RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Chemical and In Vivo Studies of the Anion oxo[N,N′-ethylenebis(2-mercaptoacetimido)]Technetate(V)

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Studies of the anionic coordination complex ^{99}Tc-oxo[N,N′-ethylene-bis(2-mercaptoacetimido)]technetate(V) ([TcO(ema)]$^-$) are described. Syntheses performed both at carrier levels (10^{-5} *M*) and with no carrier added ($<10^{-8}$ *M*) indicate that the complex is formed virtually quantitatively from pertechnetate ion over this range. Tissue distributions in normal rats are similar at both concentrations up to one hour after administration. It has been shown—using a combination of high-pressure liquid chromatography and field-desorption mass spectrometry—that the anion is excreted unchanged into both urine and bile. The effectiveness of this N_2S_2 donor set in sequestering Tc-99m, and the in vivo stability of the resulting complex, suggest that modified chelates of this structural class could provide a series of useful diagnostic agents.

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In recent years the importance of technetium in the practice of nuclear medicine has prompted more intensive studies of its basic chemistry. Although much of this work appears at first sight to be tangential to radiopharmaceutical chemistry, in fact a large proportion has been directed toward investigations of complexes formed in oxidation states that are readily accessible in aqueous media by reduction of the pertechnetate anion. An unexpected feature that has emerged is the rich and varied oxochemistry in the +5 oxidation state. The first such complexes reported also demonstrated the pronounced avidity of technetium(+5) for sulfur donor atoms (*1,2*). Since then many water-stable compounds containing a variety of complexing groups and molecules have been identified and have added to the understanding of relative ligand affinities and stereochemical requirements of the element in this oxidation state (*3–21*).

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The oxotechnetium complexes can be conveniently categorized on the basis of their cores: (a) TcO^{3+} (*1–20*); (b) trans-TcO_2^+ (*9,11,21*); and (c) linear $Tc_2O_3^{4+}$ (*9*). Depending upon the nature of the ligating group, those of type (a) can be five-coordinate, e.g. $[TcO(SCH_2COS)_2]^-$ (*1*), or $[TcOCl_4]^-$ (*5*); six-coordinate, e.g. $[TcO(CN)_5]^{2-}$ (*9,11*), pyrazolyl-$TcOCl_2$ (*4*), or trans-$[TcO(OCH_3)(CN)_4]^{2-}$ (*11*); and seven-coordinate, e.g. $[TcO(edta)]^{2-}$ (Deutsch E, Libson K, unpublished results).

The five-coordinate complexes of type (a) are based on a square pyramidal arrangement of the ligating atoms about the metal center. The unique oxygen the TcO^{3+} core is at the apex of the pyramid with the basal plane comprised of the remaining four atoms. The Tc-O distance is short, typically about 1.60–1.75 Å, implying multiple-bond character. Complexes of this type are diamagnetic (d^2 spin paired) and the multiply-bonded oxo group makes the entire configuration behave electronically like a closed shell, rendering many complexes kinetically inert in aqueous solution.

As a result of our early findings, the concept arose of

a ligand that would be capable of sequestering technetium in the (+5) state after reduction of TcO_4^- in aqueous solution, and that could be functionalized with a variety of groups and molecules to generate, conceivably, a family of radiopharmaceuticals. The ligand was designed to be tetradentate so that it would (a) exploit the geometrical preferences of the TcO^{3+} core by spanning the basal plane of a square pyramid; (b) avoid the geometrical isomerism possible with unsymmetric bidentate ligands (i.e. cis- and trans- attachment to the metal center); (c) maximize the kinetic inertness of the final complex by taking advantage of the chelate effect; and (d) provide a backbone amenable to chemical modification.

In order to avoid the difficulties associated with the synthesis of a tetradentate tetrathiolate molecule, the potential ligand [N,N′-ethylene-bis(benzoyl-2-mercaptoacetamide) was prepared. This ligand is analogous to two bridged ethanedithiol molecules (*2*) with two of the thiolate sulfur atoms replaced by amide groups to allow formation of the chelate molecule and to permit the facile deprotonation of the nitrogen atoms necessary for binding the oxotechnetium core. The benzoyl groups serve to protect the thiols, and act as leaving groups during the chelation step.

Preliminary studies of Tc-99m-labeled anion oxo[N,N′-ethylene-bis(2-mercaptoacetimido)]technetate(+5) ($[TcO(ema)]^-$) in animals have been communicated previously (*22*), as have the synthesis of this and related S_2N_2 chelates, together with the characterization of the corresponding Tc-99 complexes (*15*). The rapid urinary clearance of this complex has been confirmed by Fritzbert (*23*) and in humans by Klingensmith (*24*). In this paper we report a comparison of the chemistry and pharmacokinetics of $[TcO(ema)]^-$ at both carrier-added (CA) and no-carrier-added (NCA) levels, some details of its structural characterization, and a confirmation of its chemical stability in vivo using field-desorption mass spectrometry.

MATERIALS AND METHODS

Synthesis of $[TcO(ema)]^-$. The synthesis and chemical characterization of the bisamide bisthiolato (S_2N_2) ligand N,N′-ethylene-bis[2-mercaptoacetimido] and the corresponding Tc-99 complex have been described elsewhere (*15*). The manipulations and precautions to be taken in handling macroscopic amounts of Tc-99 have also been reported previously (*10*). Technetium-99 as ammonium pertechnetate was obtained as a gift, and the metastable radionuclide Tc-99m as sodium pertechnetate from a commercial Mo-99→Tc-99m generator.

In order to establish the equivalence of the chemistry occurring at CA ($\sim 10^{-5} M$) and NCA ($\leq 10^{-8} M$) concentrations, $^{99m}TcO_4^-$ was added as a tracer to all carrier preparations so that every step could be monitored radiometrically. A typical CA preparation was as follows: 11.33 mg (2.71×10^{-5} mole) of the benzoyl-protected ligand were placed in 8 ml ethanol containing 1 ml 1 *M* NaOH, and allowed to dissolve completely. Seven ml distilled water were added, followed by 825 μCi of Tc-99m as sodium pertechnetate (generator eluate) in 0.1 ml of saline, and 25 μl of 0.382 *M* $NH_4{}^{99}TcO_4$ (9.55×10^{-6} mole). Then, 16.77 mg (9.63×10^{-5} mole) of the reducing agent sodium dithionite were added as a powder and the resultant yellow solution heated to boiling until the volume was reduced to half of the original. The pH was adjusted to 7.0 with hydrochloric acid and the product filtered through a 0.22 μm Millex-GS unit* before further use. The sodium dithionite could also be added as a solution when dissolved in a minimum volume of 1 *N* NaOH immediately before the reduction step. In practice, the amount of $^{99m}TcO_4^-$ added was varied according to the experiment being conducted; levels of all other reactants remained in the range indicated. The NCA syntheses were performed in a similar manner except that the $NH_4{}^{99}TcO_4$ was omitted. The terminal filtration step served as a precaution against the introduction of dust and other particulates into the HPLC system, and ensured sterility before in vivo injections.

Mass-spectral studies. The studies with field-desorption mass spectroscopy (FDMS) were done using a MAT 731 double-focusing mass spectrometer† fitted with a combination EI/FI/FD ion source operating in either positive- or negative-ion mode. Mass assignments were based on the instrument's mass markers, which had been calibrated with tris(pentadecafluoroheptyl)-1,3,5-triazine and tris(pentafluoroethyl)-1,3,5-triazine. Accurate atomic masses taken from the 1961 Nuclidic Mass Tables (*25*).

The salt tetra-n-butylammonium oxo[N,N′-ethylene-bis(2-mercaptoacetimido)]technetate(V) ([n-Bu_4N][TcO(ema)]) was used in all mass-spectral studies reported here. Normally, 0.5 ml of a $10^{-3} M$ solution of the complex in methylene chloride was prepared and loaded on the carbon-activated tungsten field ion emitters by dipping. Details of the technique have been published (*26*).

X-ray structural studies. For the single-crystal x-ray structural determination, the salt methyl-triphenylarsonium oxo[N,N′-ethylene-bis(2-mercaptoacetimido)]technetate(V) [$MePh_3As$][Tco(ema)] was obtained by metathesis from Na[TcO(ema)] using [n-Bu_4N]Cl and [$MePh_3As$]Cl, followed by slow evaporation of an acetone/water solution of the pure Tc-99 complex.

Crystal data for $C_{25}H_{26}N_2O_3S_2AsTc$: $M_r = 640.5$; monoclinic, $P2_1/c$; $a = 10.203(2)$, $b = 13.449(2)$, $c = 16.140(4)$ Å, $\beta = 110.37(1)°$, $Z = 4$; $d_{cald} = 1.65$ g cm^{-3}, $d_{meas} = 1.64(1)$ g cm^{-3}. Intensity data were collected using a diffractometer‡ with Mo Kα radiation. The Patterson map was analyzed by assuming two heavy atoms in the unit cell. Each of the eight possible solutions

was tested and one selected for further refinement. The remaining nonhydrogen atoms were located on an electron-density difference map. Full matrix least-squares refinement with anisotropic temperature factors for all noncarbon atoms converged to a conventional R value of 0.058 for 3539 reflections with $I > 3\sigma(I)$.

High-pressure liquid chromatography (HPLC) studies. These analyses were performed using a chromatograph equipped with an automatic injection system and controlled by a microprocessor. All ultraviolet absorbance measurements were made at 254 nm. Simultaneous radiometric traces were obtained by placing a 2- by 2-in. cylindrical NaI(Tl) detector as close as physically possible to the end of the column. Post-column tubing (diam. 0.23 mm) was wrapped in one and five loops around the crystal, giving a choice of counting efficiency according to the activity levels in the sample injected. The crystal was coupled to modular NIM** nuclear spectroscopic equipment with count collection being controlled by a data buffer (Model M1784§) with a deadtime of 100 μs, allowing virtually continuous monitoring of the radioactivity passing out of the column. The sensitivity of the chart recorder was varied with reference to a linear/logarithmic ratemeter.

Most studies employed a μ-Bondapak C_{18} column (30 by 0.39 cm)¶, protected by a guard column of pellicular C_{18} medium^{++} to remove any impurities that might be irreversibly bound to the separation column, and also by a silica precolumn¶ (35–39 μm particle size) to saturate the mobile phase. Separations were effected using 0.005 *M* tetra-n-butylammonium phosphate§ in water (Solvent A) and in methanol** (Solvent B). Initial conditions were A:B = 80:20. Upon injection of the sample, a 10-min linear gradient to a ratio A:B = 20:80 was performed, followed by a 5-min hold at the final condition. Flow rate was 2.0 ml/min and all experiments were done at ambient temperature. The usual injection volume was 25 μl.

Distilled water was purified by passing through organic removal and ion-exchange cartridges followed by redistillation. The methanol was HPLC grade. Both solvents were filtered through 0.7 μm GF/F filters¶ before use, and all samples of technetium complexes through 0.22 μm Millex-GS filter units before injection. The retention time of the anionic complex at all concentrations was verified using purified and characterized samples of $[^{99}TcO(ema)]^-$.

Animal studies. A. *In vivo distribution studies.* Conventional tissue distribution experiments were performed in normal male Sprague-Dawley rats at both CA and NCA levels, with 25–50 μCi (0.2–0.3 ml) of $[^{99m}TcO(ema)]^-$ being injected into the saphenous vein. The animals were killed in groups of six at varying intervals up to an hour after injection by a combination of ether anesthesia and cardiac exsanguination. A small number of dynamic studies were done, also in rats, using a scintillation camera coupled to a laboratory computer. The activity levels ranged up to 1 mCi, and data were collected for 30 min after administration.

Blood clearance data were obtained in three unconditioned male dogs, with samples being taken up to 4 hr after injection.

In all cases the sample injected was the original reaction mixture, with each preparation being assayed by HPLC.

B. *Treatment of bile and urine samples for FDMS analysis.* Preliminary tissue distribution results in rats (*22*) had indicated that the bulk of the radioactivity was excreted into urine, with a small proportion passing through into the intestines. In order to identify by mass spectroscopy the chemical species involved, samples of radioactive urine and bile were collected in rats and rabbits, respectively.

Spiked CA preparations of $[^{99m,99}TcO(ema)]^-$ were injected into normal male Sprague-Dawley rats and the urine recovered after 30 min. This was filtered through a Millex-GS filter unit, and 14 successive 100 μl aliquots injected onto the HPLC column. The predominant radioactive species (usually representing >98% of all radioactivity) was collected in each case, and the fractions pooled and evaporated to dryness. The residue was dissolved in 5 ml of water and passed through a C_{18} SEP-PAK.** Excess buffer was removed by rinsing with 15 ml of water, and the desired radioactive product eluted with 20 ml of a methanol/water mixture (35/65, v/v). The eluate was evaporated to dryness and treated as described above before being analyzed by FDMS.

Samples of radioactive bile (from male New Zealand albino rabbits) were obtained by catheterization. Under anesthesia, a midline incision was made from the xiphoid process to the pubic symphysis and the abdominal viscera retracted. Two small hemostats were fastened to the caudal serosal surface of the gall bladder and an incision made between them for introduction of the catheter (i.d. 10 mm) into the lumen. The proximal end of the catheter was held by a purse-string suture and the hemostats removed. After the catheter was secured, the common and cystic bile ducts were ligated, the viscera replaced, and the abdomen sutured closed.

Again, spiked CA samples of $[TcO(ema)]^-$ were injected intravenously and the bile collected. Samples totaling 9 ml in volume were analyzed sequentially by HPLC in 500-μl aliquots, using the same mobile phase described previously but under the following conditions: initial, 100% A; after injection, a 10-min linear gradient to 100% B; flow rate 2.0 ml/min. Pooled radioactive fractions were brought to dryness and a 10-ml solution of the residue in water passed through a C_{18} SEP-PAK. A 20-ml water rinse was followed by a 10-ml methanol elution of the radioactive material. The sample was treated before mass-spectral analysis as outlined previously.

RESULTS

Synthesis of [TcO(ema)]$^-$. As previously stated, [TcO(ema)]$^-$ is produced quantitatively at carrier levels without evidence for the formation of $TcO_2 \cdot xH_2O$ (*15*). Crystals of the material, with a variety of counter-ions, are air-stable and yellow or gold in color. Solutions of the complex at carrier levels reported here are also yellow and again show no evidence of TcO_2 by inspection or by ITLC.

High-pressure liquid chromatographic traces of a carrier-added synthesis are shown in Fig. 1A, and of a no-carrier-added preparation in Fig. 2A. The lower trace in each case is the radiometric output while the upper (delayed on the chart with respect to the lower one) is the uv absorption at 254 nm. In both cases the major component corresponds to a material with the same retention time (10.95 ± 0.04 min) as the purified and characterized Tc-99 complex, with activity integrating to at least 98% of that present in the injected sample. The major peak is cut off at the sensitivity depicted in order to exaggerate any minor peaks present. In Figs. 1A and 2A, following the major peak is an unidentified impurity or impurities occasionally seen as either one or two peaks. These have never been found to account for more than 0.5–2.0% of the total activity at any of the concentrations used in this work. In Fig. 1A, the peak preceding that of [TcO(ema)]$^-$ is $^{99m}TcO_4^-$, added to the sample of the complex injected to indicate the retention time of the pertechnetate ion under the separate conditions used. The uv peak seen eluting before the anion is that of benzoic acid, formed at complexation by the benzoyl leaving group.

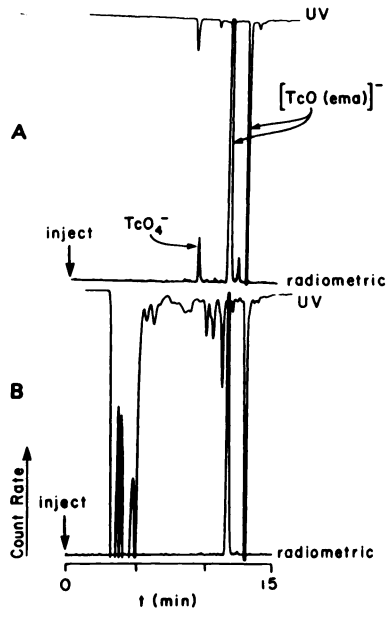

FIG. 1. HPLC scans of carrier-added samples of [^{99}TcO(ema)]$^-$ prepared from $^{99}TcO_4^-$ spiked with $^{99m}TcO_4^-$. A: chromatogram obtained after synthesis; B that of radioactivity collected from rat urine. In each, lower trace is radiometric output, and upper one (offset) the uv absorption monitored at 254 nm. Retention times of major component are 11.90 and 11.92 minutes, respectively. In A, $^{99m}TcO_4^-$ was added after synthesis to indicate its time of elution in this system (9.99 min). Note complexity of upper trace in B, due to uv-active (but nonradioactive) materials in urine.

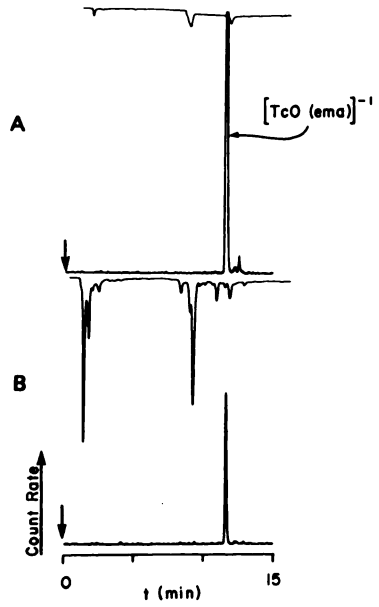

FIG. 2. HPLC scans of no-carrier-added samples of [^{99m}TcO(ema)]$^-$ prepared using generator eluant. A: chromatogram obtained after synthesis; B that of the radioactivity collected from rat urine. Retention times of major component are 11.98 and 11.99 min, respectively. In B, sensitivities permit complete peaks to be seen, in contrast to Fig. 1, where gain used exaggerates minor components. No uv absorption corresponding to [TcO(ema)]$^-$ is observed at NCA levels.

In our studies no evidence has been found under any analytical conditions of the large secondary peak representing 10–15% of the activity present as reported by Fritzberg (*23*). Occasional problems encountered in the synthesis of this complex have been traced to degraded samples of the reducing agent, sodium dithionite.

FDMS studies. Figure 3 shows FDMS of the purified complex [n-Bu_4N][^{99}TcO(ema)] recorded in positive-ion detection mode. The spectrum is simple and readily interpretable, the assignments being given in Table 1. The observation of cluster formation and of cationization by cation attachment is consistent with that found with other Werner-type technetium coordination compounds (*26*). No evidence has been seen for fragmentation of the anion.

The most intense peak in the spectrum is due to the n-butylammonium cation at m/z = 242, followed by that at m/z = 321, representing the molecular technetium anion associated by cationization with two protons. A weaker peak (m/z = 561) represents the molecular cation. The empirical formula of the technetium complex is readily derived from the differences in these values. The remaining peaks demonstrate dimerized entities of both the cation and the molecular anion.

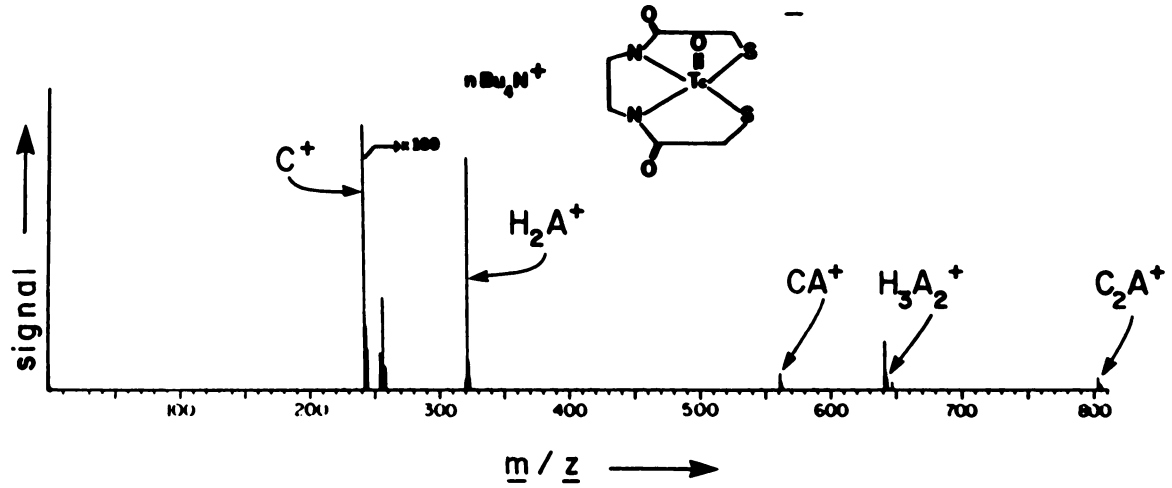

FIG. 3. Positive-ion field-desorption mass spectrum of the salt [n-Bu_4N][TcO(ema)] in *m/z* region up to 800. Species observed are identified and correspond to those shown in Table 1. In this form of mass spectrometry, cationic species are desorbed from the sample under influence of a strong field. Thus C^+ represents tetrabutylammonium cation, and CA^+ the molecular cation (M^+).

Single-crystal x-ray determination. The single-crystal x-ray structure determination of the salt [$MePh_3As$]-[TcO(ema)] provides an accurate assessment of the stereochemistry and dimensions of the technetium anion. The structure is shown in Fig. 4. The technetium atom is seen to be coordinated, in the manner predicted (*22*), to an oxygen atom and to two sulfur and two nitrogen atoms of the N,N′-ethylene-bis(2-mercaptoacetimido) ligand, forming a distorted square pyramid with the oxygen at the apex. The Tc-O bond length is 1.679(5)Å, almost identical to the 1.672(8)Å seen in *cis*-[$TcO(S\text{-}CH_2COS)_2]^-$ (*1*). The distance of the technetium atom above the square plane is also similar, 0.771(5)Å vs. 0.791(8)Å, respectively.

There is nothing remarkable in the other bond lengths but there are some differences between angles that should be equivalent. This is undoubtedly due to buckling in the five-membered rings surrounding the metal center. Thus the S(1)C(1)C(2)N(1), N(1)C(3)C(4)N(2), and N(2)C(5)C(6)S(2) torsion angles show values of 6.7(7)°, 30.5(7)°, and 29.6(7)°, respectively, demonstrating clearly the difference between the two SCCN rings. (The figure in parentheses indicates the standard deviation in the last significant figure). The distortion is even reflected in the ligand's square plane: N(1) is 0.0863(6) Å above and N(2) 0.076(6) Å below the best NSSN plane in which the two sulfur atoms lie. The rings thus have $\delta\delta\lambda$ or $\lambda\lambda\delta$ conformation [for C(1)C(2), C(3)C(4), C(5)C(6)] and the asymmetry in the anion is due to the conformational requirements of the chelate ring.

Complete details of the structure will be described in a separate communication.

Animal distribution studies. The partial distribution data obtained in normal rats at both CA and NCA concentrations are shown in Table 2. As reported previously in a preliminary form (*22*) and later confirmed by Fritzberg (*23*), the main feature is the rapid urinary clearance of the anion. This fact is underlined by the high levels of activity observed in the kidneys during the first 10 min. The rapid decrease in muscle and liver activity tends to suggest that both reflect the amount of material in blood. This is borne out by the similarity of the profile of the activity appearing in the intestines to that in the bladder, both essentially levelling out at approximately

TABLE 1. PEAK ASSIGNMENTS FOR POSITIVE- AND NEGATIVE- ION FIELD-DESORPTION MASS SPECTRA OF [n-Bu_4N]-[TcO(ema)]

	m/z	Assignment*
Positive-ion mode:	242	C^+
	321	H_2A^+
	561	$CA^+ = M^+$
	641	$H_3A_2^+$
	803	C_2A^+
Negative-ion mode:	319	A^-

* C^+ = [n-Bu_4N]$^+$: FW 242.
A^- = [TcO(ema)]$^-$: FW 319.

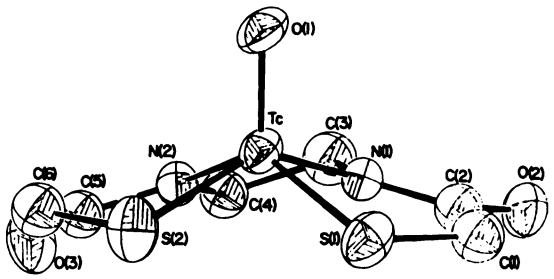

FIG. 4. Single-crystal x-ray structure of [^{99}TcO(ema)]$^-$. Salient details are given in text.

TABLE 2. TISSUE DISTRIBUTION OF [TcO(ema)]⁻ IN NORMAL RATS AT CARRIER- AND NO-CARRIER-ADDED CONCENTRATIONS

	5 min		10 min		20 min		30 min		60 min	
	CA†	NCA†	CA	NCA	CA	NCA	CA	NCA	CA	NCA
Liver	13.0 ± 1.0*	14.2 ± 1.3	7.1 ± 0.6	10.3 ± 1.2	2.6 ± 1.5	3.2 ± 1.5	2.9 ± 0.7	2.6 ± 1.6	0.6 ± 0.08	1.4 ± 0.9
Stomach	0.3 ± 0.09	0.2 ± 0.03	0.3 ± 0.1	0.2 ± 0.09	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.05	0.3 ± 0.1	0.1 ± 0.01
Kidneys	9.4 ± 1.4	14.5 ± 4.7	9.2 ± 1.6	8.8 ± 2.9	5.6 ± 0.8	5.6 ± 1.2	3.5 ± 1.2	3.1 ± 0.7	1.4 ± 0.4	3.3 ± 1.9
Bladder & urine	22.2 ± 10.3	15.3 ± 3.3	41.2 ± 4.6	20.5 ± 3.9	53.0 ± 3.9	36.9 ± 15.5	67.3 ± 2.9	59.4 ± 12.7	76.1 ± 6.1	60.5 ± 6.5
Intestines	4.3 ± 0.4	5.79 ± 0.9	5.6 ± 1.0	5.8 ± 0.4	6.8 ± 0.6	7.8 ± 1.2	7.4 ± 1.0	5.8 ± 1.1	7.9 ± 0.8	7.6 ± 3.1
Skin, fat, & fur+	10.2 ± 0.9	11.8 ± 1.6	13.2 ± 1.2	9.65 ± 2.2	6.5 ± 2.4	6.1 ± 1.3	2.6 ± 0.0	3.2 ± 0.7	1.5 ± 0.3	2.0 ± 1.1
Muscle+	20.6 ± 4.5	17.0 ± 5.5	14.0 ± 2.2	17.6 ± 3.4	4.3 ± 2.3	3.4 ± 0.4	2.8 ± 0.8	2.0 ± 0.3	1.8 ± 0.5	2.2 ± 2.4
Blood+	11.09 ± 2.0	12.9 ± 1.3	6.5 ± 1.0	10.0 ± 2.0	2.7 ± 1.2	3.5 ± 0.2	2.3 ± 0.7	1.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.2

* % ID/organ ± s.d. (n = 6 each point). + Assumed fractions of body weight: skin, fat, and fur 20%; muscle 40%; and blood 6.5%.
† CA: carrier added $^{99,99m}Tc \sim 10^{-5}$ molar.
NCA: no carrier added $^{99,99m}Tc \leq 10^{-8}$ molar.

20–30 min after injection. Gamma-camera images show clearly that the feces provide an alternative mode of excretion, and that localization or reabsorption of this activity does not occur. The studies also show no other concentrations of activity. Overall, the results at the two concentration levels are similar. The average values of urinary clearance obtained at the CA level appear to be consistently higher than those at NCA levels, but the results are not significant at 2σ.

Figure 5 shows typical time-activity curves for regions of interest set over the bladder and each of the kidneys. The figure actually depicts the results obtained when activity cleared into the urine of one rat was subjected to HPLC analysis, and the single radioactive component observed reinjected into a second animal. The plateau in urinary clearance is clearly seen to be established by approximately 25 min. Curves A and B (the activity detected over the right and left kidneys respectively) show a flattening and even a slight rise by 25–30 min, reflecting the passage of the anion through the portion of the intestines in the two fields of view.

Blood clearance data from three dogs are shown in Fig. 6. The anion passes quickly out of the blood, with levels falling in approximately 1% of the injected dose by 2 hr, and 0.4% by 4 hr. The proportion of the activity associated with red cells remains relatively constant throughout, averaging (38.0 ± 3.7%) (mean ± s.d.).

FDMS samples recovered from urine and bile. Figure 1A shows the radiometric scan of a CA preparation of the technetium anion (lower trace). The first peak eluted is pertechnetate deliberately added to the injected sample. Figure 1B indicates the result obtained on chromatographing the urine of a rat into which the preparation was injected. The same radioactive species are present and in the same integrated proportions. No evidence can be detected for the in vivo formation of pertechnetate, or for the excretion of any other species.

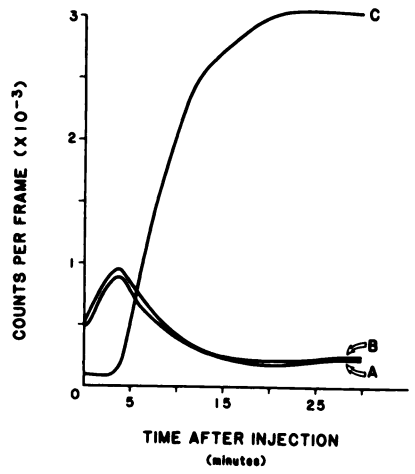

FIG. 5. Dynamic study of $[^{99m}TcO(ema)]^-$ in rat. Sample was isolated by HPLC from rat urine and subsequently reinjected. Regions of interest were set over right (A) and left (B) kidneys, and bladder (C).

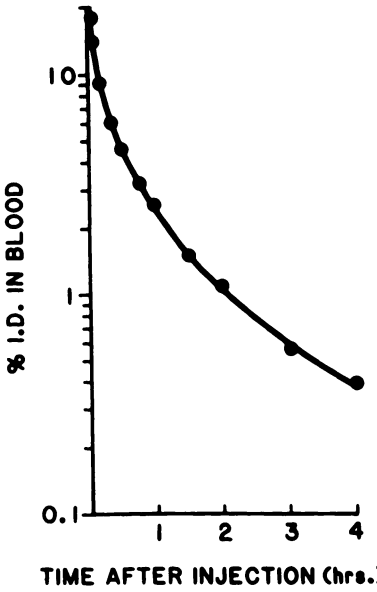

FIG. 6. Blood clearance curve for $[^{99m}TcO(ema)]^-$ in dogs (average of three animals).

Changes in the uv traces are due to other materials in urine.

Figures 2A and 2B depict the result of an analogous experiment performed at the NCA level. Again there is no evidence of radioactive species being excreted that were not present in the original synthesis. In both experiments the major radioactive component appeared at the retention time of $[^{99}TcO(ema)]^-$.

Following the pretreatment described, samples of the CA material recovered from urine were subjected to analysis by FDMS. Figure 7 shows a partial spectrum in the region m/z = 300 to 350, obtained in negative-ion detection mode. Using this mode, the spectra for the species of interest are even simpler, since all of the ion current is carried by the anion (Table 1). Note that in Fig. 3, a spectrum taken in positive-ion mode, the strongest signal is obtained from the cation. In order to achieve maximum sensitivity in the animal experiments, the former mode was used. In Fig. 7 the output signals are recorded at four different amplifications, with the most intense peak centered at m/z = 319, corresponding to the technetium anion. No evidence has been obtained of any other technetium species that might have been formed in vivo and eluted together with the known complex in the HPLC separation. The peak seen at m/z = 333 is an artifact that has also been seen in spectra of purified and fully characterized samples of the Tc-99 complex, and is thus unrelated to the passage of the complex through the body of the animal.

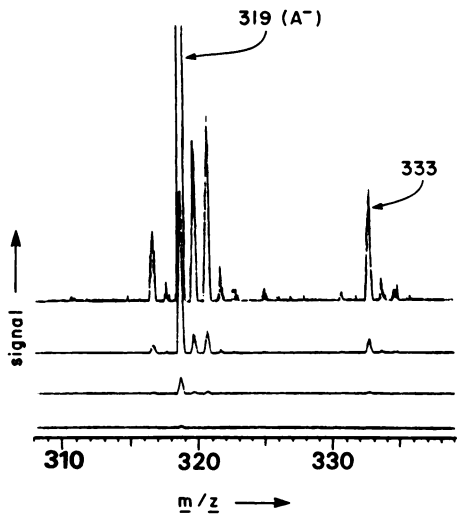

FIG. 7. Negative-ion mode field-desorption mass spectrum of CA sample isolated from rat urine, in m/z region 300–350 (anion region). In this form of FD, negatively charged species are desorbed (in contrast to positively charged entities in Fig. 3), so that the anion (m/z = 319) itself is observed. In this result, output signal is shown at four amplification levels.

The corresponding experiment with samples collected by catheterization of the gallbladder showed a single radioactive species to be present by HPLC. Under the modified separation conditions described earlier, the retention time of this material was 7.31 ± 0.03 min, the same as that of the purified sample of $[^{99}TcO(ema)]^-$. Figure 8 shows the negative-ion FD mass spectrum of the material isolated from bile in the m/z region 300 to 350. The most intense signal is that at m/z = 319, indicating the presence of the anion $[TcO(ema)]^{-1}$ in the sample.

DISCUSSION

The principal objective of this research was to investigate whether technetium complexes based on the S_2N_2 structural class would provide a means of developing a range of potentially useful Tc-99m-labeled radiopharmaceuticals. Accordingly, it had to be shown as far as possible that the prototype unmodified complex $[TcO(ema)]^-$ is stable not merely in vitro but also in

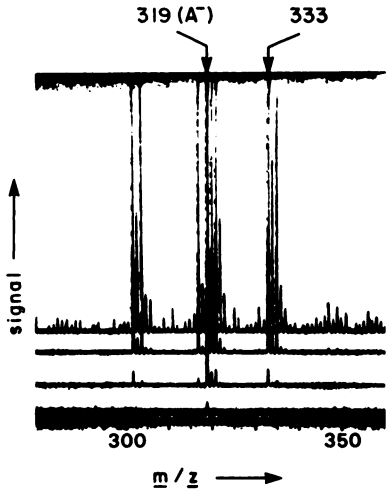

FIG. 8. Negative-ion mode field-desorption mass spectrum in anion region (m/z = 300–350) of CA sample isolated from rabbit bile. Again, result is shown at four amplification levels.

vivo, that this method of sequestering the element after its reduction yields complexes inert with respect to loss of the radiolabel. To demonstrate this, it was necessary to carry out the sequence of experiments reported here. First, the material being administered had to be fully characterized both chemically and structurally. Second, it had to be shown that the chemistry occurring at macro and at tracer concentrations is identical, and that a radiochemically pure complex could be synthesized. Next, it was essential to demonstrate that the distribution in normal animals at both concentrations is equivalent. This then permitted the final step to be taken: the use of carrier-added preparations for mass-spectroscopic analysis of radioactivity recovered from animals.

The chemical characterization of the Tc-99 complex, including the use of FDMS in positive-ion mode, has been published previously (*15*). The x-ray structural determination presented here confirms the originally predicted general form of the complex anion (*22*) but provides greater detail of its molecular geometry. This information will be a useful baseline for assessing the effect of modifications or substitutions made on the template molecule, and may possibly aid in a clearer understanding of structure-activity relationships within this class of complexes. For example, rapid clearance from nontarget areas is an important property for any potential radiopharmaceutical. Evidently some features of the $[TcO(ema)]^-$ ion promote renal clearance, and preserving these will be an important goal as modifications are made in the future.

The HPLC results show clearly that the required product is obtainable in high yield by reduction of pertechnetate in aqueous media over a concentration range of $10^{-3}M$ to less than $10^{-8}M$ in technetium. The lower limits studied are difficult to assess precisely since the technetium concentration is dependent on the generator's elution history, but are probably in the region of 10^{-10} to 10^{-11} molar (i.e. 10^{-12} mole of TcO_4^- added in the reaction). No evidence has been seen for $TcO_2 \cdot xH_2O$ formation, and the unidentified impurities seen in Figs. 1 and 2 never exceed 2% of the total activity present. When using pure characterized ligand, occasional problems in synthesis can invariably be traced back to the reducing agent, sodium dithionite.

The tissue distribution studies indicate that the behavior of the material at CA and NCA levels is essentially similar. In both cases, the bulk of the activity is cleared rapidly into the urine with a small proportion being excreted into the intestines. The results confirm our preliminary estimates (*22*) and agree with the findings of Fritzberg (*23*). The clearance rates reported for carrier-added preparations appear to be higher than those at no-carrier-added levels, but the differences does not seem to be statistically significant. Also, the data obtained by Fritzberg with Tc-99m syntheses match closely the carrier-level results here.

Chromatographic studies show that the material passing into urine has the same retention time as both the originally injected complex and the purified and characterized Tc-99 complex used as a standard. Furthermore, the major species isolated from urine by HPLC behaves like the anion when reinjected (Fig. 5). The radioactivity appearing in bile, however, raised the question as to whether there are two excretion pathways for the intact complex, or whether the anion is chemically modified in vivo and a different species is being cleared through bile. An answer to this was obviously necessary, for if the chelate were to prove unstable, further derivatization of the template molecule might prove unprofitable from the radiopharmaceutical standpoint. Separation of the radioactivity cleared into bile shows clearly that this is again one component with the same retention time on the column as both the injected anion and the purified Tc-99 standard.

The final proof of the nature of the chemical excreted appears in the results of mass spectrometry where the instrument is used in negative-ion mode. The spectra (Figs. 7 and 9) clearly show only the presence of $[TcO(ema)]^-$ in the samples isolated from both bile and urine.

There is at present little evidence that the pharmacokinetics of a new class of complex can be accurately predicted, and $[TcO(ema)]^-$ is no exception. Despite the fortuitousness of the discovery, the rapid renal clearance of this complex is nevertheless interesting. Fritzberg's comparison of this and ortho-iodohippurate (OIH) shows that these materials do not behave similarly (*23*). The urinary clearance of the technetium anion is slightly slower and cumulatively less over the time tested, and it is also excreted into bile, whereas OIH is not. Furthermore, the clearance is markedly affected by a depression of renal function, a fact confirmed clinically by Klingensmith (*24*). It therefore seems a worthwhile enterprise to search for a suitably modified form of the basic ligand that would give a complex with properties that are diagnostically useful.

FOOTNOTES

* Millipore Corporation, Bedford, MA 01730.
† Finnegan MAT, Sunnyvale, CA 94086.
‡ Syntex $P2_1$.
§ Camberra Industries, Meriden, CT 06450.
¶ Whatman Incorporated, Clifton, NJ 07104.
** Waters Associates Incorporated, Milford, MA 01757.

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