0.015
Kidney	5.9 ± 0.3	1.1 ± 0.2	0.85 ± 0.06
Stomach	2.8 ± 1.1	0.6 ± 0.3	0.4 ± 0.3
Intestines	3.3 ± 0.8†	3 ± 2	0.50 ± 0.09
Heart/blood ratio	1.6	3.2	4.5
Heart/lung ratio	1.2	1.5	1.8
Heart/liver ratio	0.10	0.34	0.55

*Table entries are the average % injected dose per gram of organ at the listed time of assay. The quoted error is ± 1 s.d. (n = 5). The radiopharmaceutical was injected in 0.3 ml of normal saline solution.

†n = 4.

TABLE 5
Tissue Distribution of [^{99m}Tc(en)-PEt_3]$^+$ in Rats*

	10 min	45 min	90 min
Blood	0.85 ± 0.19†	0.58 ± 0.04	0.48 ± 0.07
Heart	1.32 ± 0.12	1.24 ± 0.14	1.27 ± 0.17
Lung	5 ± 1	2.6 ± 0.5	1.9 ± 0.5
Liver	4.7 ± 0.5	3.8 ± 0.8	2.21 ± 0.22
Spleen	7 ± 1	7 ± 1	6.9 ± 0.7
Kidney	8.8 ± 0.7	10 ± 2	10.2 ± 0.9
Stomach	1.1 ± 0.4	0.90 ± 0.04‡	1.2 ± 0.6
Intestines	0.99 ± 0.14‡	3 ± 1†	2.4 ± 0.2‡
Heart/blood ratio	1.6	2.2	2.7
Heart/lung ratio	0.27	0.53	0.66
Heart/liver ratio	0.28	0.33	0.57

* Table entries are average % dose gram of organ at the listed time of assay. The quoted error is ± 1 s.d. (n = 5). The radiopharmaceutical was injected in 0.4 ml of a 5% ethanol/normal saline solution.
† n = 3.
‡ n = 4.

in humans (Fig. 5); the value of this ratio after 30 min is a factor of 5 larger for humans than for the dog. Table 6 summarizes the ratios of counts in the myocardial wall to counts in the blood pool, to counts in the lung and to counts in the liver for both dog and human (at 10 min, 60 min, and 5 hr after injection). In the dog, uptake in the myocardial wall is evident at 10 min (myocardium/blood pool ratio of 11), the lung uptake is low, and there is extensive uptake in the liver. Activity in the liver moves quickly to the gall bladder allowing good visualization of the heart wall at 1 hr after injection (Fig. 3, ^{99m}Tc(en)-PMe_3^+). In humans, myocardial perfusion images can be obtained only 1 hr after injection (myocardium/blood pool ratio is 0.8 at 10 min and 1.1 at 1 hr) but uptake by the heart wall is low and remains constant from 1 to 5 hr after injection. There is no detectable myocardial washout of [^{99m}Tc(en)-PMe_3]$^+$. Visualization of the heart wall is also hindered by liver activity which is only slowly cleared through the biliary system.

DISCUSSION

Chemistry

The inorganic chemistry of the mixed ligand Tc(III) complexes, *tr*-[$TcL(Y)_2$]$^+$, has been fully developed using macroscopic amounts of ^{99}Tc (*9*). Most importantly, all of these complexes are very stable in the Tc(III) oxidation state, and can be reduced to Tc(II) only at potentials that are well outside the range accessible to biologic systems (*9,16*). Thus, in the context of radiopharmaceutical development, the *tr*-[$^{99m}TcL(Y)_2$]$^+$ complexes are accurately described as nonreducible technetium-99m(III) cations.

The major chemical problem facing this study was the preparation and isolation of the [TcL(O)]$^+$ technetium(V) intermediate from generator produced, "no carrier added" $^{99m}TcO_4^-$, i.e., at concentrations of technetium in the range 10^{-6}–$10^{-8}M$ (*17*). Previous preparations (*8*) had utilitized the ^{99}Tc(V) complex $^{99}TcOCl_4^-$, at concentrations $>10^{-3}M$, as a starting material. Synthesis of the [^{99m}TcL(O)]$^+$ intermediates was accomplished by Sn(II) reduction of $^{99m}TcO_4^-$ in the presence of excess L. Systematic studies showed that the concentration of Sn(II), concentration of L, and pH must all be carefully controlled in order to obtain an acceptable yield of the ^{99m}Tc(V) intermediate. These studies also showed that the tetradentate Schiff base ligands L form relatively weak complexes with the Tc(V) center, and can be readily displaced from technetium by ligands such as ascorbate, tartrate, and citrate. Isolation of the cationic [^{99m}TcL(O)]$^+$ species is

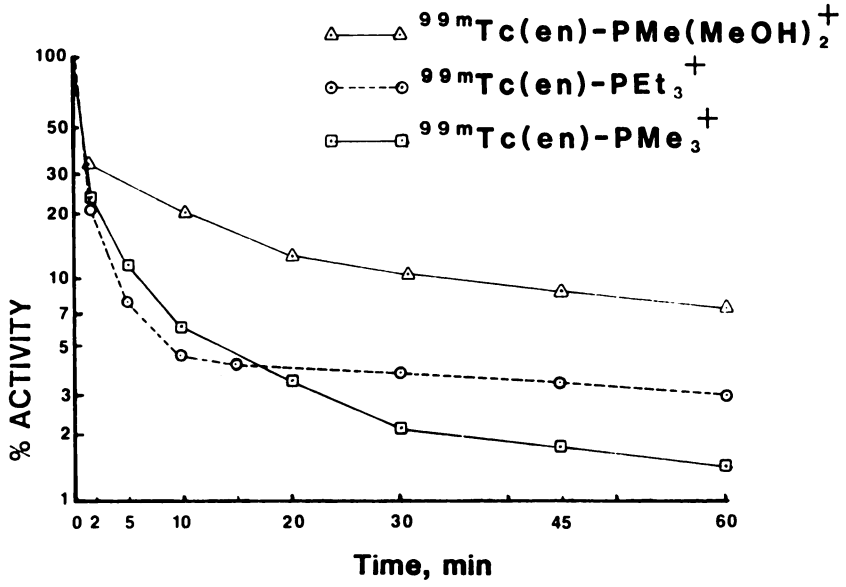

FIGURE 2
Blood clearance curves (semilog) in dogs (n = 1) for three analogous ^{99m}Tc(en) agents.

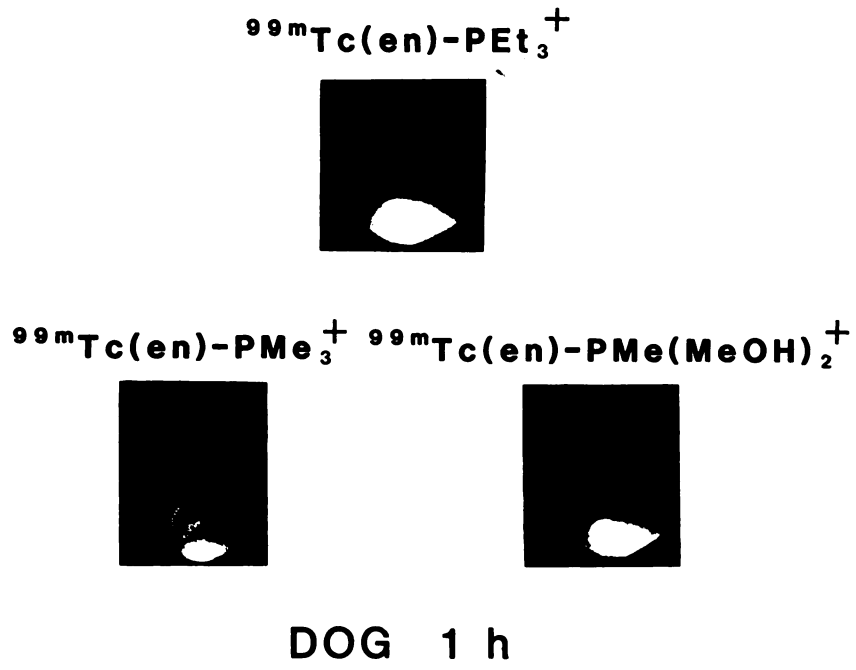

FIGURE 3
Comparative scintiphotographs of dogs imaged with three analogous ^{99m}Tc(en) agents at 1 hr after injection, demonstrating the effect of lipophilicity on biodistribution.

most effectively accomplished by extracting into dichloromethane the ion pair formed with trifluoromethane sulfonate, $F_3CSO_3^-$. This yields a >95% radiochemically pure solution of the intermediate in a solvent that can be either readily removed by evaporation or used as a reaction medium for the subsequent conversion of Tc(V) to Tc(III).

When Y is a reducing ligand (such as an alkyl phosphine or phosphite), conversion of the Tc(V) intermediate into the final Tc(III) radiopharmaceutical is readily accomplished by simply adding an excess of Y that functions as both a reductant and a ligand:

$$Tc^{V}L(O) + 3Y \rightarrow tr\text{-}Tc^{III}L(Y)_2^{+} + O{=}Y \quad (1)$$

Y = reducing ligand.

If Y by itself does not have significant reducing power, e.g., an isonitrile, then a separate, noncoordinating reductant, R, can be added to effect the conversion of Tc(V) to Tc(III):

$$Tc^{V}L(O)^{+} + 2Y + R \rightarrow tr\text{-}Tc^{III}L(Y)_2^{+} + O{=}R \quad (2)$$

Y = nonreducing ligand.

In this work we have used Sn(II) as a pharmaceutically acceptable R. Reactions 1 and 2 are of much broader scope and utility than is indicated by their use to prepare Tc(III) Schiff base complexes—many different types of Tc(V)-ligand complexes can be converted to classes of analogous Tc(III)-ligand-Y complexes, the chemical and biologic properties of which can be readily and systematically varied by changing the nature and

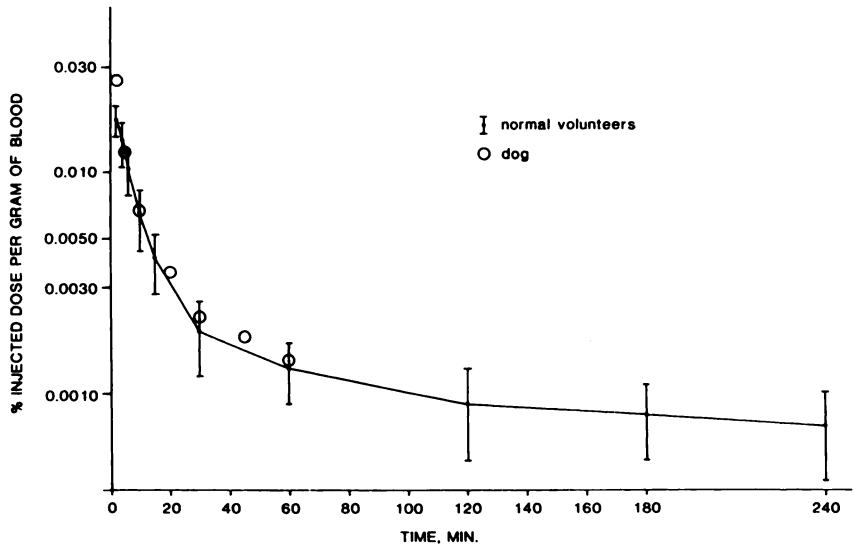

FIGURE 4
Blood clearance data (semilog) for [^{99m}Tc(en)-PMe$_3$]$^+$ in a dog (n = 1) and in normal volunteers (mean ± s.d.; n = 6).

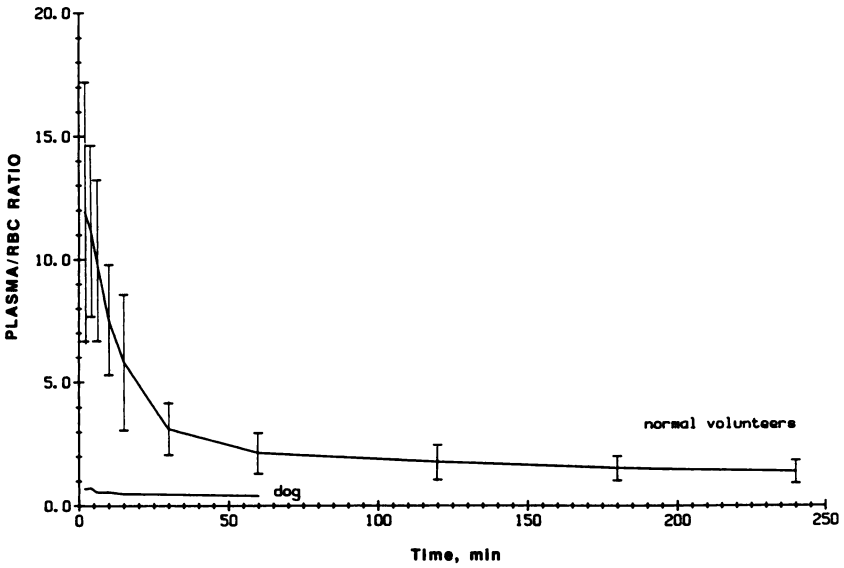

FIGURE 5
Time dependent plasma/red blood cell ratio for $[^{99m}Tc(en)\text{-}PMe_3]^+$ in a dog (n = 1) and in normal volunteers (mean ± s.d.; n = 6).

properties of Y. This ability to expand and elaborate the properties of Tc-ligand combinations should greatly assist in the design and development of new, more precisely controlled, ^{99m}Tc radiopharmaceuticals.

HPLC analysis using simultaneous uv and radiometric detection, as well as authentic samples of the *tr*-$[^{99}TcL(Y)_2]^+$ complexes (*9*), establishes the chemical identity of the technetium-99m(III) agents. HPLC also characterizes these agents by means of capacity factors (k′) which, in reversed phase chromatography, are related to the lipophilicities of the agents (*18*). The data of Table 1 show that the (pn) complexes are, as expected, slightly more lipophilic than the (en) analogs, but that much larger ranges in lipophilicities are engendered by varying the properties of the monodentate Y ligands. Simple variations along an analogous series such as Y = PMe_2MeOH, PMe_3, PEt_3, cause the octanol/saline partition coefficients of the *tr*-$[^{99m}TcL(Y)_2]^+$ complexes to increase about two orders of magnitude even though there are only two Y ligands per complex.

HPLC analysis also shows that the Tc(III) complexes containing the alkyl phosphines PEt_3, PMe_3, PMe_2MeOH or $PMe(MeOH)_2$ are stable in aqueous solution for up to 5 hr. Even after 12 hr, <15% decomposition occurs in vitro. The *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ complex appears to be correspondingly stable in vivo in rats—it is essentially (>95%) the only species found in myocardial tissue, and it is eliminated unchanged in the urine. A small amount (10%) of metabolite can be detected in kidney tissue. These observations are important in that they demonstrate that it is the biologic fate of *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ itself, and not that of some metabolite, that is being monitored in the biodistribution and scintigraphic imaging experiments.

In Vivo Studies

Biodistribution studies in rats for the four related complexes $[^{99m}Tc(en)\text{-}PMe_3]^+$, $[^{99m}Tc(pn)\text{-}PMe_3]^+$, $[^{99m}Tc(en)\text{-}PMe_2MeOH]^+$ and $[^{99m}Tc(en)\text{-}PEt_3]^+$ (Tables 2-5) reveal several interesting characteristics of these agents. Most importantly, for all four agents there is no detectable myocardial washout for up to 90 min after injection. This observation is consistent with our basic premise that nonreducible technetium(III) cations would not suffer myocardial washout. Good myocardial images can be obtained in a rat injected with $[^{99m}Tc(en)\text{-}PMe_3]^+$ even 4 hr after injection. The highest myocardial uptakes are observed for the two PMe_3 complexes; the PEt_3 complex exhibits significantly less myocardial uptake (1.3 versus 3% dose/g heart), while the PMe_2MeOH exhibits essentially no myocardial uptake at all. All four agents clear to some extent through the hepatobiliary system, leading to liver uptake values that continually decrease during the course of the study, and variable intestinal uptake values which represent the radioactivity that is in transit through the particular section of intestine sampled at that particular time point. As expected, the most lipophilic complex, $[^{99m}Tc(en)\text{-}PEt_3]^+$, exhibits the highest liver uptake. The two PMe_3 complexes are cleared to a large extent through the kidney and appear in the urine essentially unaltered. The more lipophilic PEt_3 complex appears to bind irreversibly to the kidney, while the more hydrophilic PMe_2MeOH complex undergoes the fastest renal clearance of the four agents evaluated. HPLC analysis of urine indicates that the PMe_2MeOH complex suffers some metabolism during its passage through the rat renal system. Of the four agents, only the lipophilic PEt_3 derivative exhibits an unacceptably high residue in the blood. This could arise from the fact that

$[^{99m}Tc(en)\text{-}PEt_3]^+$ is injected in a matrix containing 5% ethanol and the initial radiopharmaceutical bolus could cause blood proteins to be denatured and irreversibly bind some of the PEt_3 derivative. This phenomenon could also give rise to the unusually high lung uptake values observed only for the PEt_3 agent. The target/nontarget organ uptake ratios listed in Tables 2–5 indicate that either of the two PMe_3 derivates are suitable candidates for further evaluation as myocardial perfusion imaging agents.

Qualitative imaging studies in dogs and rats support and extend the conclusions drawn from the quantitative biodistribution studies. Images of rats obtained with $[^{99m}Tc(en)\text{-}PMe_3]^+$ clearly show the absence of myocardial washout, as well as accumulation of activity in the intestines and urinary bladder. Of the five related PMe_3, $PMe_2(MeOH)$ and $PMe(MeOH)_2$ agents, the best myocardial images in rats are obtained with the two PMe_3 derivatives, while all the agents undergo both heptobiliary and renal clearance. Figure 3 dramatically illustrates the effect of lipophilicity on the images obtained in a dog one hour after injection. While the $[^{99m}Tc(en)\text{-}PMe_3]^+$ complex produces a good myocardial image, the more lipophilic PEt_3 analog, and the more hydrophilic $PMe(MeOH)_2$ analog, do not yield any detectable myocardial image. This observation implies that, at least for this series of related complexes, there is a window of lipophilicity within which agents must fall in order to undergo significant myocardial uptake. From Table 1 this window of lipophilicity is defined as D_o values ranging from 0.04 to 20 (or k′ values ranging from 0.79 to 6.9). On this basis, mixed ligand complexes containing even the very lipophilic TBIN species should exhibit myocardial uptake since the entire complex exhibits a lipophilicity which falls within the window. This is indeed the case, and myocardial uptake occurs in dogs and rats for $[^{99m}Tc(pn)\text{-}TBIN]^+$ which has a D_o value of 5.5.

Qualitative blood clearance studies show that the TBIN derivatives clear much more slowly than do the phosphine derivatives, while among the phosphine derivatives the PMe_3 agent clears most rapidly (Fig. 2). On the basis of the combined biodistribution, imaging, and blood clearance studies in animals it was decided to carry one of the PMe_3 derivatives on to a Phase I clinical study. Because the (en) agent exhibits slightly superior target to nontarget organ uptake ratios (Tables 2 and 3), and because the (en) ligand is chemically simpler than the (pn) ligand which is composed of d and l isomers, it was decided to evaluate $[^{99m}Tc(en)\text{-}PMe_3]^+$ as a myocardial perfusion imaging agent in humans. Studies in five normal volunteers established that while $[^{9m}Tc(en)\text{-}PMe_3]^+$ does exhibit myocardial uptake in humans, it is not a clinically useful agent because of (i) relatively slow blood clearance which allows visualization of the heart only 1 hr after injection, (ii) relatively low uptake in the myocardial wall, and (iii) extensive liver uptake with only slow excretion of activity through the biliary system. Moreover, exercise does not significantly improve the lung/liver/blood biodistribution patterns of this agent for up to 5 hr after injection (Table 6).

One of the main reasons that $[^{99m}Tc(en)\text{-}PMe_3]^+$ is not an effective myocardial imaging agent in humans is that it clears from the blood slowly, and this in turn results from its high affinity for human blood plasma. This is qualitatively the same situation encountered with the three Tc(I) agents $[^{99m}Tc(DMPE)_3]^+$, $[^{99m}Tc(POM\text{-}POM)_3]^+$ and $[^{99m}Tc(TMP)_6]^+$ (*15*). For none of these four agents were preliminary studies in dogs predictive of this high affinity for human plasma; in fact, all four agents clear quite rapidly from dog blood. A retrospective analysis of dog and human blood data for six ^{99m}Tc cations of various structures and properties (Table 7) reveals that the affinity of all these cations for human plasma (expressed as their equilibrium in vivo plasma/RBC ratio) is consistently greater than the corresponding affinity for dog plasma. In fact, even though the individual human plasma/RBC ratios range from 1.4 to 32 for the six cations, the human/dog ratio of plasma/RBC values is remarkably constant at 6.7 ± 1.6 (mean ± s.d.; n = 6). The constancy of this ratio is especially noteworthy considering the inherent variability in the type, age and condition of the mongrel

TABLE 6
Biodistribution of *tr*-$[^{99m}Tc(en)(PMe_3)_2^+]$ in Human Volunteers

	Injection dose (mCi)	Blood clearance ($t_{½}$, min)	*Myocardium* / Blood pool 10 min	Blood pool 60 min	Blood pool 5 hr	*Myocardium* / Lung 10 min	Lung 60 min	Lung 5 hr	*Myocardium* / Liver 10 min	Liver 60 min	Liver 5 hr
Dog	3.8	—	1.1	1.2		2.2	2.3		0.5	0.7	
Normal volunteer											
G.A.	8.9	3.0	0.8	0.9	1.0	1.3	1.6	1.7	0.1	0.2	0.2
A.R.	8.4	6.0	0.8	1.1	1.1	1.8	2.5	2.4	0.1	0.2	0.2
S.L.	10.0	6.0	0.7	1.0	0.9	1.0	1.4	1.7	0.2	0.2	0.5
C.M.	10.8	5.0	0.8	1.0	1.0	1.5	2.3	2.2	0.2	0.3	0.3
T.A. (stress study)	12.8	5.0	0.8	1.1	1.3	1.3	1.8	1.8	0.2	0.3	0.4

TABLE 7
Comparison of In Vivo, Equilibrium Plasma/RBC Ratios in Human and Dog Blood for Various Technetium-99m Cations

Cation	Class	Human	Dog	Human/Dog	Ref.
$[^{99m}Tc(TBIN)_6]^+$	Tc(I)	1.4	0.26	5.4	*,†
$[^{99m}Tc(DMPE)_3]^+$	Tc(I)	6.2	1.0	6.1	‡,§
$[^{99m}Tc(POM\text{-}POM)_3]^+$	Tc(I)	7.2	1.0	7.1	¶,†
$[^{99m}Tc(TMP)_6]^+$	Tc(I)	7.7	0.79	9.8	**,†
tr-$[^{99m}Tc(DMPE)_2Cl_2]^+$	Reducible Tc(III)	32	5.3	6.0	††
tr-$[^{99m}Tc(en)(PMe_3)_2]^+$	Nonreducible Tc(III)	1.8	0.30	6.0	‡‡

* TBIN represents tert-butylisonitrile, $CN\text{-}C(CH_3)_3$.
† Data from Ref. 15.
‡ DMPE represents 1,2-bis(dimethylphosphino)ethane, $(CH_3)_2PCH_2CH_2P(CH_3)_2$.
§ Data from the study reported in Ref. 6.
¶ POM-POM represents 1,2-bis(dimethoxyphosphino)ethane, $(CH_3O)_2PCH_2CH_2P(OCH_3)_2$.
** TMP represents trimethylphosphite, $(CH_3O)_3P$.
†† Data from the study reported in Ref. 1.
‡‡ This work.

dogs used in these studies, and the range of structures and properties of the six ^{99m}Tc cations studied. This observation of a constant human/dog ratio of plasma/RBC values for different cationic ^{99m}Tc agents may make it feasible to use the dog as a model to predict human plasma/RBC ratios for new ^{99m}Tc cations. This would greatly facilitate the development of ^{99m}Tc myocardial imaging agents since, in our experience, accurate plasma/RBC ratios in human blood cannot be determined in vitro because of interferences from anticoagulants.

CONCLUSIONS

The nonreducible $^{99m}Tc(III)$ cations *tr*-$[^{99m}TcL(Y)_2]^+$ do not suffer myocardial washout in either animals or humans, in contradistinction to the reducible $^{99m}Tc(III)$ cation *tr*-$[^{99m}Tc(DMPE)_2Cl_2]^+$ that does suffer myocardial washout in animals and humans (*1,3*). This observation provides strong support for the hypothesis that it is in vivo reduction of $^{99m}Tc(III)$ cations to the neutral $^{99m}Tc(II)$ form which is primarily responsible for myocardial washout. Thus, nonreducible $^{99m}Tc(III)$ cations would seem to provide a class of potential myocardial imaging agents that will be at least as fruitful as the $^{99m}Tc(I)$ cations have proven to be. Moreover, the synthetic procedures developed in this study are quite general and can be used to generate many different classes of $^{99m}Tc(III)$ complexes. These procedures also allow the chemical and biologic properties of the members of any particular class of $^{99m}Tc(III)$ complexes to be readily and systematically varied.

Several *tr*-$[^{99m}TcL(Y)_2]^+$ cations, including *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ give excellent myocardial images in rats and dogs. However, the prototypical agent *tr*-$[^{99m}Tc(en)(PMe_3)_2]^+$ yields only poor myocardial images in humans, its primary deficiencies being slow blood clearance and high liver uptake. The slow blood clearance is related to the tight binding of this agent to human plasma; this tight binding does not occur in dog plasma. A retrospective analysis of equilibrium plasma/RBC ratios observed in vivo in dogs and humans for six ^{99m}Tc cations of widely varying structures and properties shows that even though the human plasma/RBC ratio varies from 1.4 to 32, the human/dog ratio of plasma/RBC ratios remain remarkably constant at 6.7 ± 1.6. This type of systematic biologic information can be combined with the synthetic flexibility inherent in the nonreducible $^{99m}Tc(III)$ cations in attempts to design and develop a second generation agent that will exhibit rapid blood clearance and high myocardial uptake without suffering myocardial washout.

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NOTES

* Strem Chemical Company, Newburyport, MA.
† Aldrich Chemical Company, Milwaukee, WI.
‡ Jones Chromatography, Inc., Columbus, OH.
§ Hamilton Company, Reno, NV.
¶ Hewlett-Packard Company, Palo Alto, CA.
** Sorin Biomedica, Italy.
†† Medi-Physics, Richmond, CA.
‡‡ Pharmacia Inc., Piscataway, NJ.
§§ Waters Chromatography Div., Milford, MA.
¶¶ Gelman Sciences, Inc., Ann Arbor, MI.

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