Use of High Performance Liquid Chromatography for the Structural Identification of Technetium-99m Radiopharmaceuticals at the NCA Level

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Technetium-99m (^{99m}Tc) complexes of dithiooxalate (dto) and maleonitriledithiolate (mnt) were prepared by ligand substitution of $^{99m}TcNCl_4^-$, dithionite reduction of $^{99m}TcO_4^-$, and ligand jexchange of[^{99m}Tc]gluconate (Sn). All complexes were characterized by high performance liquid chromatography (HPLC) on a C18 column using gradient elution with a tetrabutylammonium phosphate buffer (40–85% methanol). By performing HPLC of mixtures of the ^{99m}Tc complexes with structurally characterized ^{99}Tc complexes of the ligands, it has been shown that at the no carrier added (NCA) level, $^{99m}TcNCl_4^-$ labeling leads to the production of $[^{99m}TcN(dto)_2]^{2-}$ and $[^{99m}TcN(mnt)_2]^{2-}$. Dithionite reduction and ligand exchange of ^{99m}Tc(Sn)-gluconate lead to the production of $[^{99m}Tc(mnt)_3]^{2-}$ and not of $[^{99m}TcO(mnt)_2]^-$ which would be the expected product from the results of studies with ^{99}Tc. The product formed by dithionite reduction in the presence of dto was not identified, but it was clearly not $[TcO(dto)_2]^-$, the product expected from ^{99}Tc studies. Studies in mice showed that the biologic behavior of the ^{99m}Tc complexes is altered by the presence of the Tc-nitrido group. HPLC results indicate that caution must be taken when extrapolating results obtained using carrier quantities of Tc to the carrier free situation.

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The widespread use of the radionuclide technetium-99m (^{99m}Tc) for diagnostic imaging in nuclear medicine has led to an upsurge of interest in the chemistry and structure of technetium compounds. Many studies have been performed using the long-lived radionuclide ^{99}Tc and a large number of technetium compounds have been prepared and characterized structurally by x-ray crystallography (*1*). A long-term aim of these studies has been to obtain a better understanding of the chemistry of ^{99m}Tc radiopharmaceuticals and to provide a more rational basis for their design.

Preparation of ^{99m}Tc radiopharmaceuticals is normally performed using ^{99m}Tc to which no carrier technetium has been added (NCA). The concentration of technetium in these preparations is typically of the order of 10^{-8} *M*. The ligand concentrations used are usually several orders of magnitude greater than that of Tc. Structural and chemical studies of technetium complexes using ^{99}Tc are typically performed using Tc concentrations of the order 10^{-1}–10^{-3} *M* with ligand concentrations generally being of the same order of magnitude. Thus, results obtained in these studies at the carrier added (CA) level may not be directly applicable to the chemistry of technetium at the NCA level. Deutsch and Libson (*2*) have discussed the effect of reaction kinetics on the applicability of CA results to the NCA situation. They pointed out that if the critical product forming step is first-order in technetium concentration and the ligand is present in large excess, then the chemistry at the CA level should be readily applicable. However, if the critical step is second order in technetium concentration or involves polymer formation or disproportionation, then the results may not be directly applicable.

In this article, a study has been made of the ^{99m}Tc products formed with the ligands maleonitriledithiolate (mnt) and dithiooxalate (dto) (Fig. 1). Labeling systems investigated include $^{99m}TcO_4^-$ reduction by stannous tin, sodium borohydride, sodium dithionite and the

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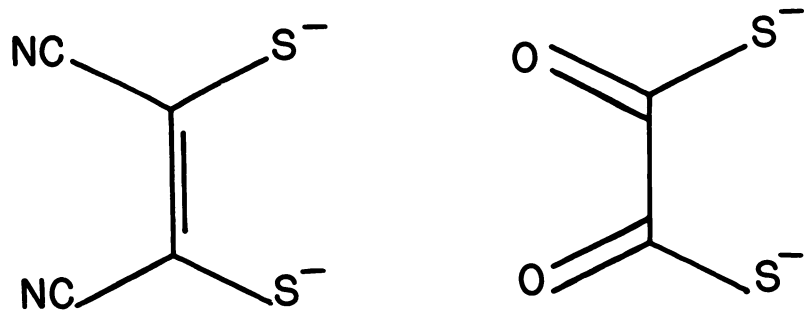

FIGURE 1
Structures of dithiooxalate (dto) and maleonitriledithiolate (mnt) anions.

^{99m}Tc-nitrido labeling technique where ^{99m}TcN radiopharmaceuticals are produced by a substitution reaction of $^{99m}TcNCl_4^-$ (*3*). These ligands were selected because salts of the following complex anions have been characterized chemically and/or structurally by x-ray crystallography and were available for study: $[TcO(mnt)_2]^-$ (*4–6*); $[Tc(mnt)_3]^{2-}$ (*7*); $[TcN(mnt)_2]^{2-}$ (*8*); $[TcO(dto)_2]^-$ (*4,6,9*); and $[TcN(dto)_2]^{2-}$ (*8,9*). The biologic behavior in mice of the various preparations is also reported.

MATERIALS AND METHODS

Chemicals

Dithiooxalic acid, dipotassium salt was obtained from Eastman Kodak Co., Rochester, NY. Maleonitriledithiolate, disodium salt was prepared using the procedure of Davison and Holm (*10*). All other chemicals were of analytic reagent grade and were obtained from commercial sources. Technetium-99 in the form of ammonium pertechnetate was obtained from Amersham International plc, Buckinghamshire, England.

Technetium-99m pertechnetate was obtained from generators manufactured by the Australian Nuclear Science and Technology Organisation, Sydney, Australia. Technetium-99m complexes of dto and mnt described in this paper were provided by Dr. S. Colmanet of the Australian Radiation Laboratory.

Preparation of ^{99m}TcN Complexes

For biodistribution studies, $^{99m}TcNCl_4^-$ was prepared using the method described previously (*3*). Eight to ten milliliters concentrated hydrochloric acid was added to 0.5 ml $^{99m}TcO_4^-$ followed by 15–20 mg solid sodium azide. After refluxing for 5 min to destroy excess azide, the solution was taken to dryness in a rotary evaporator. The dried residue was then dissolved in 3–4 ml ligand solution (mnt 5 mg/ml, dto 1 mg/ml) and, if necessary, the pH adjusted to 6–7 using 0.1*M* sodium hydroxide. Solutions were membrane filtered (0.22 μ) prior to use.

Preparation of ^{99m}Tc Complexes

Technetium-99m complexes of the ligands were prepared using sodium dithionite as the reducing agent. A 4-ml ligand solution (mnt 5 mg/ml, dto 1 mg/ml) was added to 0.5 ml $^{99m}TcO_4^-$ followed by 200 μl sodium dithionite solution (5 mg/ml in 0.1*M* sodium hydroxide). The solution was heated gently on a hotplate for 5–10 min, allowed to cool, and then membrane filtered (0.22 μ) prior to use.

Technetium-99m mnt(Sn) was also prepared by the use of a stannous gluconate cold kit. A preparation obtained by lyophilizing 20 mg sodium gluconate and 1 mg stannous chloride was reconstituted with 1 ml $^{99m}TcO_4^-$. Three milliliters mnt solution (5 mg/ml) was then added and the solution membrane filtered prior to use.

Some ^{99m}Tc preparations for high performance liquid chromatography (HPLC) were made using sodium borohydride as the reducing agent. The procedure was identical to that used with dithionite except that 200 μl of $NaBH_4$ (10 mg/ml in 1*M* NaOH) was used.

Preparation of ^{99m}Tc(*V*)-Complexes

Six to eight milliliters of 12*M* hydrochloric acid were added to 0.2–0.5 ml $^{99m}TcO_4^-$ and the solution allowed to stand for 30 min at room temperature. The solution was then taken to dryness in a rotary evaporator with a minimum of warming. The dry residue was dissolved in 3 ml ligand solution and the pH adjusted to 6–7. Labeling efficiency was usually low and a large amount of free pertechnetate was present. Heating was avoided in order to reduce the formation of ^{99m}Tc(*IV*) complexes.

Preparation of ^{99}Tc Complexes

For chromatographic studies, ^{99}Tc as $^{99}TcO_4^-$ was added to $^{99m}TcO_4^-$ for the preparations described above.

HPLC Studies

A Shimadzu SCL-6A System Controller fitted with two Shimadzu LC-6A pumps and a Waters Nova-Pak C18 Radial-PAK cartridge were used for all studies. Radioactivity in the effluent was measured by use of an Isoflo detector fitted with a gamma flow cell.

The mobile phase was prepared by dissolving 5 ml 40% tetrabutylammonium hydroxide in ~150 ml water and adjusting the pH to 7.4 using dilute phosphoric acid. After addition of the required volume of methanol, the volume was adjusted to 1 l with water and the solution membrane filtered. A linear gradient of 40–85% methanol was used for all studies (Fig. 2).

Adsorption losses on the column were measured by counting the total effluent in a gamma counter and comparing this count with the counts injected onto the column.

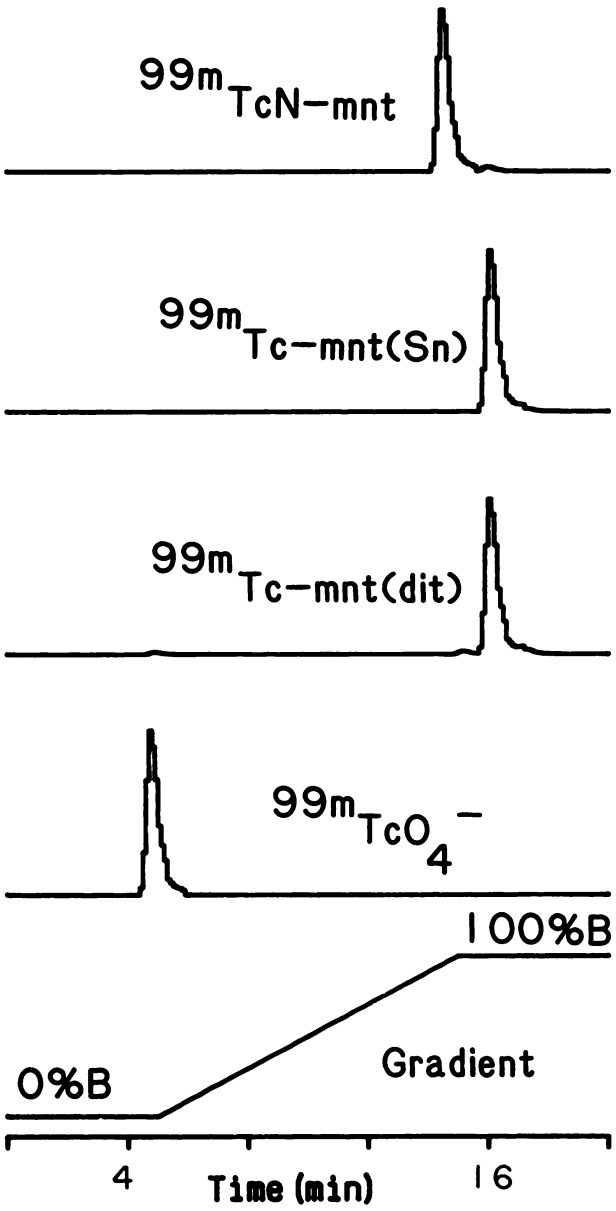

FIGURE 2
HPLC chromatograms of ^{99m}Tc-mnt complexes prepared via $^{99m}TcNCl_4^-$, stannous reduction (Sn) and dithionite reduction (dit). Solvent A: Bu_4N^+ in 40% methanol. Solvent B: Bu_4N^+ in 85% methanol. Column: Waters Nova-Pak C18 Radial-PAK cartridge. Flow rate: 2 ml/min.

Studies were performed to identify the chemical nature of the complex formed when the preparation was made without the addition of ^{99}Tc carrier. Samples of ^{99}Tc complexes were dissolved in methanol and diluted until a 100 μl aliquot gave a count rate of ~200,000 cpm when counted in a liquid scintillation counter. The solution of the ^{99}Tc complex was then spiked with a sample of a ^{99m}Tc preparation and 100–200 μl aliquots analyzed by HPLC. The effluent from the HPLC radioactivity detector was collected using a fraction collector and samples of each fraction measured for ^{99m}Tc and ^{99}Tc. Technetium-99 samples were measured by liquid scintillation counting after allowing an interval of 1 wk for decay of ^{99m}Tc.

Biologic Distribution Studies

The organ distribution of the injected activity (20–50 MBq, 0.1 ml) was measured at 0.5, 1, 2, and 6 hr after injection into the tail vein of Swiss mice (20–30 g). The injected activity was measured in a calibrated Capintec CRC-2N ionization chamber. The mice were allowed free access to food and water and three mice were killed at each time interval and dissected. Organs and the residual carcass were weighed and the activities measured in the ionization chamber. After correction of the original injected activity for activity localized in the tail (<2% in all cases), the organ distribution was calculated as the percentage of the original injected dose. Total blood activity was estimated on the assumption that the blood mass is 7% of total-body mass. At each time interval, one mouse was scanned by a Berthold LB2733 small animal scanner prior to dissection. No bone uptake was found in any scan.

Measurement of ^{99m}Tc Labeling

Aliquots (2 μl) of each preparation were chromatographed on Whatman No. 1 paper in three solvent systems: saline, 85% methanol, and ethyl methyl ketone (MEK). After development, all papers were dried and scanned using a radiochromatogram scanner (Packard 7220/21). Peaks were cut from the strips and counted for ^{99m}Tc activity in a gamma counter. All preparations contained <5% free pertechnetate.

RESULTS

HPLC Studies

mnt Complexes. HPLC chromatograms of ^{99m}Tc-mnt complexes prepared via the $^{99m}TcNCl_4^-$, dithionite, and ^{99m}Tc-gluconate(Sn) labeling procedures are shown in Figure 2. Labeling via $^{99m}TcNCl_4^-$ yielded a complex of high radiochemical purity (>97%) with a peak of retention time 14.7 min. The only contaminant was a small peak at 16.2 min. No free pertechnetate was evident. The use of ^{99m}Tc-gluconate (Sn) as the labeling intermediate gave a single peak at 16.2 min without any significant contaminant. The dithionite labeling procedure yielded a peak at 16.2 min with traces of radiochemical impurities (including pertechnetate). Borohydride reduction produced a similar chromatogram to dithionite reduction except that more free pertechnetate was evident. In no case did the addition of up to 70 μg ^{99}Tc in the preparation produce a significant alteration to the chromatogram.

The effect of varying the ligand concentration was also studied. Preparations of the ^{99m}Tc complexes were made using ligand concentrations of 1, 0.1, and 0.01 mg/ml. Using the $^{99m}TcNCl_4^-$ labeling procedure, only slight differences were observed in the chromatograms obtained. The major difference was the presence of ~5% $^{99m}TcO_4^-$ at the 0.01 mg/ml level. However, significant differences were evident with the complexes obtained via dithionite labeling—a number of peaks appeared as the ligand concentration was reduced. The complexes obtained via the $^{99m}TcNCl_4^-$ labeling procedure were stable, little change being observed even at

24 hr after preparation. In contrast, the ^{99m}Tc(dit) complexes showed significant changes with time, the most significant being the appearance of a peak at 17.3 min (~30% at 6 hr after preparation).

The tetraphenylarsonium salts of $[^{99}TcN(mnt)_2]^{2-}$, $[^{99}TcO(mnt)_2]^{-}$, and $[^{99}Tc(mnt)_3]^{2-}$ were used in the characterization of the ^{99m}Tc complexes. Histogram plots of the distribution of ^{99m}Tc and ^{99}Tc activity of the collected fractions of the various mnt complexes are shown in Figures 3–5. When a mixture of ^{99m}TcN-mnt and $[^{99}TcN(mnt)_2]^{2-}$ was passed through the HPLC system (Fig. 3), the distributions of ^{99}Tc and ^{99m}Tc activities in the collected fractions were identical. However, when a mixture of $[^{99}TcO(mnt)_2]^{-}$ and the ^{99m}Tc-mnt(dit) was passed through the HPLC system, the distributions of the ^{99}Tc and ^{99m}Tc activity in the collected fractions were different (Fig. 4) with the maximum ^{99}Tc activity being found one fraction after that containing the maximum ^{99m}Tc activity. Although there

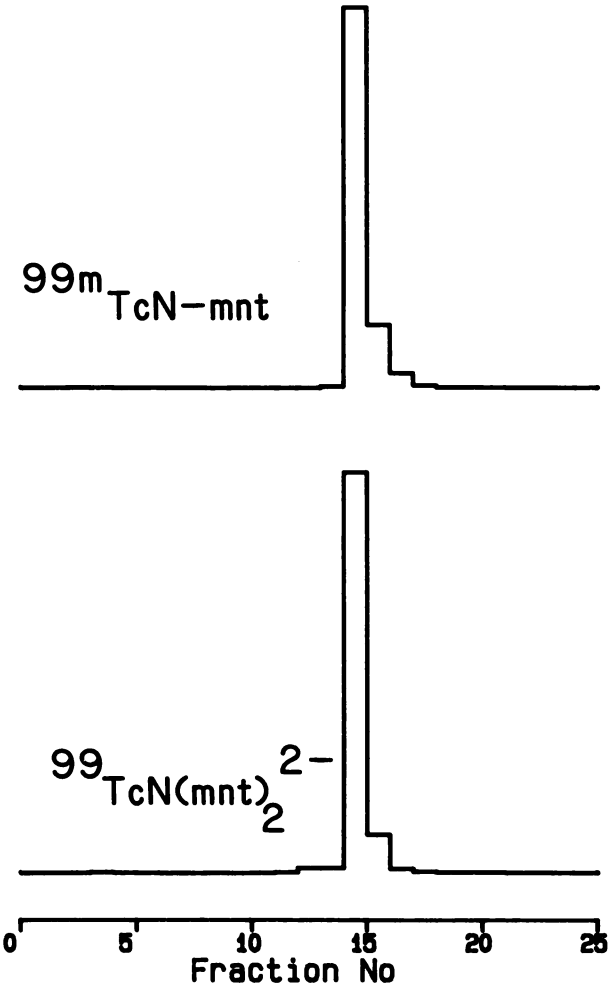

FIGURE 3
Histogram plots of the ^{99m}Tc and ^{99}Tc activity in each fraction (1 min) of the HPLC eluate following the injection of a mixture of ^{99m}TcN-mnt (prepared via $^{99m}TcNCl_4^-$) and $[^{99}TcN(mnt)_2]^{2-}$. HPLC conditions as in Figure 2.

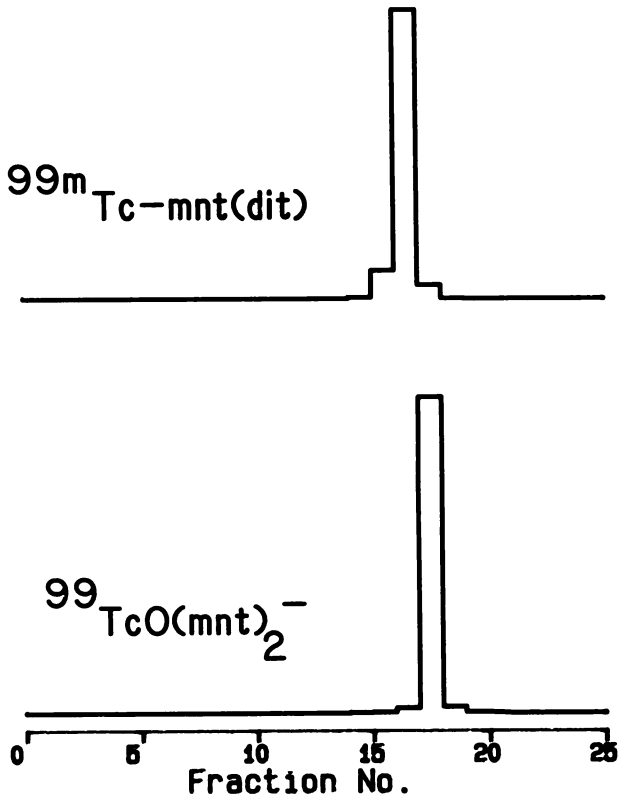

FIGURE 4
Histogram plots of the ^{99m}Tc and ^{99}Tc activity in each fraction (1 min) of the HPLC eluate following the injection of a mixture of ^{99m}Tc-mnt (prepared via dithionite reduction) and $[^{99}TcO(mnt)_2]^{-}$. HPLC conditions as in Figure 2.

is only one fraction difference in the ^{99m}Tc and ^{99}Tc peaks, the one fraction difference was observed in several runs and the result is conclusive proof that the complex being formed at the NCA level is not $[^{99m}TcO(mnt)_2]^{-}$ (see Discussion). Furthermore, the same difference in behavior was found when $[^{99}TcO(mnt)_2]^{-}$ was mixed with the ^{99m}Tc-mnt complex obtained either via ^{99m}Tc-gluconate(Sn) or by sodium borohydride reduction. However, when ^{99m}Tc-mnt labeled via $[^{99m}$Tc]gluconate(Sn) was mixed with $[^{99}Tc(mnt)_3]^{2-}$ and passed through the system, the distributions of the ^{99m}Tc and ^{99}Tc activity were essentially identical (Fig. 5). A similar result was obtained when $[^{99}Tc(mnt)_3]^{2-}$ was mixed with the ^{99m}Tc complex prepared either by borohydride or dithionite reduction. These results imply that at the NCA level $[^{99m}Tc(mnt)_3]^{2-}$ is formed when sodium dithionite, sodium borohydride, or stannous gluconate are used as the reducing agents.

dto Complexes. HPLC chromatograms of ^{99m}Tc complexes prepared via the $^{99m}TcNCl_4^-$ and sodium dithionite procedures are shown in Figure 6. The chromatogram obtained using the Tc(*V*) labeling procedure is also shown. The $^{99m}TcNCl_4^-$ labeling procedure was found to give a product of good radiochemical purity

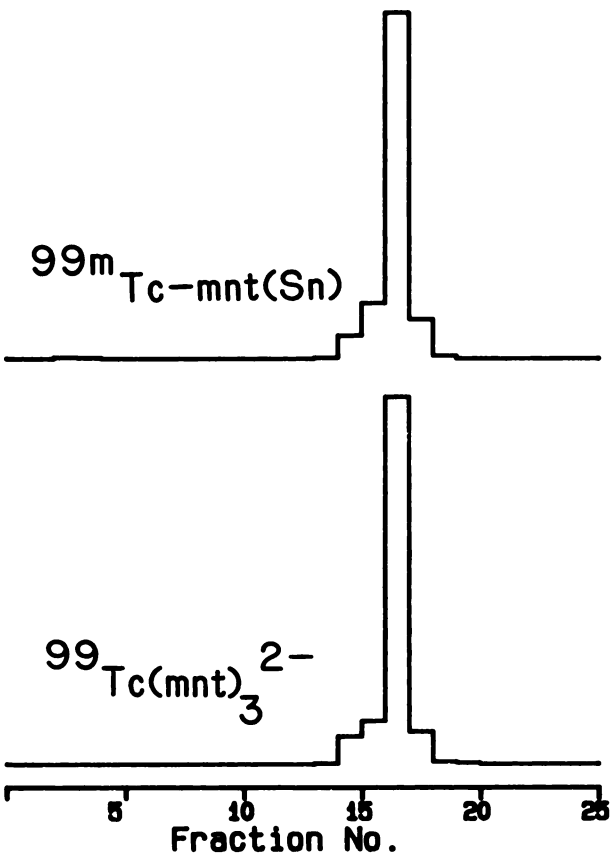

FIGURE 5
Histogram plots of the ^{99m}Tc and ^{99}Tc activity in each fraction (1 min) of the HPLC eluate following the injection of a mixture of ^{99m}Tc-mnt (prepared via stannous reduction) and $[^{99}Tc(mnt)_3]^{2-}$. HPLC conditions as in Figure 2.

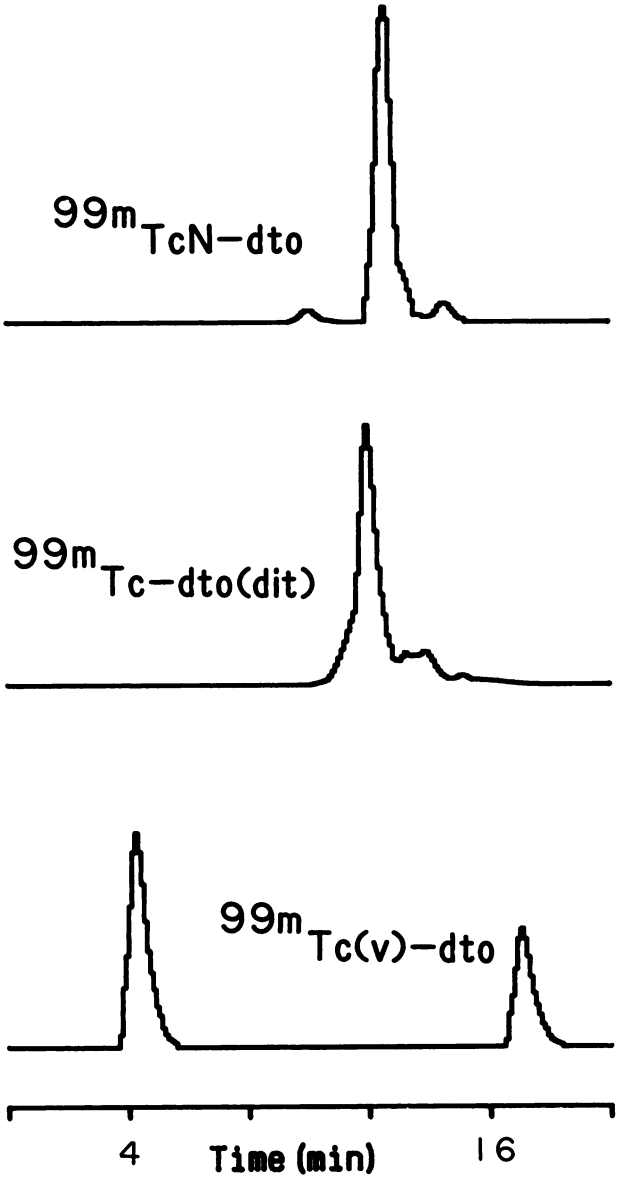

FIGURE 6
HPLC chromatograms of ^{99m}Tc-dto complexes prepared via $^{99m}TcNCl_4^-$, dithionite reduction and via $^{99m}TcOCl_4^-$. HPLC conditions as in Figure 2.

yielding a peak at 12.5 min. Sodium dithionite labeling usually produced a mixture of peaks, the relative proportion of which varied among different preparations. Radiochemical purity was usually improved by heating. While labeling efficiency with the Tc(*V*) procedure was poor (~40%), it was noted that the retention time of the complex formed (17.4 min) was different to the retention times of the complexes observed with sodium dithionite. Use of the stannous gluconate kit described failed to give satisfactory labeling because of precipitation of stannous-dto. Satisfactory labeling was achieved by the use of a kit containing a lower amount of stannous tin (17 μg $SnCl_2 \cdot 2H_2O$, 20 mg Ca gluconate). The HPLC chromatogram was similar to that shown for ^{99m}Tc-dto(dit). Addition of up to 70 μg ^{99}Tc in the $^{99m}TcNCl_4^-$ labeling procedure resulted in no change in the chromatogram. However, in the dit labeling procedure, increased amounts of a complex eluting at 17.4 min were produced as the amount of ^{99}Tc was increased.

Use of ligand concentrations of 0.1 mg/ml and 0.01 mg/ml had little effect on the product of the $^{99m}TcNCl_4^-$ labeling procedure. The only significant difference was the presence of ~8% $^{99m}TcO_4^-$ in the preparation obtained using 0.01 mg/ml dto. However, varying the dto concentration in the dithionite labeling procedure led to the appearance of additional peaks in the chromatogram.

Histogram plots of the fractions collected when a mixture of ^{99m}TcN-dto and $[^{99}TcN(dto)_2]^{2-}$ was passed through the HPLC system (Fig. 7), show the ^{99}Tc and ^{99m}Tc activity distributions to be identical, suggesting that the complex formed at the NCA level is $[^{99m}TcN(dto)_2]^{2-}$. The plots obtained when a mixture of $[^{99}TcO(dto)_2]^-$ and ^{99m}Tc-dto(dit) was passed through the system (Fig. 8), indicate that $[^{99m}TcO(dto)_2]^-$ is not formed at the NCA level using the preparation conditions described. The $[^{99}TcO(dto)_2]^-$ peak, however, ap-

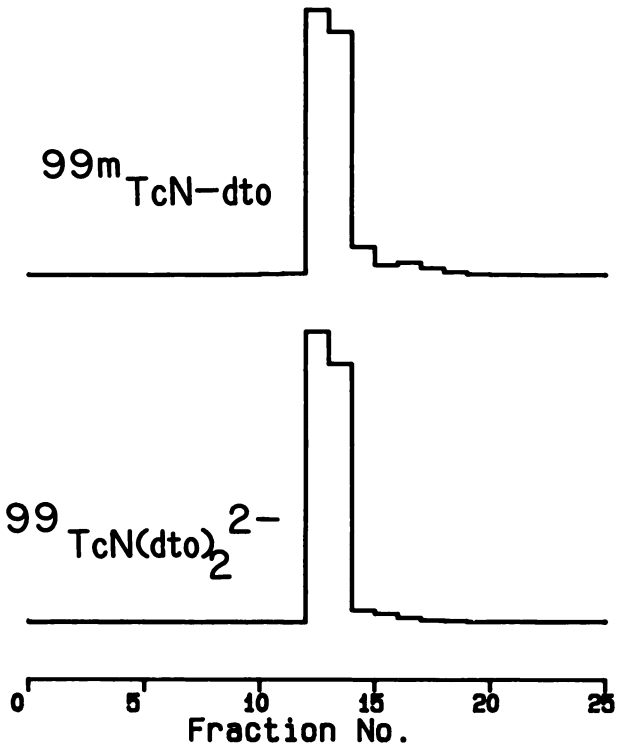

FIGURE 7
Histogram plots of the ^{99m}Tc and ^{99}Tc activity in each fraction (1 min) of the HPLC eluate following the injection of a mixture of ^{99m}TcN-dto (prepared via $TcNCl_4^-$) and $[^{99}TcN(dto)_2]^{2-}$. HPLC conditions as in Figure 2.

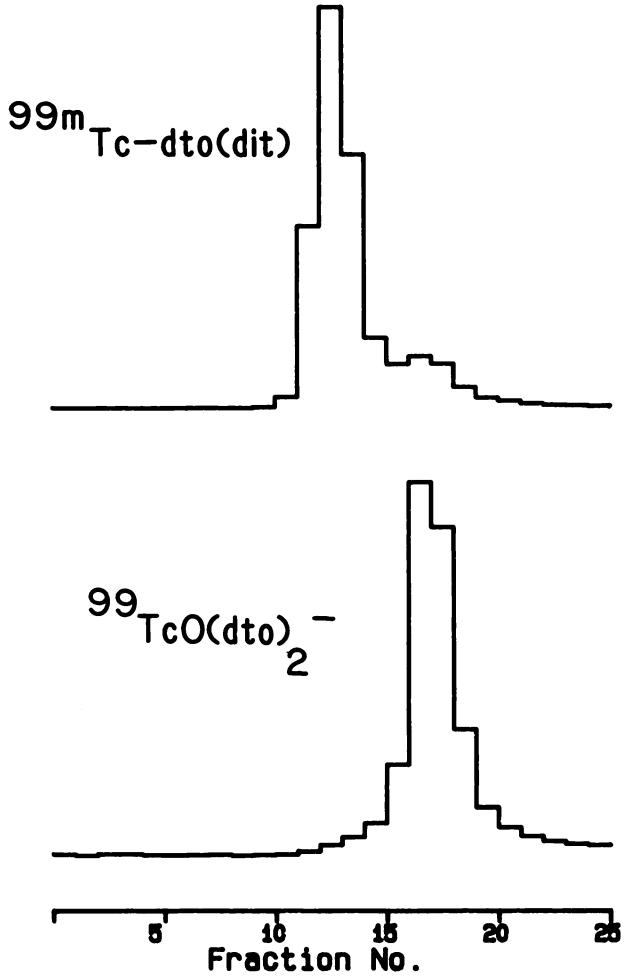

FIGURE 8
Histogram plots of the ^{99m}Tc and ^{99}Tc activity in each fraction (1 min) of the HPLC eluate following the injection of a mixture of ^{99m}Tc-dto (prepared via dithionite reduction) and $[^{99}TcO(dto)_2]^-$. HPLC conditions as in Figure 2.

pears to correlate with the ^{99m}Tc-complex peak observed in the Tc(*V*) labeling procedure.

In order to demonstrate that exchange was not taking place between ^{99m}Tc and ^{99}Tc during HPLC, the following mixtures were passed through the HPLC system and 1 min fractions collected and counted: ^{99m}TcN-mnt/$[^{99}TcO(mnt)_2]^-$, ^{99m}TcN-mnt/$[^{99}Tc(mnt)_3]^{2-}$, ^{99m}Tc-mnt(dit)/$[^{99}TcN(mnt)_2]^{2-}$, ^{99m}Tc-mnt(Sn)/$[^{99}TcN(mnt)_2]^{2-}$, ^{99m}TcN-dto/$[^{99}TcO(dto)_2]^-$ and ^{99m}Tc-dto(dit)/$[^{99}TcN(dto)_2]^{2-}$. In all cases there was no evidence of ^{99m}Tc-^{99}Tc exchange and the respective chromatograms were the same as the preparations run singly.

Biologic studies

Biologic distribution data in mice for the ^{99m}Tc-mnt preparations are given in Tables 1–3. ^{99m}TcN-mnt (Table 1) localized mainly in the liver where the activity remained essentially unaltered over 6 hr. The ^{99m}Tc-mnt preparations obtained both by sodium dithionite reduction (Table 2) and stannous gluconate reduction (Table 3) behaved identically in vivo, as would be expected from the HPLC results. With ^{99m}Tc-mnt(dit), 84% of the injected activity localized in the liver-intestines by 30 min with 66% being present in the liver. Liver activity cleared slowly into the intestines with 14% of the injected activity remaining in the liver after 6 hr. An unusual feature of the preparation was the slow increase in lung activity from 1.8% at 30 min to 3.3% at 6 hr. Blood activity also showed a small rise after 30 min.

In vivo differences between ^{99m}TcN-dto and ^{99m}Tc-dto were less marked (Tables 4, 5). Both complexes showed significant hepatobiliary clearance (^{99m}TcN-dto 35%, ^{99m}Tc-dto 42%) and both cleared from the liver at a similar rate. ^{99m}TcN-dto showed greater renal localization at 30 min after injection (11% vs. 4%) and showed far greater urinary clearance. Blood levels of ^{99m}Tc-dto were a factor of ~2 greater at all time intervals.

DISCUSSION

A major reason for the study of the chemistry and structure of technetium complexes using ^{99}Tc is to provide information regarding the chemical nature of the complexes formed by ^{99m}Tc at the concentrations

TABLE 1
Biologic Distribution of ^{99m}TcN-mnt In Mice

Time after injection	% Injected dose/organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	0.3 (0.1)*	0.3 (0.1)	0.2 (0.0)	0.3 (0.1)
Liver	79.6 (3.2)	82.6 (2.3)	83.5 (3.2)	79.8 (2.8)
Spleen	0.1 (0.0)	0.1 (0.1)	0.1 (0.0)	0.0 (0.0)
Stomach	0.1 (0.1)	0.3 (0.1)	0.5 (0.4)	0.2 (0.1)
Kidneys	4.9 (1.2)	4.2 (0.7)	2.8 (1.3)	2.7 (0.1)
Intestines	3.5 (0.3)	2.8 (0.7)	4.3 (0.4)	6.8 (1.9)
Blood	2.3 (0.3)	1.4 (0.5)	1.0 (0.0)	0.7 (0.1)
Urine	1.2 (0.6)	1.2 (0.8)	1.7 (0.2)	

Time after injection	% Injected dose/g organ			
	0.5 hr	1 hr	2 hr	6 hr
Liver	35.6 (1.8)	35.8 (1.4)	37.3 (5.6)	37.1 (3.3)
Kidneys	7.8 (0.6)	7.3 (0.7)	4.7 (1.1)	4.1 (0.3)
Blood	1.0 (0.1)	0.6 (0.2)	0.5 (0.1)	0.3 (0.0)

* Standard deviation in brackets.
n = 3.

TABLE 3
Biologic Distribution of ^{99m}Tc-mnt (Sn-gluconate) in Mice

Time after injection	% Injected dose/organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	1.9 (0.1)*	1.8 (0.3)	2.5 (0.6)	2.8 (0.0)
Liver	57.0 (4.2)	46.3 (1.5)	27.3 (3.1)	13.9 (1.1)
Spleen	0.2 (0.0)	0.1 (0.1)	0.1 (0.0)	0.1 (0.0)
Stomach	0.2 (0.1)	0.4 (0.3)	0.5 (0.2)	0.3 (0.0)
Kidneys	2.5 (0.2)	1.7 (0.4)	1.9 (0.0)	1.1 (0.1)
Intestines	19.5 (3.5)	29.1 (2.4)	45.6 (4.7)	30.3 (3.2)
Blood	5.0 (0.4)	4.7 (0.5)	5.7 (0.6)	4.4 (0.6)
Urine	2.9 (1.4)	3.3 (1.5)	3.9 (1.5)	

Time after injection	% Injected dose/g organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	7.0 (1.7)	6.0 (2.5)	7.9 (2.2)	9.9 (1.1)
Liver	27.9 (5.5)	21.3 (5.5)	12.0 (1.2)	6.4 (0.4)
Kidneys	3.8 (0.7)	2.5 (0.3)	2.7 (0.3)	1.7 (0.3)
Blood	2.0 (0.3)	1.8 (0.5)	2.0 (0.1)	1.7 (0.3)

* Standard deviation in brackets.
n = 3.

normally found in radiopharmaceuticals. Several workers (*11,12*) have used HPLC to characterize ^{99m}Tc complexes by using two detectors in series—a radiometric detector to detect ^{99m}Tc and a spectral detector to monitor added ^{99}Tc complexes. These studies have generally been performed to establish that the ^{99m}Tc complex is the same compound as a ^{99}Tc complex of known structure. The assumption is made that if the ^{99m}Tc complex and the ^{99}Tc complex elute at the same time, they are chemically identical. Technetium complexes can be formed in several valency states and at varying stages of hydrolysis and, in most cases, it is difficult to obtain a range of the possible complexes between Tc and a particular ligand. It is thus not possible to conclusively characterize the ^{99m}Tc complex on HPLC evidence alone, unless samples of the possible

TABLE 2
Biologic Distribution of ^{99m}Tc-mnt(dit) in Mice

Time after injection	% Injected dose/organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	1.8 (0.0)*	1.9 (0.0)	2.0 (0.4)	3.3 (0.7)
Liver	65.9 (3.8)	54.5 (0.7)	30.2 (5.7)	14.4 (0.4)
Spleen	0.5 (0.2)	0.2 (0.0)	0.1 (0.1)	0.1 (0.0)
Stomach	0.5 (0.2)	0.9 (0.3)	0.5 (0.0)	0.3 (0.3)
Kidneys	2.2 (0.3)	1.6 (0.3)	1.5 (0.4)	1.0 (0.2)
Intestines	19.1 (4.2)	25.4 (1.5)	47.3 (3.9)	42.1 (21.0)
Blood	3.1 (0.5)	4.1 (0.4)	4.1 (0.2)	2.7 (0.5)
Urine	0.0 (0.0)	0.0 (0.0)	1.6 (0.7)	

Time after injection	% Injected dose/g organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	6.2 (1.2)	6.5 (1.1)	7.9 (2.6)	12.7 (1.9)
Liver	30.6 (3.3)	26.8 (1.9)	12.0 (2.2)	6.6 (0.2)
Kidneys	3.5 (0.2)	2.7 (0.4)	2.2 (0.6)	1.6 (0.2)
Blood	1.3 (0.2)	1.7 (0.1)	1.5 (0.1)	1.1 (0.3)

* Standard deviation in brackets.
n = 3.

TABLE 4
Biologic Distribution of ^{99m}TcN-dto in Mice

Inter after injection	% Injected dose/organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	0.8 (0.3)*	0.8 (0.1)	0.9 (0.3)	0.3 (0.1)
Liver	24.7 (2.8)	20.2 (0.4)	15.6 (0.8)	10.8 (0.4)
Spleen	0.1 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.0)
Stomach	0.4 (0.1)	0.4 (0.2)	0.7 (0.1)	0.3 (0.0)
Kidneys	10.8 (1.7)	8.8 (2.7)	4.1 (1.0)	2.5 (0.1)
Intestines	10.9 (0.7)	12.0 (1.2)	16.1 (1.1)	8.9 (1.7)
Blood	10.8 (1.1)	8.3 (0.4)	6.4 (0.4)	5.6 (0.1)
Urine	29.1 (4.9)	37.8 (3.1)	46.2 (1.6)	

Time after injection	% Injected dose/g organ			
	0.5 hr	1 hr	2 hr	6 hr
Liver	15.3 (0.6)	12.5 (0.7)	9.8 (0.4)	7.7 (1.1)
Kidneys	22.5 (1.1)	17.4 (4.7)	9.3 (1.3)	5.7 (0.2)
Blood	5.4 (0.6)	4.2 (0.2)	3.6 (0.3)	3.1 (0.2)

* Standard deviation in brackets.
n = 3.

TABLE 5
Biologic Distribution of ^{99m}Tc-dto(dit) in Mice

Time after injection	% Injected dose/organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	1.9 (0.4)*	2.2 (0.3)	1.2 (0.1)	0.9 (0.2)
Liver	31.4 (1.6)	26.8 (1.8)	19.6 (1.8)	13.2 (0.6)
Spleen	0.4 (0.1)	0.3 (0.0)	0.3 (0.1)	0.2 (0.0)
Stomach	1.4 (0.3)	2.0 (0.2)	2.6 (0.6)	1.1 (0.2)
Kidneys	3.7 (0.6)	4.4 (0.2)	5.1 (0.1)	4.3 (0.3)
Intestines	10.8 (3.1)	16.4 (1.9)	23.4 (2.0)	23.0 (4.6)
Blood	19.5 (2.9)	19.3 (1.7)	16.7 (2.1)	12.3 (1.8)
Urine	8.0 (3.3)	13.4 (2.1)	21.3 (2.4)	

Time after injection	% Injected dose/g organ			
	0.5 hr	1 hr	2 hr	6 hr
Lung	10.9 (1.8)	10.2 (1.5)	7.0 (0.7)	6.0 (1.0)
Liver	27.5 (4.9)	22.5 (2.2)	18.5 (2.7)	14.0 (2.1)
Kidneys	11.0 (1.6)	14.8 (1.3)	16.4 (2.0)	16.4 (1.6)
Blood	14.0 (3.0)	13.9 (1.2)	12.0 (1.6)	9.9 (2.0)

* Standard deviation in brackets.
n = 3.

alternative ^{99}Tc complexes are available for use in the system. The availability of a range of Tc complexes of mnt and dto, gave us the opportunity to examine, by HPLC, the chemical composition of the complexes formed with mnt and dto at the NCA level.

At present, it is not possible to simultaneously monitor the ^{99m}Tc and ^{99}Tc activity of the effluent of a HPLC column. The common practice is to use a radiometric detector to monitor ^{99m}Tc and to measure the ^{99}Tc complex by absorption spectroscopy using either a uv or a diode array rapid scanning spectrophotometric detector. As the two detectors must be connected in series, there is a small time delay between the two detectors and there is also the possibility of band broadening if the volume of the detector cell of the second unit is significantly larger than that of the first. Rather than adopt this approach, we elected to use a fraction collector and measure the ^{99m}Tc and ^{99}Tc content of each fraction. Since there cannot be any chemical isotope effects between the two radionuclides ^{99m}Tc and ^{99}Tc, there cannot be any fractionation between identical complexes of the two radionuclides. As the counts injected on to the column were of the order of 200,000 cpm for ^{99}Tc and far greater for ^{99m}Tc, counting errors for the peak fractions were <1%. Thus, in the cases where the ^{99m}Tc peak fraction did not correspond with the ^{99}Tc peak fraction, because ^{99m}Tc and ^{99}Tc were measured in the same fraction, the result was conclusive proof that the predominant ^{99m}Tc complex was different to the ^{99}Tc complex. Where the ^{99}Tc complex and the ^{99m}Tc complex are identical, then the respective profiles should overlap exactly. It should be noted that profiles of histograms may vary between different runs because of variability in sample changing times of a fraction collector. The accuracy and precision of the method are based on the measurement of ^{99m}Tc and ^{99}Tc in the same fraction and the absence of chemical isotope effects between the two radionuclides. Technetium-99m and ^{99}Tc profiles are only compared in each run. Calculations show that if the fraction size is less than one-half the width of the peak, small differences in the peak retention times for ^{99m}Tc and ^{99}Tc peaks of the same shape would be readily detected. As discussed earlier in the paper, there is always some uncertainty in basing the identification of a ^{99m}Tc complex solely on HPLC data. Nevertheless, provided the fraction sizes are kept smaller than the peak widths obtained in the chromatogram, then the activity profile procedure is comparable in sensitivity to dual detection systems and may be the more accurate method where the molar absorptivity coefficient of the ^{99}Tc complex has a low value or other strongly absorbing complexes are present.

The HPLC system was one commonly used for the separation of anions: tetrabutylammonium buffer in methanol with a reverse phase column. Gradient elution yielded sharp peaks for most preparations. Adsorption losses on the column were measured by collecting the total effluent and were found to be <5%. Absence of exchange between ^{99m}Tc and added ^{99}Tc was demonstrated by running chromatograms of a wide range of mixtures of ^{99m}Tc complex and a known ^{99}Tc complex. If complete exchange takes place, then the distribution of ^{99m}Tc activity should be the same as the distribution of the ^{99}Tc activity. Thus, in this situation, the ^{99m}Tc trace obtained in HPLC should reflect that due to the ^{99}Tc complex added and the activity profiles of the ^{99m}Tc and ^{99}Tc content of the collected fractions would be identical. Partial exchange would result in the appearance of a peak at the elution time of the ^{99}Tc complex. In our experiments, in no case was the ^{99m}Tc chromatogram altered by the addition of a ^{99}Tc complex. The comparison of the ^{99m}Tc and ^{99}Tc profiles together with the absence of any change in the ^{99m}Tc trace from the chromatogram is thus a valid procedure for determining the structural identity of the ^{99m}Tc complex. However, as with other HPLC procedures, it is not conclusive proof of the identity of the ^{99m}Tc complex.

Use of the $TcNCl_4^-$ labeling procedure with mnt leads to the formation of $[TcN(mnt)_2]^{2-}$ at both the CA and NCA levels. $TcNCl_4^-$ has been shown to produce stable nitrido complexes at both the NCA and CA levels (*3,13*), and the result reported here is not unexpected. However, the results obtained using sodium dithionite and stannous tin (via gluconate) as reductants were entirely unexpected. The HPLC evidence clearly establishes that at the NCA level, the product formed is $[^{99m}Tc(mnt)_3]^{2-}$ and not $[^{99m}TcO(mnt)_2]^{2-}$. Spies et al.

have shown (*6,14*) that the use of ^{99}Tc-gluconate(Sn) as a labeling agent leads to the production of TcO complexes when used with a range of dithiols (including mnt). Indeed, they found no traces of the tris complex in their preparation of $[TcO(mnt)_2]^{2-}$.

The identity of the ^{99m}Tc complexes formed with dto is not as well defined. HPLC data indicate that $[^{99m}TcN(dto)_2]^{2-}$ is formed when $^{99m}TcNCl_4^-$ is used as the labeling agent. The product formed when sodium dithionite was used as the reducing agent has not been identified. Davison et al. (*4*) found that $TcOS_4^-$ complexes were formed when TcO_4^- was reacted with dithionite in the presence of thiols. Our results indicate that, at the NCA level, the product is not $[^{99m}TcO(dto)_2]^-$. However, we found that when 70 μg ^{99}Tc was added to the $^{99m}TcO_4^-$, significant quantities (40–60%) of a complex eluting at 17.0 min were obtained. It is probable that this peak is due to $[TcO(dto)_2]^-$, indicating that its formation is favored by CA levels of technetium. Because of the number of possible complexes that can be formed, it would be unwise in the absence of further evidence, to speculate on the composition of the ^{99m}Tc-dto product.

The biologic distribution data confirm earlier observations (*3*) which show that the biologic behavior of ^{99m}Tccomplexes is altered by the presence of the Tc-nitrido group. The difference between ^{99m}TcN-mnt and ^{99m}Tc-mnt(dit) is quite marked while that between ^{99m}TcN-dto and ^{99m}Tc-dto(dit) is not as well defined. One feature of the biologic data is the unusual behavior with respect to lung uptake observed with the ^{99m}Tc-mnt(dit) and ^{99m}Tc-mnt(Sn-gluconate) preparations. In these preparations the lung uptake increased from 1.8% injected dose at 30 min to 3.3% injected dose at 6 hr with the result that the lung became the dominant organ on scanning. The reason for this behavior is not apparent but it may be associated with a resorption of the complex into the blood from the G.I.T. (*15*).

In summary, it has been shown that HPLC is a useful tool in the identification of complexes formed at the NCA level. The results obtained indicate that care must be taken when extrapolating results from the CA level to the NCA level.

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REFERENCES

1. Melnik M, Van Lier JE. Analyses of structural data of technetium compounds. *Coord Chem Rev* 1987; 77:275–324.
2. Deutsch E, Libson K. Recent advances in technetium chemistry: bridging inorganic chemistry and nuclear medicine. *Comments Inorg Chem* 1984; 3:83–103.
3. Baldas J, Bonnyman J. Substitution reactions of $^{99m}TcNCl_4^-$—a route to a new class of ^{99m}Tc-radiopharmaceuticals. *Int J Appl Radiat Isot* 1985; 36:133–139.
4. Davison A, Orvig C, Trop HS, Sohn M, DePamphilis BV, Jones AG. Preparation of oxobis(dithiolato) complexes of technetium(*V*) and rhenium(*V*). *Inorg Chem* 1980; 19:1988–1992.
5. Colmanet SF, Mackay MF. Preparation and structure of tetraphenylarsonium bis[1,2-dicyanoethenedithiolato]oxotechnetate(*V*). *Aust J Chem* 1988; 41:151–155.
6. Spies H, Johannsen B. Preparation and characterization of tetraethylammonium bis(1,2-dicyanoethylenedithiolato)oxotechnetate(*V*). *Inorg Chim Acta* 1979; 33:L113.
7. Colmanet SF, Mackay MF. Preparation and structure of tetraphenylarsonium tris(1,2-dicyanoethenedithiolato)technetate(*IV*). *Aust J Chem* 1988; 41:1127–1131.
8. Abram U, Spies H, Gorner W, Kirmse R, Stach J. Lipophilic technetium complexes. Chelate complexes of technetium(*V*) containing the Tc-nitrido core. *Inorg Chim Acta* 1985; 109:L9–L11.
9. Colmanet SF, Mackay MF. Crystal structures of $(AsPh_4)[TcO(SCOCOS)_2]$ and $(AsPh_4)_2[TcN(SCOCOS)_2]$. A comparison of the Tc^VO^{3+} and Tc^VN^{2+} cores in the same coordination environment. *Inorg Chim Acta* 1988; 147:173–178.
10. Davison A, Holm RH. Sodium *cis*-1,2-dicyano-1,2-ethylenedithiolate. *Inorg Synth* 1967; 10:11–13.
11. Jones AG, Davison A, LaTegola MR, et al. Chemical and in vivo studies of the anion oxo[N,N′-ethylenebis(2-mercaptoacetimido)]technetate (V). *J Nucl Med* 1982; 23:801–809.
12. Deutsch E, Vanderheyden J, Gerundini P, et al. Development of nonreducible technetium-99m (III) cations as myocardial imaging agents: initial experience in humans. *J Nucl Med* 1987; 28:1870–1880.
13. Baldas J, Bonnyman J, Williams GA. Studies of technetium complexes. 9. Use of the tetrachloronitridotechnetate(*VI*) anion for the preparation of nitrido complexes of technetium. Crystal structure of bis(8-quinolinethiolato)nitridotechnetium(V). *Inorg Chem* 1986; 25:150–153.
14. Spies H, Johannsen B. Oxotechnetium(*V*)bis(dithiolato)complexes. *Inorg Chim Acta* 1981; 255–258.
15. Loberg MD, Porter DW, Ryan JW. Review and current status of hepatobiliary agents. In: *Radiopharmaceuticals II: Proceedings of the 2nd International Symposium on Radiopharmaceuticals.* New York: The Society of Nuclear Medicine, 1979: 519–543.