Chemical and Biological Studies of Tc-99m N,N′-Bis(mercaptoacetamido)-ethylenediamine: A Potential Replacement for I-131 Iodohippurate

Alan R. Fritzberg, William C. Klingensmith III, Wayne P. Whitney, and Christopher C. Kuni

University of Colorado Health Sciences Center and Veterans Administration Medical Center, Denver, Colorado

The tetradentate chelating agent N,N′-bis(benzoylmercaptoacetamido)ethylenediamine was synthesized for evaluation as a potential technetium-99m renal-function radiopharmaceutical. Complexes were prepared using different reducing agents and analyzed by high-performance liquid chromatography. Biological studies were performed in mice, rats, and rabbits and indicated that the new agent is cleared by the kidneys significantly faster than Tc-99m DTPA ($p < 0.01$) and slightly slower than I-131 *o*-iodohippuric acid ($p > 0.05$). There was no evidence of significant renal retention. Renal excretion in all species studied was 70–75% of the injected dose in 30 min; biliary excretion in rats was 7% in animals with normal renal function and 18% in 90 min in the absence of renal function. We conclude that limited clinical trials are warranted.

J Nucl Med 22: 258–263, 1981

Ideally, renal function should be evaluated with a single radiopharmaceutical that possesses a high extraction efficiency, such as I-131 *o*-iodohippuric acid (OIH), and is labeled with a radionuclide having good physical properties such as technetium-99m. Currently two agents are commonly used in the evaluation of renal function, OIH and Tc-99m DTPA. Renal perfusion is evaluated by rapid serial imaging during the first circulation after bolus injection of Tc-99m DTPA. OIH cannot be used for this purpose because the iodine-131 label limits the amount of radioactivity that can be injected. Renal clearance can be evaluated with either Tc-99m DTPA or OIH, but since Tc-99m DTPA is limited to clearance by glomerular filtration, the maximum extraction efficiency is 20% (*1,2*). Secretion of OIH by the renal tubular cells, in addition to some filtration, results in an extraction efficiency of about 67% (*3*). The higher extraction efficiency of OIH increases the kidney-to-background image ratio, thus increasing the sensitivity of OIH for detection and evaluation of reduced renal function.

Received Aug. 4, 1980; revision accepted Nov. 3, 1980.

For reprints contact: Alan R. Fritzberg, PhD, Dept. of Radiology, Univ. of Colo. Health Sciences Ctr., 4200 E. 9th, Denver, CO 80262.

Davison and coworkers have recently (*4,5*) described the structure of Tc-99 N,N′-bis(mercaptoacetamido)-ethylenediamine (Tc-99m DADS) (Fig. 1). In addition to forming an apparently pure radiochemical complex with Tc-99m, it was also found to be rapidly excreted by the kidneys, like OIH. With an interest in finding a Tc-99m radiopharmaceutical that would replace OIH, we synthesized the complex and compared its behavior with those of OIH and Tc-99m DTPA in several animal species.

MATERIALS AND METHODS

General. Elemental analyses were obtained commercially.* Proton magnetic resonance spectra were obtained† and high-performance liquid chromatographic analyses were done.‡

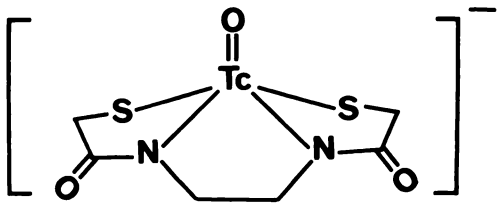

FIG. 1. Structure of Tc-N,N′-bis(mercaptoacetamido)ethylenediamine (Tc-99m DADS) as proposed by Davison et al. (*4*).

Carrier synthesis. The sulfhydryl-group-protected carrier precursor, N,N′-bis(benzoylmercaptoacetamido)ethylenediamine ($PhCOSCH_2CONHCH_2CH_2$-; $NHCOCH_2SCOPh$), was synthesized in two steps. Five milliliters of tetrahydrofuran (THF), containing 1.35 g (22 millimols) of ethylenediamine, were added dropwise under nitrogen to a solution of 5.95 g (30 millimols) of methyl mercaptoacetate in 30 ml of THF. After stirring 30 min at room temperature, 10 ml of dimethylformamide (DMF) were added to maintain solution, and the reaction mixture was refluxed for an additional hour. The THF was removed under a stream of nitrogen, then excess methylmercaptoacetate was removed by washing the residue three times with 50-ml portions of hexane. The residue was dissolved by the addition of 10 ml of pyridine and another 10 ml of DMF. Then 7.3 g (0.052 mol) of benzoyl chloride was added dropwise in 5 ml of pyridine. After stirring under nitrogen overnight, the product was isolated by adding to 300 ml of ice water. Filtration gave 8.76 g (94%) of crude product. This was purified by recrystallization, once in isopropyl alcohol and twice in methyl ethyl ketone: m.p. 192–194°C; nmr (DMSO-d_6,$CDCl_3$) δ 3.10–3.45 (complex, 2H), 3.75 (S, 2H), δ 7.3 to 8.05 (complex, 5H), and δ 8.20 (broad S, IH). Anal: calcd. for $C_{20}H_{20}N_2O_4S_2$: C 57.69, H 4.81, N 6.73, S 15.38; found: C 57.60, H 4.94, N 6.75, S 15.39.

Radiolabeling with Tc-99m. This was done with three reducing agents. Initially 20 mg of the carrier was dissolved in 0.5–1 ml of ethanol or propylene glycol and 0.2 to 0.4 ml of 1 *M* NaOH with heating. After dissolution, the pH was adjusted to 12. When dithionite was used as the reducing agent, the pertechnetate (Tc-99m) was added in a volume to give 2.5 ml. Finally, 3 mg of freshly prepared dithionite in 0.1 ml was added. After 5 min, the pH was readjusted to 8 and the volume adjusted to 3 ml with water. When formamidine sulfinic acid (FSA) was used, 1.5 mg of recrystallized FSA in 0.1 ml was added to the solution of carrier and pertechnetate, and the mixture was heated at 70–75°C for 10 min. The pH was readjusted to 8 and the volume to 3 ml. When stannous chloride was used, 0.3 mg of $SnCl_2 \cdot 2H_2O$ in 0.1 ml was added to the carrier solution, followed by the pertechnetate. HPLC analysis of the stannous-ion preparation was done without pH adjustment. Kits for I-131 *o*-iodohippuric acid and DTPA kits were obtained commercially.

The preparations (Tc-99m DADS) were analyzed for reduced hydrolyzed technetium on silica gel thin-layer strips.[‖] The complex had R_f values of 1 in both methyl ethyl ketone and 0.9% NaCl. Analysis for soluble-species purity was performed on HPLC with 0.01 *M* phosphate, pH 5.8, and acetonitrile as solvents. Typical chromatograms with conditions are shown in Fig. 2.

Studies of protein binding were carried out using 4 mg/ml human serum albumin (HSA) and plasma.

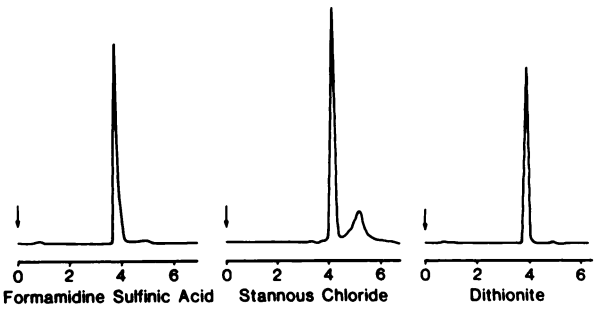

FIG. 2. High-performance liquid chromatograms of Tc-99m DADS (*1*) prepared with different reducing agents. Solvents were 0.01 *M* phosphate, pH 5.8, (A) and acetonitrile (B). Gradient was 10–40% solvent B in 10 min; and flow rate 2.3 ml/min on an octadecasilyl (ODS) column. Alteration of conditions to a 16–27% B gradient at 1.8 ml/min results in appearance of a shoulder peak of about 15% of radioactivity.

About 200 μCi in 0.2 ml were added to 2 ml of HSA or 4 ml of plasma. After mixing, the mixture was placed in a filter cone[§] and centrifuged. Separations of protein-free filtrate (0.1–0.3 ml) were made at various times from 2–60 min after mixing. Samples of filtrate and unfiltered mixture were counted to determine concentrations in each compartment. The binding of OIH in plasma was determined simultaneously.

Samples of urine and bile obtained from rats injected with 2.0 mCi of Tc-99m DADS were analyzed by HPLC. The samples were injected without manipulation through a 20-μl injection loop.

Animal studies. Animal studies were carried out in mice, rats, and rabbits. In the interest of economy, the toxicity and the time course of organ distributions were determined in mice. Rats were used for measurement of blood disappearance and intestinal excretion of the radiochemical, since we already had developed procedures for rapid blood sampling and timed bile collection (*6*). Rabbits were used for measurements of renal excretion, since a time-activity curve for the urinary bladder could be obtained by placement of a region of interest over the bladder on a scintigram. The use of the different species also allowed evaluation of the comparability of renal agents in the different species.

Initial qualitative rabbit-imaging studies with preparations made with the various reducing agents appeared to have similar in vivo behavior. In the interest of minimizing variations, however, all animal studies of Tc-99m DADS used dithionite as the reducing agent.

The time course of organ distribution was followed in groups of six Swiss Webster albino male mice. They were injected with 0.10 ml (0.5 μCi) of the preparations with propylene glycol as cosolvent. For comparison purposes, 0.2 μCi OIH was added to each injection. The mice were placed in metabolic cages to collect excreted urine. At indicated intervals after injection, the penis was ligated and the mice killed with chloroform vapor. The organs were removed and counted in a dual-channel counter

with correction for I-131 crossover into the Tc-99m channel.

Blood disappearance rates and biliary excretion were determined in male Sprague-Dawley rats. For blood studies the animals were anesthetized with sodium pentobarbital, line placed in a femoral vein for tracer injections and hydration, and another in a carotid artery for blood sampling. About 20 μCi (0.25 ml) each of OIH and the Tc-99m complex under study was injected, and 0.1- to 0.2-ml samples of blood taken at 1, 2, 3, 4, 5, 6, 9, 12, 15, 20, 25, 30, 45, 60, 75, and 90 min after injection. Biliary excretion was determined by cannulating the common bile duct and collecting bile in 4-min fractions for 90 min. This was done first with normal renal function. After an additional 30 min, the renal pedicles were tied off and a second dose was given to determine the biliary excretion in the absence of renal function. The radioactivity in the urine from the first run was also measured to determine renal excretion in 90 min.

The rate of renal excretion was measured in New Zealand albino male rabbits. They were anesthetized with ketamine and xylazine and placed on a gamma camera provided with digital storage. After injection of 1-1.5 mCi of the Tc-99m complex containing 0.5 μCi of OIH, images were collected on tape for 45 min. At 35 min after injection, urine was expelled from the bladder and its percentage dose contained in the expelled urine determined. Accumulation of bladder radioactivity was monitored by placing a region of interest over the bladder. The drop in the radioactivity in the bladder after expelling the urine sample (50-80% of the bladder radioactivity) allowed calibration of the bladder time-activity curve in terms of percent injected dose. The amounts determined for OIH excretion were based on the change in bladder radioactivity due to Tc-99m and amount of I-131 in the urine sample. These values correlated well with runs made with OIH alone, in which 250 μCi were injected and the bladder radioactivity monitored for I-131. The values obtained at 35 min after injection were 81.3 ± 10.2 (s.d.) percent for OIH when the Tc-99m DADS time-activity curve was used, (82.5 ± 6.4)% when the Tc-99m DTPA curve was used, and (85.2 ± 9.1)% when I-131 radioactivity from OIH was monitored.

Acute toxicity studies were performed in 71 HA I Charles River female mice. The formulation using propylene glycol as cosolvent and dithionite as reducing agent was given in doses covering a range of 100 to 400 mg/kg. Results were analyzed by the method of Litchfield and Wilcoxin (*7*).

RESULTS

In vitro studies. High-performance liquid chromatography analysis of Tc-99m DADS prepared with different reducing agents is shown in Fig. 2. Preparations using dithionite or FSA showed a single radiochemical form predominating under the conditions in Fig. 2. More recent analyses using a slower flow rate and smaller solvent gradient have revealed a shoulder peak of about 10-15% of the main peak. Although the ratio is somewhat variable, varying the reducing agent or the initial pH during the reduction step has not appeared to alter the shoulder component reproducibly. The chromatogram from the stannous-ion preparation chromatogram contained an additional later peak. Conditions were not optimized for stannous-ion reduction, so it may be possible to reduce the later peak by adjusting preparation conditions. Once formed, little change of the HPLC pattern with time was noted over a 5-hr period. No

TABLE 1. BIODISTRIBUTION TIME COURSE OF RENAL AGENTS IN MICE*

Time (min)	Blood	Kidneys	Liver	Muscle	Stomach	Intestines	Urine
				Tc-99m DADS			
5	5.15 ± 0.99	12.63 ± 6.39	8.40 ± 1.13	5.61 ± 1.52	0.25 ± 0.11	6.70 ± 0.84	28.54 ± 8.90
10	1.85 ± 0.90	4.25 ± 2.83	2.24 ± 0.64	3.35 ± 0.51	0.24 ± 0.34	4.69 ± 0.85	60.90 ± 5.72
15	1.17 ± 0.28	4.13 ± 2.03	2.24 ± 0.35	2.16 ± 0.76	0.07 ± 0.03	6.00 ± 0.56	66.60 ± 4.70
30	0.50 ± 0.03	2.88 ± 2.03	2.67 ± 0.54	1.80 ± 1.18	0.07 ± 0.03	10.44 ± 2.12	64.74 ± 4.96
60	0.22 ± 0.04	1.46 ± 1.88	1.13 ± 0.12	1.18 ± 1.26	0.10 ± 0.09	5.50 ± 1.11	72.16 ± 9.42
120	0.33 ± 0.02	0.68 ± 0.15	2.44 ± 0.32	0.64 ± 0.13	0.49 ± 0.82	9.55 ± 3.02	72.00 ± 9.74
240	0.24 ± 0.03	0.30 ± 0.08	1.46 ± 0.26	0.70 ± 0.36	1.24 ± 1.61	6.55 ± 2.16	70.14 ± 7.96
				I-131 *o*-iodohippurate			
5	9.64 ± 1.25	7.85 ± 3.65	3.71 ± 0.66				42.02 ± 6.45
10	3.97 ± 1.29	2.44 ± 1.36	1.66 ± 0.44				68.51 ± 6.63
15	1.92 ± 0.44	1.64 ± 0.18	0.86 ± 0.19				78.24 ± 1.13
60	0.53 ± 0.12	0.23 ± 0.08	0.38 ± 0.06				90.69 ± 7.44
120	1.24 ± 1.40	0.47 ± 0.40	0.28 ± 0.16				92.92 ± 7.23

* Values are mean and standard deviation for five or more mice.

change was observed before and after pH adjustments. With all reducing agents, silica-gel, thin layer chromatography indicated negligible reduced hydrolyzed Tc-99m.

Analysis of urine and bile samples of rats, using HPLC conditions that resulted in the shoulder peak, indicated no change in the chromatographic pattern of the urine specimen, and in the bile specimen at least 80% of the radioactivity was present at the same retention times as the original preparation. The other 20% of radioactivity in the bile samples consisted of at least two components with retention times of 0.5–1 min, indicating pertechnetate or more polar complexes. In albumin solution, the protein-bound values of Tc-99m DADS increased with time, with 60% bound at 5 min, 62% at 15 min, and 72% at 22 and 30 min. In plasma, 88% was bound at 5 min, 92% at 10 min, and 95% from 30–60 min. OIH averaged 52.6% bound in plasma, with no change over 10–60 min. The value for OIH can be compared with the 70% determined by use of collodion (*8*) and 64–70% by equilibrium dialysis or ultrafiltration (*9*).

In vivo studies. Table 1 shows the organ distribution of Tc-99m DADS in mice from 5 min to 2 hr. Blood disappearance was rapid, with <1% remaining by 30 min. High renal extraction efficiency was indicated by 12.6% in the kidneys at 5 min. Negligible renal retention occurred, since the percent dose in both kidneys decreased to <1% at 120 min. Liver uptake was apparent at 5 min, then decreased to ~1% by 60 min. Intestinal radioactivity appeared by 5 minutes, then remained relatively constant with time at 6–10%. The rapidity of renal excretion was demonstrated by finding 60% of the injected dose in the urine in 10 min. However, the urinary excretion leveled off at 72% whereas that of OIH exceeded 90%.

Values obtained with OIH are also shown for comparison. Blood and kidney radioactivities for both agents are similar over the first 15 min, while liver radioactivity is lower. Urinary values for OIH, however, are somewhat higher. Urinary values of Tc-99m DTPA were also determined similarly and were 28 ± 3.5 (s.d.)% at 5 min (43.3 ± 6.2)% at 10 min and (55.3 ± 2.0)% at 15 min. All were significantly less than the urinary values for Tc-99m DADS at those times.

Blood disappearance rates were compared in rats (Fig. 3). As expected, OIH radioactivity in the blood was lowest at all times. Surprisingly, Tc-99m DADS radioactivity did not drop below that of Tc-99m DTPA until after 3 min. From that time until 45 min, the disappearance slopes of OIH and Tc-99mm DADS were similar. The higher blood levels of Tc-99m DADS shortly after injection may be due to a smaller volume of distribution, which would be consistent with the relatively high fraction of Tc-99m DADS bound to plasma proteins.

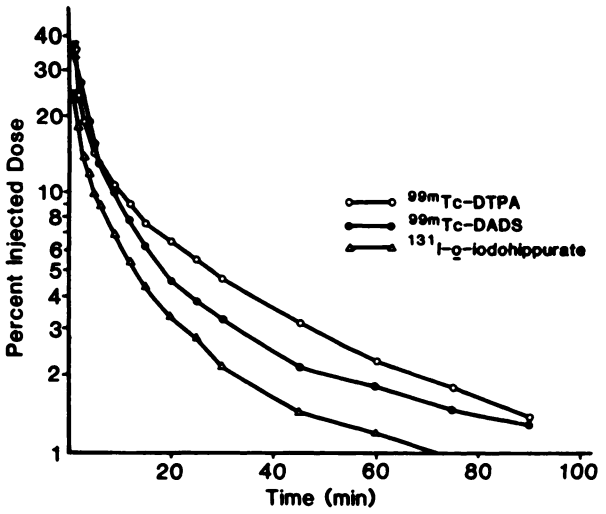

FIG. 3. Blood disappearance curves for renal agents in rats. Data are plotted as mean for five or more rats at each point. After 3 min, slope of disappearance curve of Tc-99m DADS is similar to that of OIH, reflecting greater renal clearance of Tc-99m DADS.

The rates of appearance of radioactivity in the urine were compared in rabbits (Fig. 4). The shape of the appearance curve of Tc-99m DADS is similar to that of OIH, but Tc-99m DADS approached a lower maximum. The values at 35 minutes were 73.9 ± 6.0 (s.d.) percent of the injected dose in the urine for Tc-99m DADS, (42.9 ± 12.8)% for Tc-99m DTPA, and (85.2 ± 9.1)% for OIH. The difference between Tc-99m DADS and Tc-99m DTPA is significant ($p < 0.01$); the difference between Tc-99m DADS and OIH is not. Simultaneous determination of Tc-99m complex and OIH resulted in ratios of 0.91 ± 0.10 for Tc-99m DADS to OIH and 0.51 ± 0.13 for Tc-99m DTPA to OIH at 35 min.

The biliary excretion of Tc-99m DADS was determined in rats. With normal renal function, the biliary excretion rose to 5% in the first 20 min, then approached a plateau at 7% (range 3.9–10.6%) by 90 min (Fig. 5).

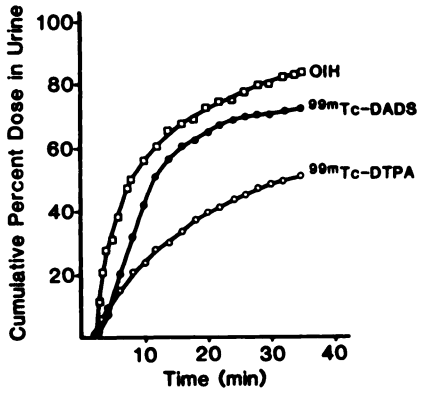

FIG. 4. Renal excretion of agents in rabbits. Curves are representative and scaled to mean value at 35 min from four or more runs for each agent. Tc-99m DADS curve shows rate of appearance in urine similar to that of OIH, and much faster than Tc-99m DTPA.

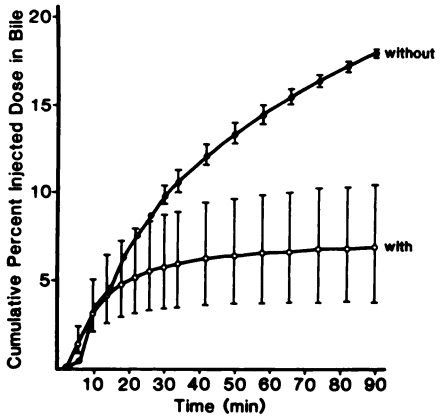

FIG. 5. Biliary excretion of Tc-99m DADS in rats, with and without renal function. Data are plotted as mean and range for three animals at each condition. With kidneys tied off biliary excretion increases. Although only 18% at 90 min, bile radioactivity is still increasing at that time.

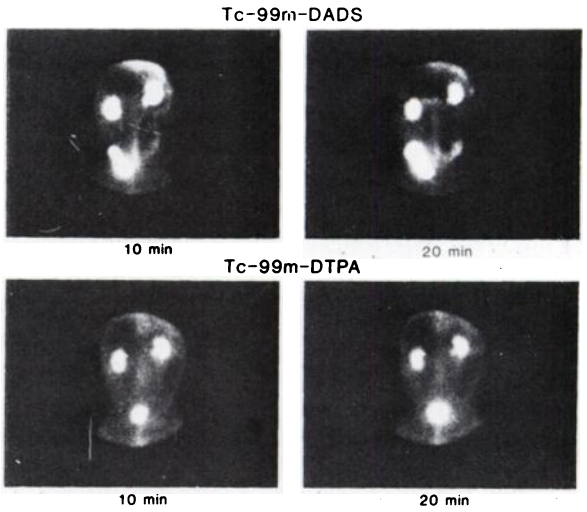

FIG. 6. Images of Tc-99m DADS and Tc-99m DTPA in rabbits. Tc-99m DADS demonstrates greater urine radioactivity and kidney-to-background ratio at both times. At 10 min a small amount of liver radioactivity is seen, and at 20 min some biliary radioactivity is seen in Tc-99m DADS images.

In the absence of renal function, biliary excretion climbed more steadily, reaching 18% at 90 min. Bile radioactivity, however, was still increasing at this point. Determination of radioactivity in the urine of the rats at 90 min after injection indicated 72% of the injected dose, in agreement with the values obtained in mice and rabbits.

Comparative Tc-99m DADS and Tc-99m DTPA images in rabbits are shown in Fig. 6. Technetium-99m DADS demonstrates greater urinary radioactivity and kidney-to-background ratio at 10 and 20 min. Liver radioactivity is visible above the kidneys at 10 min, and biliary radioactivity at 20 min.

Acute toxicity studies in mice yielded a minimum lethal dose of 200 mg/kg and an LD_{50} of 256 mg/kg. If half of the present formulation (10 mg carrier DADS, 1.5 mg dithionite) was injected into a 70-kg person, the safety factor would be 1,350.

DISCUSSION

The results of the chemical studies indicate that Tc-99m DADS is predominantly a single radiochemical and is generally the same when prepared with different reducing agents. It is stable with respect to time, and appears unaltered over a range of pH after preparation. Although dithionite efficiently provides a technetium complex of reproducible radiochemical purity, its potential for mass kit production is untested. For these studies it was used immediately, since precipitation of the dithionite solution occurred a few minutes after preparation. Recently FSA was proposed for use in the preparation of Tc-99m glucoheptonate (*10*), but it also has not been adapted to long-term kit shelf life. Stannous ion may be effective, but more work is needed on conditions for its use since several radiochemical forms resulted during initial evaluations.

The biological studies clearly demonstrated rapid renal excretion of Tc-99m DADS. Although it was excreted significantly faster than Tc-99m DTPA, it was slightly slower than OIH. Seventy-five percent of the injected dose of Tc-99m DADS appeared in the urine compared with 90% of OIH. Biliary excretion of Tc-99m DADS in 90 min amounted to 7% in rats with normal renal function, and 18% in the absence of renal function. Potential interference from biliary excretion will require clinical evaluation. While it is a significant percentage, it may possibly be less in humans since species variations in biliary excretion are well known (*11*). It is of interest that at least 80% of the bile activity appeared to be unchanged. The chromatographic results, and the observed increase in biliary excretion in the absence of renal function, suggest that biliary excretion is a normal route for Tc-99m DADS and is not due to contaminants or metabolites.

Since Tc-99m DADS is excreted into the urine faster than Tc-99m DTPA, which is efficiently excreted by glomerular filtration, renal tubular secretion of Tc-99m DADS is suggested. The observation that Tc-99m DADS is predominantly bound to plasma protein also supports this suggestion, since plasma-protein binding prevents excretion by glomerular filtration (*12*). It has been suggested that structural requirements for renal tubular secretion are satisfied by the general formula $RCONX\text{-}(CHR')_nCOOH$, where n = 1-5 (*12*). Although structural similarities between Tc-99m DADS and the model, usually exemplified by benzoylglycines (hippuric acids), are not immediately apparent, several of the requirements appear to be satisfied. The technetium complex has a net negative charge (*4*), which is also expected for the model carboxylic acid group at physi-

ological pH. Thus the technetium-bound oxygen atom, or one of the sulfurs, may act in a binding role similar to that of the terminal carboxylic acid group. The amide carbonyl of the chelating group bears a similarity also to the amide carbonyl of the model compound. Since binding of the nitrogen or amide hydrogen is not required (*13*), the technetium nitrogen binding probably would not interfere. The lack of evidence for active tubular secretion of Tc-99m DTPA can be rationalized on the basis that no carbonyl groups are located in an appropriate manner from one or more of the carboxylate groups. The presence of a side-chain amino nitrogen, as in the DTPA molecule or complex, is not sufficient to result in active renal transport (*11*).

It is concluded that the results of animal evaluation of Tc-99m DADS warrant limited studies in humans to correlate biological distribution parameters and to determine its potential as a radiopharmaceutical. Although the specificity of Tc-99m DADS for renal excretion is not optimal, the results indicate that one can make a Tc-99m complex that will be secreted by renal tubular cells with a relatively high extraction efficiency and without significant tubular-cell retention.

FOOTNOTES

* Galbraith Laboratories, Inc., Knoxville, TN.

† Varian EM 360A and EM 390 instruments.

‡ Altex Model 312 gradient liquid chromatograph with 254 nm ultraviolet and sodium iodide scintillation detectors for mass and radioactivity. Column used was 4.6 × 150 mm with octadecylsilyl ultrasphere packing.

‖ Gelman Instrument Co., Ann Arbor, MI.

§ Amicon Corporation, Lexington, MA.

REFERENCES

1. ATKINS HL, ECKELMAN WC, HAUSER W, et al: Evaluation of glomerular filtration rate with ^{99m}Tc-DTPA. *J Nucl Med* 12: 338, 1971 (abst)
2. WINCHELL HS: Radiopharmaceuticals in evaluation of kidneys. In *Radiopharmaceuticals II.* Proceedings of 2nd International Symposium on Radiopharmaceuticals, Seattle, WA. Sodd VJ, Allen DR, Hoogland DR Eds. New York, Society of Nuclear Medicine, 1979, pp 459-464
3. STADALNIK RC, VOGEL JM, JANSHOLT A-L, et al: Renal clearance and extraction parameters of ortho-iodohippurate (I-123) compared with OIH(I-131) and PAH. *J Nucl Med* 21: 168-170, 1980
4. DAVISON A, SOHN M, ORVIG C, et al: A tetradentate ligand designed specifically to coordinate technetium. *J Nucl Med 20:* 641, 1979 (abst)
5. DAVISON A, JONES A, ORVIG C, et al: A new class of oxotechnetium (+5) chelate complexes containing a $TcON_2S_2$ core. *Inorg Chem,* in press.
6. FRITZBERG AR, WHITNEY WP, KLINGENSMITH WC: Hepatobiliary transport mechanism of Tc-99m-N,α(2,6-diethyacetanilide)-iminodicarboxylic acid (Tc-99m-diethyl-IDA). In *Radiopharmaceuticals II,* Proceedings of 2nd Intl Symp. on Radiopharmaceuticals, Seattle, WA, VJ Sodd, DR Allen, DR Hoogland, Eds., 1979, New York, Society of Nuclear Medicine, pp 557-586
7. LITCHFIELD JT, WILCOXON F: A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 96: 99-113, 1949
8. SMITH WW, SMITH HW: Protein binding of phenol red, diodrast, and other substances in plasma. *J Biol Chem* 124: 107-113, 1938
9. MAHER FT, TAUXE WN: Renal clearance in man of pharmaceuticals containing radioactive iodine. Influence of plasma binding. *JAMA* 207: 97-104, 1969
10. SCOTT JR, GARRETT GL, LENTLE BC: Preparation of technetium-99m-glucoheptonate utilizing formamidine sulfinic acid. *Int J Nucl Med Biol* 7: 71-73, 1980
11. ABOU-EL-MAKAREM MM, MILLBURN P, SMITH RL, et al: Biliary excretion of foreign compounds. Species differences in biliary excretion. *Biochem J* 105: 1289-1293, 1967
12. GOLDSTEIN A, ARANOW A, KALMAN SM: *Principles of Drug Action,* New York, John Wiley and Sons, 1974, p 163
13. DESPOPOULOS A: A definition of substrate specificity in renal transport of organic anions. *J Theoret Biol* 8: 163-192, 1965

BOOKS RECEIVED

Quality Assurance of Radiopharmaceuticals. A Guide to Hospital Practice. M. Frier, S.R. Hesslewood, Eds. Published by Chapman and Hall in association with The British Nuclear Medicine Society, 1980, 57 pp

Information to Authors 1980-1981. Editorial Guidelines reproduced from 246 Medical Journals. Compiled by Harriet R. Meiss, Doris A. Jaeger. Urban & Schwarzenberg. 1980, 665 pp, $26.00

Radionuclides in Clinical Chemistry. Phillip L. Howard, Thomas D. Trainer. Boston, Little, Brown, 1980, 154 pp, $22.50

Computers in Medicine. An Introduction. Derek Enlander. St. Louis, Toronto, London, C.V. Mosby, 1980, 124 pp, illustrated, $12.95

Pearls in Diagnostic Radiology. Harold D. Rosenbaum, New York, Edinburg, London, Melbourne, Churchill Livingstone, 1980, 238 pp, illustrated, $32.50

The Year Book of Nuclear Medicine 1980. James L. Quinn III, Ed. Chicago, London, Year Book Medical Publishers, Inc., 1980, 335 pp, $34.95