Preparation and Biological Distribution of Technetium Diphosphonate Radiotracers Synthesized Without Stannous Ion

Edward Deutsch, Karen Libson, Carolyn B. Becker, Marion D. Francis, Andrew J. Tofe, Debra L. Ferguson, and Lionel D. McCreary

University of Cincinnati and Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, Ohio

Two HEDP complexes of technetium (either Tc-99 or a mixture of Tc-99 and Tc-99m) have been prepared without the use of stannous ion. The first, Tc($NaBH_4$)-HEDP, is synthesized by reduction of TcO_4^- with $NaBH_4$ in the presence of excess HEDP; this is analogous to the preparation of Tc(Sn)-HEDP in commercial kits wherein Sn(II) functions as the reductant. The second, Tc-HEDP, is prepared by substitution of HEDP onto the pre-formed, pre-reduced, technetium center $TcBr_6^{2-}$. The HEDP-to-Tc ratio in Tc-HEDP was found to be 1.0 by double-labeling procedures (Tc-99 and [^{3}H]HEDP), implying that in solution this material is polymeric or at least dimeric. Preparations of Tc($NaBH_4$)-HEDP and Tc-HEDP with Tc-99m are excellent bone-imaging agents in both rats and dogs. Tissue distribution studies in rats show that uptake of Tc($NaBH_4$)-HEDP and Tc-HEDP by the bone is at least equivalent to that achieved by Tc(Sn)-HEDP prepared in commercial kits with Sn(II) as the reductant. Tin is therefore not necessary for the bone-seeking properties of Tc(Sn)-HEDP, and the in vivo distribution of a given HEDP radiotracer seems to depend primarily on the presence of the HEDP ligand and not on the exact nature of the technetium complex itself. Synthesis of technetium radiotracers by a substitution route, rather than by redox, is practicable; this route has the potential of introducing hitherto unattainable flexibility and subtlety into the preparation of technetium radiotracers.

J Nucl Med 21: 859–866, 1980

Diphosphonate complexes of technetium-99m have been shown to be effective bone-imaging agents (*1–3*), and have also been used widely as myocardial infarct-imaging agents (*4,5*). The clinical preparation of these tracers from commercially available kits involves the aqueous reduction of $^{99m}TcO_4^-$ with excess Sn(II) in the presence of excess diphosphonate ligand. This procedure leads to the injection of a dilute tin(II) solution into the patient. The injected tin has a long biological half-time (*6–8*) and generates several side effects (*9–11*). For this reason we thought it desirable to develop procedures for the synthesis of technetium-diphosphonate complexes that did not involve the use of stannous ion. In addition, the availability of technetium-diphosphonate complexes prepared without tin(II) will allow evaluation of the role of tin in the in vivo distribution and localization mechanisms of technetium-diphosphonate agents (*3,11*).

Tin(II) can be eliminated from radiopharmaceutical preparations by the simple expedient of replacing it with another reductant. In this work we have chosen $NaBH_4$, also used by other researchers (*12,13*), since this reagent rapidly reduces pertechnetate in dilute aqueous solution and excess $NaBH_4$ is readily hydrolyzed to boric acid, a chemically and biologically innocuous product. However, replacement of Sn(II) with another reductant does not mitigate the inherent limitations of radio-

Received July 20, 1979; revision accepted March 24, 1980.

For reprints contact: Edward Deutsch, PhD, Dept. of Chemistry, Univ. of Cincinnati, Cincinnati, OH 45221.

pharmaceutical preparations based on reduction of pertechnetate. These include lack of control over the oxidation state, coordination number, and coordination geometry of technetium in the final radiopharmaceutical, as well as lack of control over the variety and distribution of ligands coordinated to technetium in the final product. We therefore sought to develop a more controlled and more flexible route to technetium radiotracers. We have based this route on substitution rather than redox chemistry, and this work reports the synthesis of reduced technetium-diphosphonate complexes prepared by the substitution of diphosphonate ligands onto a prereduced technetium center. This substitution route is derived from the classical inorganic synthesis of technetium complexes and is quite general. We anticipate that the synthetic flexibility and control inherent in substitution reactions will eventually allow the preparation of new classes of technetium tracers wherein the chemical and biological properties of individual complexes can be precisely tailored to meet the requirements of nuclear medicine.

MATERIALS AND METHODS

Chemistry. Unless otherwise noted, all chemicals were of reagent grade. The disodiumdihydrogen and tetrahydrogen derivatives of (1-hydroxyethylidene)diphosphonate (Na_2H_2HEDP and H_4HEDP) were obtained commercially.* Technetium-99 of greater than 99% radiochemical purity was obtained as $NH_4{}^{99}TcO_4$† (either solid or in saturated aqueous solution); this material was contaminated with $^{99}TcO_2$, but could be readily purified by metathesis with KOH to $K^{99}TcO_4$ and subsequent crystallization of $K^{99}TcO_4$ from aqueous solution. $(NH_4)_2{}^{99}TcBr_6$ was prepared by treating a solution of $NH_4{}^{99}TcO_4$ with concentrated HBr and NH_4Br according to the general procedure of Dalziel et al. (*14*); the $^{99}TcO_2$ impurity in $NH_4{}^{99}TcO_4$ also reacts with concentrated HBr to yield the desired $(NH_4)_2{}^{99}TcBr_6$. [^{99m}Tc] pertechnetate solutions were obtained by elution of commercial generators with 0.9% NaCl.

Preparation of Tc(Sn)-HEDP. ^{99m}Tc(Sn)-HEDP was prepared from commercial kits according to the manufacturer's instructions. ^{99}Tc(Sn)-HEDP was prepared by reducing ~ 0.4 mmol $^{99}TcO_4^-$ with a twofold molar excess of $SnCl_2$ in an aqueous medium containing a fourfold molar excess of Na_2H_2HEDP. The brown product was precipitated by addition of concentrated $HClO_4$; successive dissolutions of this material in water, and reprecipitations with $HClO_4$, led to a material that was homogeneous to x-ray fluorescence analysis by scanning electron microscopy.†

Preparation of Tc($NaBH_4$)-HEDP. This material was prepared either with Tc-99 or with a mixture of Tc-99 and Tc-99m. A typical preparation containing both is as follows. To 0.24 ml of 0.05 *M* $K^{99}TcO_4$ were added about 100 mCi $^{99m}TcO_4^-$; this solution was then combined with 0.24 g Na_2H_2HEDP in approximately 6 ml H_2O. To this starting solution was then added 1.5 ml of a $NaBH_4$ solution that had been prepared by diluting 0.6 g $NaBH_4$ and 1.0 ml of 1.0 *M* NaOH to 25 ml with water. After stirring for about 1 min, the resulting reaction mixture was adjusted to pH 7 with 0.1 *M* $HClO_4$, one drop 30% H_2O_2 was added, and the solution was then diluted to 50 ml with 0.005 *M* Tris/$HClO_4$ buffer (pH 7.4). This diluted solution was then sorbed onto a Sephadex-QAE anion-exchange column (1.2 cm i.d. × 15 cm) and the desired brown band eluted with 0.1 *M* $NaClO_4$ in 0.005 *M* Tris/$HClO_4$ buffer (pH 7.4). The final band volume was ~10 ml. All manipulations were conducted in the presence of air; note that different complexes are formed when the manipulations are conducted under anaerobic conditions.

Preparation of Tc-HEDP. This material was prepared either with Tc-99 or with a mixture of Tc-99 and Tc-99m. A typical preparation containing both is as follows. To 0.1 ml of 0.4 *M* $NH_4{}^{99}TcO_4$ were added ~100 mCi $^{99m}TcO_4^-$, then 5 ml of concentrated HBr solution. The resulting solution was then heated in a mineral-oil bath for 30 min at 150°C (causing the HBr solution to reflux) and subsequently taken to dryness by directing an air stream at the surface of the heated solution. The dry residue was dissolved in 3 ml N,N-dimethylformamide, 30–35 mg H_4HEDP added, and the resulting solution heated for 10 min in a bath maintained at 85°C. After dilution to ~300 ml with 0.05 *M* Tris/$HClO_4$ aqueous buffer (pH 7), the reaction mixture was sorbed onto a Sephadex-QAE anion-exchange column (1.8 cm i.d. × 6 cm). The column was rinsed with about one column volume of 0.05 *M* $LiClO_4$ aqueous solution (buffered to pH 7 with Tris/$HClO_4$), then the brown-gold band was eluted with 0.2 *M* $LiClO_4$, collection being completed in a volume of <10 ml. All manipulations were conducted in the presence of air.

Preparation of Tc-(^{3}H)HEDP. In order to determine the HEDP-to-Tc ratio in the Tc-HEDP complex, a double-label experiment using Tc-99 and (^{3}H)-HEDP* was conducted as follows. $(NH_4)_2{}^{99}TcBr_6$ (7.6 μmol, 1.68 μCi/μmol) and (^{3}H)H_4HEDP (29.6 μmol, 4.26 μCi/μmol) in 0.13 ml N,N-dimethylformamide were heated at 84°C for 25 min. The reaction mixture was diluted to 25.0 ml with 0.05 *M* Tris/$HClO_4$ aqueous buffer (pH 7.4). After removal of aliquots for radiometric and chemical standardization, the remainder of the solution was adjusted to pH 7.4 with free Tris base and quantitatively transferred onto a Sephadex-QAE anion-exchange column (1.8 cm i.d. × 6 cm). To ensure complete removal of uncomplexed (^{3}H) HEDP, the column was rinsed with ~207 ml 0.05 *M* Tris/$HClO_4$ buffer (pH 7.4), the column effluent being collected in successive 20-ml portions. The desired doubly labeled product was then eluted with 10 ml 0.2 *M* $LiClO_4$ in 0.05 *M* Tris/$HClO_4$ buffer (pH 7.4). The final yield of ^{99}Tc-(^{3}H)HEDP, based on Tc-99, was ~35%. All column fractions were analyzed by beta scintillation for total Tc-99 and H-3 content.

Biology. Biological distribution and bone scintigraphic studies of the above technetium complexes were conducted in rats and dogs. Distribution studies were conducted with material prepared using only Tc-99, and with both Tc-99 and Tc-99m. The procedures detailed herein were used for materials containing both Tc-99 and Tc-99m; procedures with Tc-99 alone were essentially the same.

Rat studies. Twenty-four male Sprague-Dawley rats (200–220 g) were fasted overnight before the study. The rats were divided into six groups: Groups 1 and 2 contained two rats each and were used for scintigraphy only; Groups 3–6 contained five rats each and were used for tissue distribution studies. Doses of Tc($NaBH_4$)-HEDP and Tc-HEDP were prepared the morning of the study according to the foregoing procedures. Thin layer chromatography (TLC) was performed on each preparation before administration in order to qualitatively ensure complete complexing of the technetium; the absence of free $^{99m}TcO_4^-$ was subsequently confirmed quantitatively by Tc-99m assay of the TLC strip. All rats were anesthetized by Metofane (2,2-dichloro-1,1-difluoro-1-methoxyethane) inhalation. Groups 1, 3, and 5 received an intrajugular injection of 0.5 ml of Tc-HEDP, whereas Groups 2, 4, and 6 received 0.5 ml of Tc($NaBH_4$)-HEDP. Groups 1–4 were housed in the laboratory where the dose was given. At 5, 15, and 60 min, and 3 hr after the dose, bone images were made on the four rats in Groups 1 and 2. The gamma camera used was equipped with a high-resolution parallel collimator. Immediately following the dose, Groups 5 and 6 were moved to

the biological testing facility and housed in separate metabolism cages to facilitate quantitative collections of urine and feces. These rats received water *ad libitum*, but remained fasting for the remainder of the study. Tail cups were used to prevent coprophagy and urine-feces cross-contamination. Groups 3 and 4 were killed at 3 hr after dose; Groups 5 and 6 were killed at 24 hr. Animals from these four groups provided the biological samples necessary to determine tissue distributions of Tc-99m and Tc-99. Urine and feces were also quantitatively collected from animals in Groups 5 and 6. For each animal in Groups 3-6, right and left femurs, liver, thigh muscle, blood, spleen, kidney, heart, and stomach washed with isotonic saline were obtained. All biological samples were placed in tared scintillation vials, weighed, and assayed for Tc-99m and Tc-99, the latter by beta scintillation after decay of Tc-99m. For Groups 5 and 6, urine and feces collections were similarly analyzed.

Dog studies. Tc($NaBH_4$)-HEDP and Tc-HEDP were prepared and subjected to TLC quality control as above. One of two beagles (each ~8 kg) was injected with 1.0 ml Tc($NaBH_4$)-HEDP and the other with 3.0 ml Tc-HEDP. Injection (cephalic vein of left foreleg) of the second dog occurred ~10 min after injection of the first dog so that comparably timed blood samples could be obtained from the two dogs. An indwelling catheter was placed into the cephalic vein of the right foreleg of each dog for serial blood sampling. Blood samples (3 ml) were taken with heparinized syringes before the dose, and at 15 and 30 min, and 1, 2, and 3 hr after dose. They were placed in tared scintillation vials, weighed, and assayed for Tc-99m. After each sampling, heparinized 0.9% saline was injected to fill the catheter and ensure patency. The injected volume, plus 0.5 ml, was withdrawn and discarded before collecting subsequent blood samples. Scintiphotos of the dogs were obtained at 1, 2, and 3 hr after dose using the gamma camera.* For the 1- and 2-hr scintigrams the unanesthetized dogs were held upright in a sling (left lateral view). Before the 3-hr image, the dogs were anesthetized with sodium pentobarbital (30 mg/kg) so that several left lateral scintiphotos (right lateral recumbency) could be obtained under optimum conditions. After collection of the last blood sample, the dogs were placed in metabolism cages and urine and feces were collected at 24 and 48 hr for radioassay.

Stability of Tc-HEDP and Tc($NaBH_4$)-HEDP. In order to assess the stability of Tc-HEDP and Tc($NaBH_4$)-HEDP to ligand exchange, aqueous solutions of these complexes were incubated at 80°C with ethylenediaminetetraacetic acid (about 0.1 m*M* Tc-99 complex and 0.1 *M* EDTA in Tris/$HClO_4$ buffer, (pH 7.4), EDTA being known to have a high affinity for reduced technetium (*15,16*). The reaction mixtures were monitored spectrophotometrically over a 24-hr period.

RESULTS

Chemistry. Technetium complexes of HEDP can readily be prepared by (a) the standard aqueous tin(II) reduction of pertechnetate in the presence of excess HEDP ligand, (b) the analogous borohydride reduction of pertechnetate in the presence of excess HEDP, or (c) substitution of HEDP onto a prereduced technetium center such as $TcBr_6^{2-}$ in nonaqueous media. The three products, Tc(Sn)-HEDP, Tc($NaBH_4$)-HEDP, and Tc-HEDP, may be purified by aqueous anion-exchange procedures, and have similar, although distinct, chemical properties. All three products are anionic and exhibit an absorption maximum at ~400 nm. The two complexes investigated herein, Tc($NaBH_4$)-HEDP and Tc-HEDP, are very stable toward ligand substitution onto the reduced technetium center; no observable reaction with EDTA occurs over a 24-hr period. Macroscopically prepared Tc(Sn)-HEDP is a chemically stable species containing stoichiometric amounts of tin; in the solid state, x-ray fluorescence analysis yields Tc/Sn/P/Cl = 1/3/12 ± 4/1. Two independent double-labeling experiments with (^{3}H)-HEDP and Tc-99 show that in aqueous solution the HEDP-to-Tc ratio of Tc-HEDP is 1.0 (observed values of 0.97 and 1.10).

Biology. *Rat studies.* Tissue distributions of Tc($NaBH_4$)-HEDP and Tc-HEDP prepared with Tc-99, at 3 and 24 hr after dose, are given in Tables 1 and 2, respectively. Equivalent data for the complexes prepared with both Tc-99m and Tc-99 are given in Tables 3 and 4. There is no significant difference in distribution between the Tc-99 and Tc-99m. The biodistributions of

TABLE 1. BIOLOGICAL DISTRIBUTION OF Tc-99 IN RATS INJECTED WITH Tc($NaBH_4$)-HEDP*

	3 hr after dose		24 hr after dose	
Organ assayed	% dose/g	% dose/sample	% dose/g	% dose/sample
Right femur	1.72 (27)	1.16 (13)	1.64 (27)	0.98 (13)
Left femur	1.62 (25)	1.16 (10)	1.59 (28)	0.97 (9)
Femur muscle	<0.06		<0.05	
Heart	≤0.06†	≤0.06†	<0.05	<0.04
Liver	0.08 (1)	0.74 (10)	0.05†	0.40†
Spleen	<0.07	<0.04	<0.09	<0.04
Kidneys	0.84 (15)	1.83 (35)	1 (1)	3 (2)
Stomach	0.08 (2)	0.11 (4)	≤0.04†	≤0.06
Blood	0.04 (1)		<0.03	
Feces				3 (4)
Urine				56 (9)

* n = 5 unless otherwise noted. Standard deviation of last significant digit(s) given in parentheses.

† n = 4. Values of % dose/g are 0.04, 0.04, 0.09, 0.05, and <0.03. Values of % dose/sample are 0.30, 0.34, 0.58, 0.39, and <0.24.

TABLE 2. BIOLOGICAL DISTRIBUTION OF Tc-99 IN RATS INJECTED WITH Tc-HEDP*

Organ assayed	3 hr after dose		24 hr after dose	
	% dose/g	% dose/sample	% dose/g	% dose/sample
Right femur	2.05 (22)	1.31 (3)	1.97 (24)	1.30 (7)
Left femur	1.97 (24)	1.29 (5)	1.99 (22)	1.29 (4)
Femur muscle	<0.04		<0.05	
Heart	≤0.040	≤0.03	<0.03	<0.03
Liver	≤0.07	≤0.85	0.05 (1)	0.35 (11)
Spleen	<0.05	<0.03	<0.06	<0.03
Kidneys	0.77 (5)	1.74 (27)	0.62 (5)	1.22 (8)
Stomach	0.08 (4)	0.11 (5)	≤0.04	≤0.04
Blood	0.050 (2)		<0.01	
Feces				1.61 (27)
Urine				38 (20)

* n = 5. Standard deviation of last significant digit(s) given in parentheses.

Tc-99m HEDP and Tc-99m HEDP are graphically compared in Fig. 1; those for Tc-99m HEDP, ^{99m}Tc(Sn)-HEDP, and ^{99m}Tc($NaBH_4$)-HEDP in Fig. 2. Scintiphotos obtained at 3 hr after dose using each of the three agents are compared in Fig. 3.

Dog studies. Data comparing the urinary and fecal excretion of Tc($NaBH_4$)-HEDP, Tc-HEDP, and Tc(Sn)-HEDP are presented in Table 5. Blood clearance curves are compared in Fig. 4, and 3-hr scintiphotos in Fig. 5.

DISCUSSION

Whereas the preparations of Tc(Sn)-HEDP and Tc($NaBH_4$)-HEDP are based on the usual reduction of aqueous pertechnetate in the presence of excess ligand, the synthesis of Tc-HEDP described in this paper involves a fundamentally different route based on substitution chemistry rather than redox. Prior reduction of pertechnetate, with isolation of a stable intermediate containing reduced technetium, minimizes potential interference by the reducing agent or its reaction products. In our procedure concentrated HBr serves (a) as the reducing agent that converts Tc(VII) to Tc(IV), (b) as a source of bromide ligands to stabilize the Tc(IV) as $TcBr_6^{2-}$, and (c) as a source of acid to keep the reduced technetium from hydrolyzing to TcO_2. Concentrated HBr is also a convenient reagent in that it is easily removed by evaporation after reduction has occurred. Substitution of a variety of ligands onto $TcBr_6^{2-}$ is readily accomplished in aqueous or nonaqueous media; we used N,N-dimethylformamide as a reaction solvent since both $(NH_4)_2TcBr_6$ and H_4HEDP are readily soluble in this medium. In general, use of a nonaqueous solvent provides a synthetic advantage in that lipophilic ligands, not soluble in water, may readily be used to prepare radioagents that cannot be easily synthesized in water.

Preparations of Tc(Sn)-HEDP, Tc($NaBH_4$)-HEDP, and Tc-HEDP contain several components that may be

TABLE 3. BIOLOGICAL DISTRIBUTION OF Tc-99m IN RATS INJECTED WITH Tc($NaBH_4$)-HEDP*

Organ assayed	3 hr after dose		24 hr after dose	
	% dose/g	% dose/sample	% dose/g	% dose/sample
Right femur	1.84 (33)	1.24 (14)	1.70 (27)	1.02 (13)
Left femur	1.74 (28)	1.24 (11)	1.66 (33)	1.02 (11)
Femur muscle	0.009 (2)	0.003 (2)	0.002 (1)	0.002 (1)
Heart	0.024 (6)	0.023 (6)	0.007 (2)	0.006 (1)
Liver	0.092 (16)	0.81 (14)	0.050 (16)	0.37 (10)
Spleen	0.025 (4)	0.015 (3)	0.017 (6)	0.007 (2)
Kidneys	0.98 (29)	2.15 (66)	1.22 (76)	3 (2)
Stomach	0.081 (20)	0.117 (34)	0.032 (32)	0.043 (10)
Blood	0.050 (3)	0.135 (33)	0.009 (4)	
Feces				3 (4)
Urine				54 (9)

* n = 5. Standard deviation of last significant digit(s) given in parentheses.

TABLE 4. BIOLOGICAL DISTRIBUTION OF Tc-99m IN RATS INJECTED WITH Tc-HEDP*

Organ assayed	3 hr after dose		24 hr after dose	
	% dose/g	% dose/sample	% dose/g	% dose/sample
Right femur	2.19 (19)	1.41 (3)	2.14 (21)	1.42 (5)
Left femur	2.14 (27)	1.40 (6)	2.16 (24)	1.41 (6)
Femur muscle	0.0090 (4)	0.008 (2)	0.004 (1)	0.003‡
Heart	0.025 (2)	0.022 (3)	0.009 (2)	0.0070 (8)
Liver	0.071 (7)	0.67 (12)	0.044 (4)	0.33 (3)
Spleen	0.027 (1)	0.016 (1)	0.022 (2)	0.0100 (9)
Kidneys	0.85 (8)	1.9 (3)	0.66 (4)	1.30 (8)
Stomach	0.064†	0.082†	0.1 (2)	0.029 (10)
Blood	0.054 (12)	0.160 (38)	0.007‡	
Feces				1.8 (8)
Urine				36 (22)

* n = 5 unless otherwise noted. Standard deviation of last significant digit(s) given in parentheses.

† n = 4. Values of % dose/g are 0.069, 0.093, 0.052, and 0.044. Values of % dose/sample are 0.103, 0.071, 0.085, and 0.070.

‡ Standard deviation less than one in the last significant digit.

separated by anion-exchange chromatography. This separation markedly reduces the number of different chemical species in the final product, and therefore makes at least possible the rational study of structure-activity relationships. The column chromatographic procedures outlined above are not prohibitive in terms of time: the entire $Tc(NaBH_4)$-HEDP preparation requires about 30 min, and that for Tc-HEDP ~2 hr. The number of product components in the $Tc(NaBH_4)$-HEDP preparation may be decreased by mild oxidation with O_2 or H_2O_2; vigorous oxidation reconverts the reduced technetium to pertechnetate. The original reductions with Sn(II), $NaBH_4$, or HBr are very efficient in that there is no detectable pertechnetate in the reaction mixture. Of the several components that can be separated from the $Tc(NaBH_4)$-HEDP and Tc-HEDP preparations, we have arbitrarily taken the predominent one (under the conditions outlined above) as the designated radioagent. However, the column behavior of these components indicates that each product is not a single chemical species, but is more probably a mixture of polymeric entities involved in time-dependent hydrolytic equilibria that interconvert the various polymeric forms. This view is supported by several lines of evidence.

1. The HEDP-to-Tc ratio of 1.0 observed for Tc-HEDP in solution implies a dimeric or polymeric structure, since there is no way that a single HEDP li-

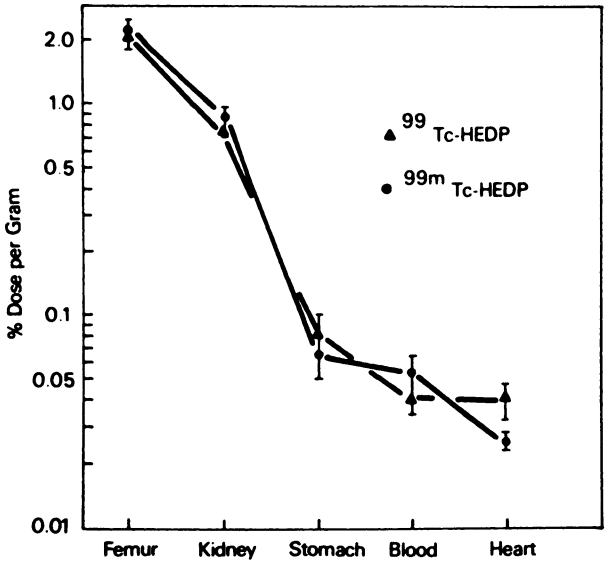

FIG. 1. Comparative biodistributions of Tc-99 HEDP and Tc-99m HEDP in Sprague–Dawley rats (n = 5) at 3 hr after i.v. dose.

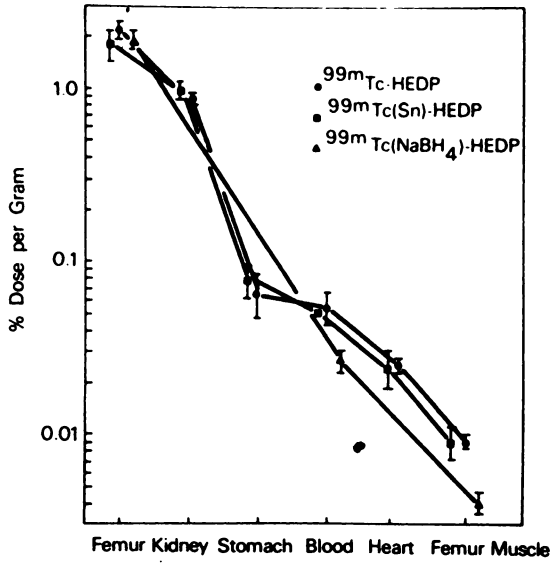

FIG. 2. Comparative biodistributions of Tc-99m HEDP, $^{99m}Tc(Sn)$-HEDP, and $^{99m}Tc(NaBH_4)$-HEDP in Sprague–Dawley rats (n = 5) at 3 hr after i.v. dose.

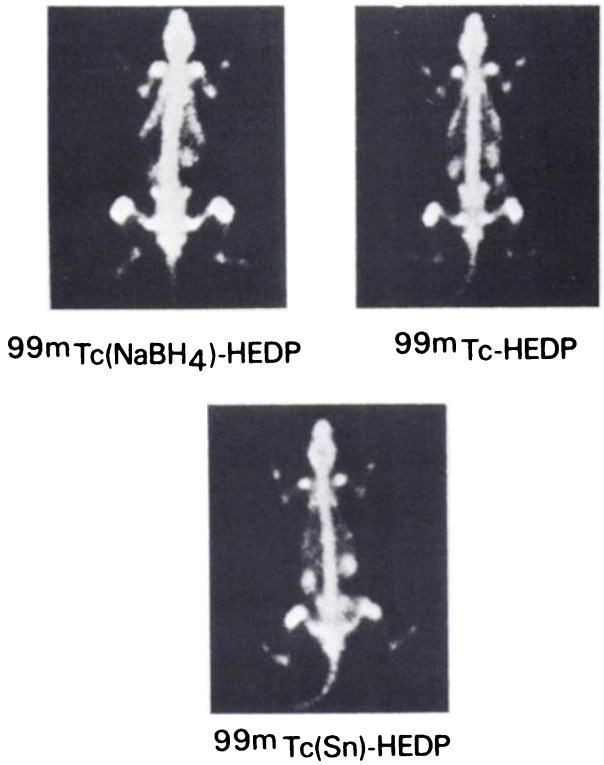

FIG. 3. Comparative scintiphotos of Sprague–Dawley rats imaged with ^{99m}Tc(NaBH$_4$)-HEDP, ^{99m}Tc-HEDP, and ^{99m}Tc(Sn)-HEDP at 3 hr after i.v. dose.

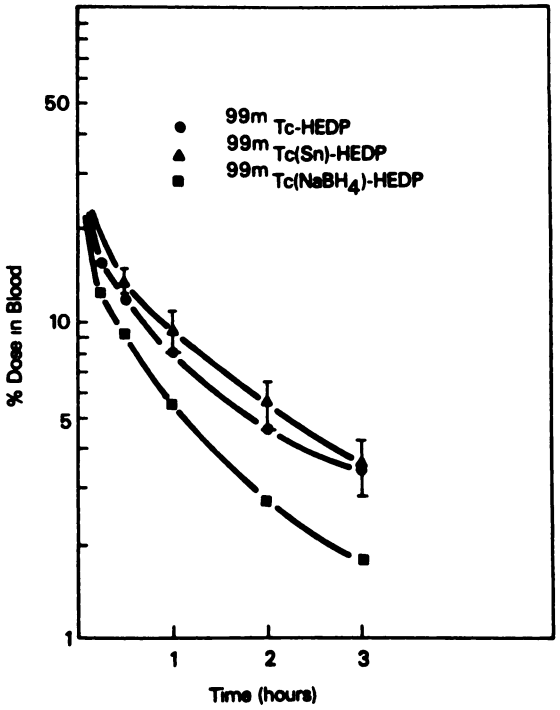

FIG. 4. Comparative blood clearances of Tc-99m HEDP, ^{99m}Tc(Sn)-HEDP, and ^{99m}Tc(NaBH$_4$)-HEDP in beagle dogs.

gand can satisfy the coordination requirements of reduced technetium within a monomeric complex.

2. A recent single crystal x-ray structural determination of Tc-MDP (MDP = methylenediphosphonic acid) (*17*) shows that this analogous complex exists in the solid state as a polymeric array with MDP/Tc = 1.0, each MDP bridging two technetium centers.

3. Recent work on the analogous Tc(Sn)-PO_4 system (*18*) shows that the components of this formulation are multinuclear and are involved in time-dependent equilibria.

Further work will be needed to determine whether these polymeric species are also important at the low Tc-99 concentrations present in extant radiopharmaceutical preparations.

Even though initial preparations appear to consist of interconverting polymeric forms, the results of EDTA substitution experiments indicate that, once a given HEDP-technetium preparation has achieved equilibrium with respect to polymeric forms, the reduced-technetium chromophore is inert to substitution by EDTA. This could occur because the HEDP complexes are thermodynamically more stable than Tc-EDTA, but this hypothesis must await further experiments. The behavior

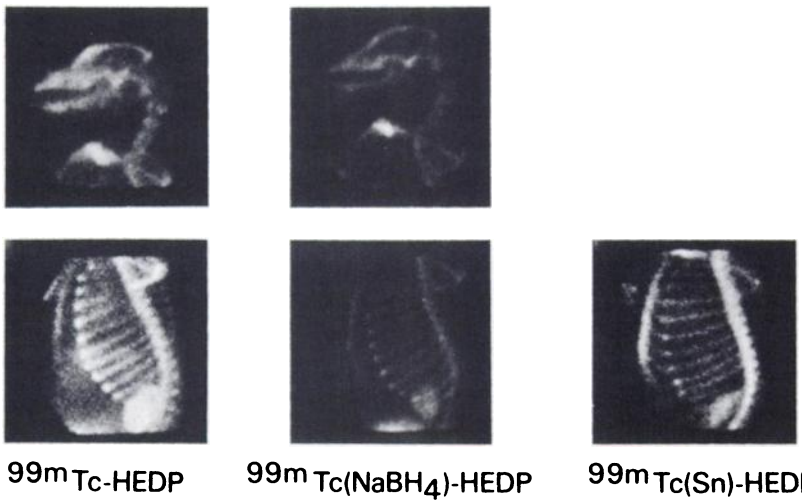

FIG. 5. Comparative scintiphotos of beagle dogs imaged with Tc-99m HEDP, ^{99m}Tc(NaBH$_4$)-HEDP, and ^{99m}Tc(Sn)-HEDP at 3 hr after i.v. dose.

TABLE 5. PERCENT DOSE OF Tc-99 AND Tc-99m EXCRETED FROM BEAGLE DOGS FOR Tc($NaBH_4$)-HEDP, Tc-HEDP, AND Tc(Sn)-HEDP

	Tc($NaBH_4$)-HEDP		Tc-HEDP		Tc(Sn)-HEDP
	99	99m	99	99m	99m
Urine					
0–24 hr	70.6	67.0	56.3	56.8	~70
24–48 hr	8.8	8.0	3.3	1.8	~5
Total	79.4	75.0	59.6	58.6	~75
Feces					
0–48 hr	1.7	0.8	0.2	<0.4	—

of the HEDP complexes toward EDTA substitution is in contrast to that of the Tc(Sn)-HIDA formulation, which reacts with EDTA under fairly stringent conditions to yield the Tc-EDTA complex (*15*).

Macroscopically prepared Tc(Sn)-HEDP definitely contains chemically bonded tin, confirming implications from previous in vivo (*8*) and chemical (*19*) studies; in fact it contains more tin than technetium (Sn/Tc = 3). It is thus likely that many technetium tracers prepared by stannous reduction of pertechnetate contain tin as an integral part of their chemical structure (*20*), and it will be of interest to determine whether analogous radioagents prepared without tin have different chemical and biological properties. For the HEDP tracers investigated in this work, the chemically bonded tin of Tc(Sn)-HEDP does *not* cause its biological distribution to differ significantly from that of Tc($NaBH_4$)-HEDP or Tc-HEDP, which necessarily do not contain tin (*vide infra*). It thus appears that the biological distribution—and indeed much of the chemistry—of Tc(Sn)-HEDP, Tc($NaBH_4$)-HEDP, and Tc-HEDP is determined by the HEDP ligand, and the presence of tin in Tc(Sn)-HEDP is not of serious consequence in determining the biochemistry of this species.

The tissue distribution data given in Tables 1–4 and illustrated in Figs. 1 and 2 show that Tc(Sn)-HEDP, Tc($NaBH_4$)-HEDP, and Tc-HEDP all have essentially the same biodistribution, being effective bone-seeking agents. Uptake of Tc($NaBH_4$)-HEDP and Tc-HEDP by the bone is at least equivalent to that achieved by Tc(Sn)-HEDP; hence tin is not a necessary ingredient for bone-imaging agents. The equivalent biodistributions of these three agents are reflected in the equivalent scintiphotos shown in Figs. 3 and 5. The bulk of the material not deposited on bone appears in the kidneys and urine, indicating that, as expected, these three tracers occur in the blood stream as small, hydrophilic, ionic molecules that are not tightly bonded to proteins and are readily filtered through the glomeruli. Consistent with this view, very little technetium appears in the feces, and thus these materials are not appreciably handled through the biliary system, which tends to accumulate lipophilic molecules (*21–24*) (see Table 5). The very low uptake of technetium in the liver and spleen indicates that there is no detectable in vivo production of TcO_2 or of large (0.1–5 μ) aggregates, consistent with the observed chemical stability of these agents. Tc($NaBH_4$)-HEDP is cleared from the blood (Fig. 4), and through the kidneys (Tables 1 and 3), significantly faster than Tc-HEDP; hence the in vivo system can distinguish between these similar, but chemically distinct, species.

The results of this study show that effective bone imaging HEDP complexes of technetium can be prepared without the use of stannous ion and therefore tin is not necessary for the bone seeking properties of Tc(Sn)-HEDP. Since the in vivo tissue distributions of Tc(Sn)-HEDP, Tc($NaBH_4$)-HEDP, and Tc-HEDP are similar, it is likely that distribution is primarily dependent on the presence of the HEDP ligand and not on the exact chemical nature of the technetium complex itself. This study also shows that technetium-diphosphonate complexes probably have polymeric, or at least dimeric, structures, and that the synthesis of technetium tracers by a substitution route, rather than by redox, is practicable (*25*). This substitution route has the potential of introducing hitherto unattainable flexibility and subtlety into the preparation of technetium radiopharmaceuticals.

FOOTNOTES

* Procter & Gamble Co.

† Oak Ridge National Laboratories, Oak Ridge, TN.

‡ SEM experiments were kindly performed by Dr. Charles J. Weschler of Bell Laboratories, Holmdel, NJ, using an AMR-1000 apparatus.

ACKNOWLEDGMENTS

E.D. gratefully acknowledges financial support by the National Institutes of Health (Grant No. HL-21276) and the Procter and Gamble Co. The authors also thank Dr. Charles J. Weschler of Bell Laboratories for performing the scanning electron microscope analyses.

REFERENCES

1. TOFE AJ, FRANCIS MD: Optimization of the ratio of stannous tin: ethane-1-hydroxy-1,1-diphosphonate for bone

scanning with ^{99m}Tc pertechnetate. *J Nucl Med* 15: 69-74, 1974
2. ECKELMAN WC, LEVENSON SM: Radiopharmaceuticals labelled with technetium. *Int J Appl Radiat Isot* 28: 67-82, 1977
3. JONES A, FRANCIS MD, DAVIS MA: Bone scanning: radionuclidic reaction mechanisms. *Semin Nucl Med* 6: 3-18, 1976
4. BUJA LM, TOFE AJ, KULKARNI PV, et al: Sites and mechanism of localization of technetium-99m phosphorus radiopharmaceuticals in acute myocardial infarcts and other tissues. *J Clin Invest* 60: 724-740, 1977
5. DAVIS MA, HOLMAN BL, CARMEL AN: Evaluation of radiopharmaceuticals sequestered by acutely damaged myocardium. *J Nucl Med* 17: 911-917, 1976
6. HILES RA: Adsorption, distribution and excretion of inorganic tin in rats. *Toxicol Appl Pharmacol* 27: 366-379, 1974
7. FURCHNER JE, DRAKE GA: Comparative metabolism of radionuclides in mammals XI. Retention of ^{113}Sn in the mouse, rat, monkey and dog. *Health Phys* 31: 219-224, 1976
8. ZALUTSKY MR, RAYUDU GV, FRIEDMAN AM: The biological behavior of tin following administration of nine ^{99m}Tc-Sn complexes. *Int J Nucl Med Biology* 4: 224-230, 1977
9. ANCRI D, LONCHAMPT MF, BASSETT JY: The effect of tin on the tissue distribution of ^{99m}Tc-sodium pertechnetate. *Radiology* 124: 445-450, 1977
10. PAVEL DG, ZIMMER AM, PATTERSON VN: In vivo labelling of red blood cells with ^{99m}Tc: A new approach to blood pool visualization. *J Nucl Med* 18: 305-308, 1977
11. SRIVASTAVA SC, MEINKEN G, SMITH TD, et al: Problems associated with stannous 99m Tc-radiopharmaceuticals. *Int J Appl Radiat Isot* 28: 83-95, 1977
12. SUBRAMANIAN G, MCAFEE JG: Use of sodium borohydride in labeling biologically useful compounds with ^{99m}Tc. *J Nucl Med* 10: 443, 1969 (abst)
13. ECKELMAN W, MEINKEN G, RICHARDS P: Chemical state of 99m-Tc in biomedical products. *J Nucl Med* 12: 596-600, 1971
14. DALZIEL J, GILL NS, NYHOLM RS, et al: Technetium. Part I. The preparation and properties of potassium hexahalogenotechnetates. *J Chem Soc:* 4012-4016, 1958
15. LOBERG MD, FIELDS AT: Stability of 99m Tc-labeled N-substituted iminodiacetic acids: ligand exchange reaction between 99m-Tc-HIDA and EDTA. *Int J Appl Radiat Isot* 28: 687-692, 1977
16. GORSKI B, KOCH H: Über die komplexbildung von technetium mit chelatbildenden liganden. II. *J Inorg Nucl Chem* 32: 3831-3836, 1970
17. LIBSON K, DEUTSCH E, BARNETT BL: Structural characterization of a 99-Tc-diphosphonate complex. Implications for the chemistry of 99m-Tc skeletal imaging agents. *J Am Chem Soc* 102: 2476-2478, 1980
18. STEIGMAN J, MEINKEN G, RICHARDS P: The reduction of pertechnetate-99 by stannous chloride. II. The stoichiometry of the reaction in aqueous solutions of several phosphorus(V) compounds. *Int J Appl Radiat Isot* 29: 653-660, 1978
19. DEUTSCH E, ELDER RC, LANGE B, et al: Structural characterization of a bridged 99-Tc-Sn-dimethylglyoxime complex: implications for the chemistry of ^{99m}Tc-radiopharmaceuticals prepared by the Sn(II) reduction of pertechnetate. *Proc Natl Acad Sci USA* 73: 4287-4289, 1976
20. DEUTSCH E: Inorganic radiopharmaceuticals. In *Radiopharmaceuticals II.* New York, Society of Nuclear Medicine, 1979, pp 129-146
21. JOHANNSEN B, SYHRE R, SPIES H, et al: Chemical and biological characterization of different Tc complexes of cysteine and cysteine derivatives. *J Nucl Med* 19: 816-824, 1978
22. HEINDEL ND: Principles of target tissue localization of radiopharmaceuticals. In *The Chemistry of Radiopharmaceuticals,* Heindel ND, Burns HD, Honda T, Brady LW, eds, New York, Masson, 1978, pp 11-33
23. BURNS HD, WORLEY P, WAGNER HN Jr, et al: Design of technetium radiopharmaceuticals. In *The Chemistry of Radiopharmaceuticals,* Heindel ND, Burns HD, Honda T, Brady LW, eds, New York, Masson, 1978, pp 269-289
24. MCAFEE JG: Radioactive diagnostic agents: current problems and limitations. In *Radiopharmaceuticals,* Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds, New York, Society of Nuclear Medicine, 1975, pp 3-14
25. KUBIATOWICZ DO, BOLLES TF, NORA JC, et al: Localization of low molecular weight ^{99m}Tc-labeled dimercaptodicarboxylic acids in kidney tissue. *J Pharmacol Sci* 68: 621-623, 1979