# Localization of Technetium 99m–Ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic Acid and Technetium 99m–*N*-(2-Mercapto-1-oxopropyl)glycine in Hepatobiliary System

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Received September 17, 1979, from the *Division of Nuclear Medicine and Radiopharmacology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61801, and the ‡College of Chemistry, University of Wisconsin at Eau Claire, Eau Claire, WI 54701. Accepted for publication January 4, 1980.

**Abstract** □ Two radiopharmaceuticals, technetium 99m–*N*-(2-mercapto-1-oxopropyl)glycine ($^{99m}$Tc-I) and technetium 99m–ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid ($^{99m}$Tc-II), were prepared and evaluated in dogs, rabbits, and rats. Both agents gave good scintigraphic images of the liver and gallbladder in dogs. The cumulative amount of $^{99m}$Tc-I and $^{99m}$Tc-II excreted in the bile of dogs and the physiological disposition data in rats revealed slight, inconclusive differences in their distributions. However, the scintigraphic images and physiological disposition data in rabbits revealed gross differences in the distribution pattern of the two agents. The observed similarities in the biliary excretion of both agents in dogs and rats were attributed to the fact that these species are relatively good biliary excretors, and both agents therefore were excreted extensively. However, rabbits, which are poor biliary excretors relative to dogs and rats, excreted $^{99m}$Tc-II more extensively than $^{99m}$Tc-I because of the favorable molecular characteristics of $^{99m}$Tc-II.

**Keyphrases** □ Radiopharmaceuticals—technetium 99m complexes of *N*-(2-mercapto-1-oxopropyl)glycine and ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid, localization in hepatobiliary system □ Technetium 99m—complexes with *N*-(2-mercapto-1-oxopropyl)glycine and ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid, localization in hepatobiliary system □ Scintigraphic imaging agents—complexes of technetium 99m with *N*-(2-mercapto-1-oxopropyl)glycine and ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid, localization in hepatobiliary system

External scintigraphic diagnosis of gallbladder pathology has been the subject of several investigations. Application of the intravenous radionuclide cholangiogram has saved time in routine hepatobiliary function tests, demonstrating the patency of the cystic duct in patients with suspected acute cholecystitis, and also in evaluating the patency of surgically devised drainage pathways of the biliary system, particularly after liver transplantation and after the Kusai procedure for biliary atresia (1).

Complexes of technetium 99m with mercaptoisobutyric acid (2), dihydrothioctic acid (3), pyridoxylidene glutamate (4), pyridoxal phenylalanine (5), methyliminodiacetic acid (6), and *N*-[[(2,6-dimethyl)phenylcarbamoyl]-methyl]iminodiacetic acid (6) have been used for the intravenous radionuclide cholangiogram in humans and for replacing [$^{131}$I]rose bengal, which has undesirable radiation characteristics. Complexes of technetium 99m with *N*-(2-mercapto-1-oxopropyl)glycine (7) and ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid (8) have been proposed as possible agents for the intravenous radionuclide cholangiogram.

The purpose of this study was to use animals to explore the relative merit of technetium 99m–*N*-(2-mercapto-1-oxopropyl)glycine ($^{99m}$Tc-I) and technetium 99m–ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic acid ($^{99m}$Tc-II) as diagnostic radiopharmaceuticals in humans. The criteria for selecting the best agent were the quality of the scintigraphic images of the hepatobiliary system in dogs and rabbits and the tissue distribution of both agents in rats and rabbits.

## EXPERIMENTAL

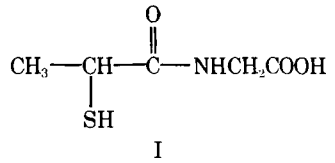
***N*-(2-Mercapto-1-oxopropyl)glycine[1] (I)**—Compound I was used without further purification.

**Ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic Acid[2] (II)**—A solution of *p*-bromophenol (22.82 g, 0.1319 mole), sodium dichloroacetate (19.92 g, 0.1319 mole), and ethylenediamine (3.96 g, 0.066 mole) in 10 ml of methanol and 6 ml of water was heated to 85° and stirred for 24 hr under nitrogen. The reaction mixture pH was maintained at 9.5 by adding 6 *N* NaOH. After cooling to room temperature, the pH was adjusted carefully to 6.0 with 6 *N* HCl. The precipitated *p*-bromophenol was removed by extraction with ether (3 × 35 ml).

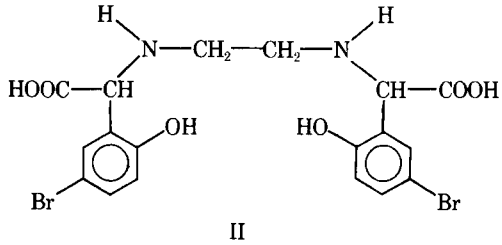
The aqueous solution pH was lowered to 4.0 with 6 *N* HCl, whereupon an orange solid precipitated. The dark supernate was carefully decanted, and the residue was washed repeatedly with methanol and acetone. The solvent finally was removed *in vacuo*, leaving a yellow solid (9.57 g, 30% yield), mp 245° dec. The compound could be purified further by column chromatography with silica gel and methanol–ammonium hydroxide as the eluent; IR (KBr): 3400–2400 (hydrogen-bonded OH) and 1660 (C=O) $cm^{-1}$; NMR ($CF_3COOH$–tetramethylsilane): δ 10.56 (s, 4H), 7.0 (s, 4H), 6.34–6.48 (d, 2H), 4.75 (s, 2H), and 3.4 (m, 4H).

*Anal.*—Calc. for $C_{18}H_{18}Br_2N_2O_6$: C, 41.72; H, 3.50; Br, 30.84; N, 5.4. Found: C, 41.72; H, 3.52; Br, 28.17; N, 6.18.

**Technetium 99m-*N*-(2-Mercapto-1-oxopropyl)glycine ($^{99m}$Tc-I)**—The technetium 99m complex of I was prepared by a modification of a reported method (7). Three milliliters of a saline eluate of sodium pertechnetate (III) (1–5 mCi), received from a molybdenum 99-technetium 99m generator[3], was mixed with 0.5 ml of a stock solution of stannous chloride–I, followed by 5–10 min of incubation at room temperature under a nitrogen atmosphere. The stock solution was prepared by mixing 40 ml of 0.15 *M* I in 1 *M* $NaHCO_3$ with 40 ml of $8.4 \times 10^{-3}$ *M* $SnCl_2 \cdot 2H_2O$ in 0.1 *M* HCl. The stock solution pH was adjusted



I



II

[1] A D and L mixture, Calbiochem, La Jolla, Calif.
[2] A D, L, and *meso* mixture.
[3] Mallinckrodt Chemical, St Louis, Mo.

0022-3549/80/0500-0581$01.00/0

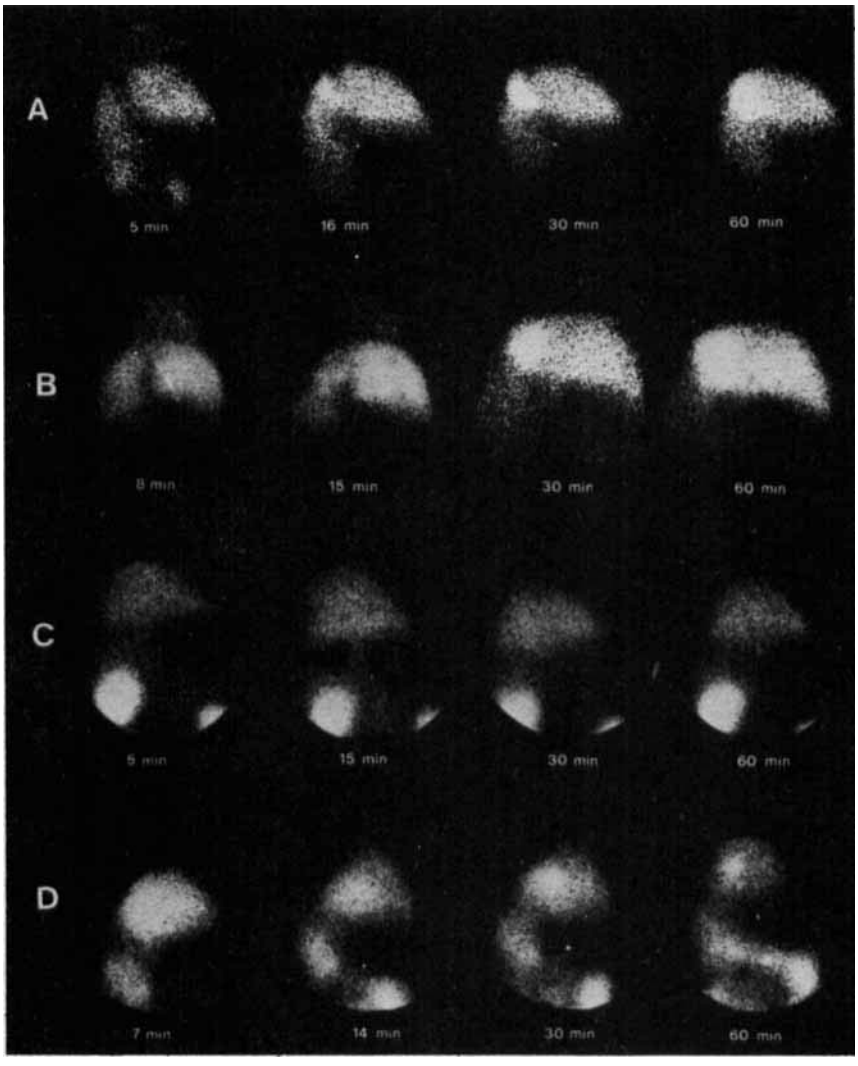



**Figure 1**—*Successive scintigraphic ventral images of the liver and gallbladder of* $^{99m}$*Tc-I (A and C) and* $^{99m}$*Tc-II (B and D) in beagles (A and B) and rabbits (C and D).*

to 7.0 with 5 *M* HCl, and it then was filtered through a 0.22-$\mu$m membrane filter into a sterile glass container.

All solutions were purged with nitrogen and were prepared fresh prior to each experiment. The molar ratio of I to tin was 18:1.

**Technetium 99m-Ethylenediamine-*N,N'*-bis(*α*-2-hydroxy-5-bromophenyl)acetic Acid ($^{99m}$Tc-II)**—The technetium 99m complex of II was prepared under a nitrogen atmosphere by mixing 2 ml of III (1–5 mCi) with 1 ml of a stock solution of stannous chloride–II, followed by 5–10 min of incubation at room temperature. The stock solution was prepared by mixing 100 ml of $2.5 \times 10^{-2}$ *M* II in 0.1 *M* NaOH with $2.0 \times 10^{-4}$ mole of stannous chloride dihydrate. After the stannous chloride was dissolved completely, the pH was adjusted to 8.8 with 1 *M* HCl, and the solution was filtered through a 0.22-$\mu$m membrane filter into a sterile glass container.

All solutions were purged with nitrogen and were prepared fresh prior to each experiment. The molar ratio of II to tin was 12.5:1.

**Labeling Efficiency of $^{99m}$Tc-I and $^{99m}$Tc-II**—Prior to administration, $^{99m}$Tc-I was analyzed for unreacted III by TLC on cellulose plates[4] with acetone as the developing solvent. The carrier I and the complex $^{99m}$Tc-I remained at the origin, while the unreacted III moved with the solvent front. The unreacted III was always <3%.

The $^{99m}$Tc-II was chromatographed on cellulose TLC plates[4] sprayed with 1% (w/v) $BaCl_2$. The developing solvent was prepared by mixing five parts of *n*-butanol with four parts of water and one part of acetic acid in a separator and retaining the organic layer. The $R_f$ values of III and $^{99m}$Tc-II were 0.43 and 0.94, respectively. The labeling efficiency was higher than 95%.

**Distribution Studies in Animals**—An 8-kg fasted beagle was injected intravenously with $^{99m}$Tc-I ($5 \times 10^{-4}$ mmole of I, 1 mCi). The same beagle was injected intravenously 15 days later with $^{99m}$Tc-II ($6.6 \times 10^{-4}$ mmole of II, 1 mCi). In both instances, the animal was anesthetized with an intramuscular dose of the dissociative anesthetic fentanyl and droperidol[5] (0.12 cm$^3$/kg); the animal was immobilized on a restrainer and placed under a $\gamma$-camera[6]. A series of ventral scintigraphic images was taken using a high-resolution pinhole collimator (Fig. 1).

**Table I—Percent of Injected Dose Recovered in Dog Bile**[a]

| $^{99m}$Tc-I | $^{99m}$Tc-II |
|---|---|
| 70.1[a] | 48.6 |
| 55.9 | 64.1 |

[a] Obtained 75 min postinjection; result of one measurement.

For the bile recovery studies, two groups of two beagles each were injected in the forelimb vein with $^{99m}$Tc-I ($1 \times 10^{-3}$ mmole, 1 mCi) and $^{99m}$Tc-II ($1 \times 10^{-3}$ mmole, 1 mCi), respectively. The animals were anesthetized with pentobarbital sodium (30 mg/kg), the common bile duct was catheterized with a polyethylene catheter, and bile was collected for 75 min (Table I). The cystic duct was ligated.

Two groups, each containing 21 albino New Zealand rabbits (2–3 kg), received by injection into the ear vein freshly prepared $^{99m}$Tc-I ($6.6 \times 10^{-4}$ mmole of I/kg, 1 mCi) and $^{99m}$Tc-II[7] ($6.6 \times 10^{-4}$ mmole of II/kg, 1 mCi), respectively. Groups of three animals were sacrificed at 0.25, 0.5, 1, 2, 3, 5, and 24 hr after injection of a lethal dose of pentobarbital. Blood samples were obtained by cardiac puncture immediately after death. The blood weight was considered to be 7% of the body weight. Certain organs were excised, wet weighed, and counted by a shielded scintillation crystal

[4] No. 6064, Eastman Kodak.

[5] Innovar, Pitman-Moore, Washington Crossing, N.J.

[6] Model II C, Picker Dyna camera.

[7] Reconstituted from lyophilized II provided by R. E. Boyd, Australian AEC.

**Table II—Distribution of Technetium 99m–*N*-(2-Mercapto-1-oxopropyl)glycine in Rabbits [a]**

| Organ | 0.25 hr | 0.50 hr | 1.00 hr | 2.00 hr | 3.00 hr | 5.00 hr | 24.00 hr |
|---|---|---|---|---|---|---|---|
| Liver | 0.07 ± 0.01[a] | 0.08 ± 0.02 | 0.09 ± 0.01 | 0.07 ± 0.02 | 0.08 ± 0.00 | 0.06 ± 0.02 | 0.03 ± 0.00 |
| Kidneys | 0.79 ± 0.27 | 1.23 ± 0.04 | 1.29 ± 0.09 | 1.19 ± 0.04 | 1.08 ± 0.19 | 0.68 ± 0.21 | 0.25 ± 0.03 |
| Gallbladder | 0.10 ± 0.06 | 0.14 ± 0.06 | 0.41 ± 0.03 | 0.37 ± 0.06 | 0.25 ± 0.13 | 0.09 ± 0.07 | 0.00 ± 0.01 |
| Blood | 1.34 ± 0.19 | 0.69 ± 0.10 | 0.89 ± 0.09 | 0.50 ± 0.04 | 0.29 ± 0.03 | 0.25 ± 0.02 | 0.02 ± 0.04 |
| Intestines | 0.03 ± 0.02 | 0.04 ± 0.00 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.03 ± 0.01 | 0.02 ± 0.00 |
| Urine and bladder | 0.71 ± 0.13 | 3.50 ± 3.53 | 3.69 ± 1.70 | 4.21 ± 1.59 | 3.10 ± 1.90 | 2.58 ± 1.25 | 5.35 ± 3.57 |

[a] Percent of dose per gram of organ (±*SD*); average results from three replications.

**Table III—Distribution of Technetium 99m–Ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic Acid in Rabbits [a]**

| Organ | 0.25 hr | 0.50 hr | 1.00 hr | 2.00 hr | 3.00 hr | 5.00 hr | 24.00 hr |
|---|---|---|---|---|---|---|---|
| Liver | 0.32 ± 0.05[a] | 0.18 ± 0.02 | 0.17 ± 0.01 | 0.12 ± 0.02 | 0.13 ± 0.01 | 0.09 ± 0.02 | 0.04 ± 0.00 |
| Kidneys | 0.72 ± 0.11 | 0.63 ± 0.05 | 0.61 ± 0.10 | 0.64 ± 0.17 | 0.61 ± 0.17 | 0.33 ± 0.03 | 0.20 ± 0.05 |
| Gallbladder | 0.57 ± 0.46 | 0.30 ± 0.23 | 1.23 ± 0.92 | 0.94 ± 0.28 | 0.92 ± 0.50 | 0.86 ± 0.39 | 0.00 ± 0.00 |
| Blood | 0.53 ± 0.09 | 0.30 ± 0.00 | 0.30 ± 0.04 | 0.31 ± 0.02 | 0.35 ± 0.08 | 0.27 ± 0.09 | 0.10 ± 0.06 |
| Intestines | 0.07 ± 0.00 | 0.09 ± 0.02 | 0.08 ± 0.00 | 0.11 ± 0.00 | 0.13 ± 0.04 | 0.13 ± 0.02 | 0.13 ± 0.01 |
| Urine and bladder | 1.34 ± 1.00 | 1.14 ± 0.20 | 1.49 ± 0.07 | 1.40 ± 0.57 | 1.20 ± 0.34 | 1.51 ± 0.70 | 4.63 ± 2.4 |

[a] Percent of dose per gram of organ (±*SD*); average results from three replications.

**Table IV—Distribution of Technetium 99m–*N*-(2-Mercapto-1-oxopropyl)glycine in Rats [a]**

| Organ | 0.50 hr | 1.00 hr | 2.00 hr | 3.00 hr | 5.00 hr | 24.00 hr |
|---|---|---|---|---|---|---|
| Liver | 15.1 ± 1.1[a] | 12.5 ± 2.0 | 9.3 ± 0.5 | 6.8 ± 1.0 | 4.9 ± 0.3 | 1.1 ± 0.7 |
| Kidneys | 11.5 ± 0.6 | 16.0 ± 2.0 | 15.1 ± 0.3 | 14.7 ± 2.3 | 16.1 ± 3.1 | 7.7 ± 2.6 |
| Blood | 9.0 ± 0.8 | 8.0 ± 0.8 | 4.8 ± 0.7 | 3.2 ± 0.9 | 1.3 ± 0.3 | 0.7 ± 0.4 |
| Urine and bladder | 15.9 ± 3.0 | 16.1 ± 3.0 | 18.1 ± 2.5 | 15.9 ± 3.3 | 23.4 ± 2.5 | 38.0 ± 3.7 |
| GI tract and feces | 30.1 ± 4.0 | 35.1 ± 2.0 | 40.1 ± 3.6 | 48.4 ± 2.7 | 52.1 ± 3.9 | 49.3 ± 6.1 |

[a] Percent of total (±*SD*); average results from three replications.

**Table V—Distribution of Technetium 99m–Ethylenediamine-*N,N'*-bis(α-2-hydroxy-5-bromophenyl)acetic Acid in Rats [a]**

| Organ | 0.50 hr | 1.00 hr | 2.00 hr | 3.00 hr | 5.00 hr | 24.00 hr |
|---|---|---|---|---|---|---|
| Liver | 20.5 ± 1.3 | 15.6 ± 2.0 | 13.5 ± 0.9 | 10.9 ± 0.8 | 6.7 ± 1.0 | 2.9 ± 0.7 |
| Kidneys | 8.9 ± 0.3 | 7.0 ± 0.1 | 7.7 ± 0.3 | 5.9 ± 0.4 | 4.7 ± 0.7 | 3.1 ± 0.1 |
| Blood | 5.7 ± 0.5 | 3.0 ± 0.3 | 3.2 ± 0.2 | 1.9 ± 0.3 | 1.5 ± 0.0 | 1.0 ± 0.0 |
| Urine and bladder | 4.4 ± 0.3 | 5.5 ± 1.0 | 5.9 ± 0.7 | 8.1 ± 1.2 | 8.4 ± 0.9 | 10.9 ± 3.9 |
| GI tract and feces | 45.9 ± 3.7 | 49.7 ± 4.3 | 59.7 ± 2.8 | 61.3 ± 5.0 | 74.9 ± 3.9 | 80.6 ± 5.4 |

[a] Percent of total (±*SD*); average results from three replications.

of fixed geometry linked to a counter[8,9]. The results were reported as the percent of total injected radioactivity per gram of organ (Tables II and III). Prior to sacrifice, several scintigraphic images of the abdominal area were taken with a γ-camera[6] shielded with a pinhole collimator (Fig. 1).

Two groups, each containing 18 white rats, received by injection into the tail vein freshly prepared $^{99m}$Tc-I ($1.3 \times 10^{-3}$ mmole of I, 0.5 mCi) and $^{99m}$Tc-II[10] ($1.3 \times 10^{-3}$ mmole of II, 0.5 mCi), respectively. Prior to experimentation, the animals were housed in groups of three for 3 days. They were allowed free access to food and tap water. The animals were sacrificed in groups of three at 0.5, 1, 2, 3, 5, and 24 hr postinjection. Certain organs were excised and counted in a well counter[9]. The results are reported as the percent of total injected radioactivity (Tables IV and V).

## RESULTS AND DISCUSSION

The radioactive sodium pertechnetate (III) reacted with an excess of I. The complexation took place at room temperature in the presence of the oxidizable salt stannous chloride and under a nitrogen atmosphere. Since trace quantities of cations could interfere with the stability of the thiol (I), highly pure reagents and diluents were used. Although the ligand (I) to tin ratio varied from 9:1 to 18:1, this variation did not affect the extent of the complexation with III, as was evident from TLC–radiochromatographic monitoring of the extent of chelation of III.

Ligand II was soluble at pH values lower than 4.0 and higher than 8.0. The complex was formed by reducing III with stannous chloride in the presence of II at pH 8.5. The solutions were purged thoroughly with nitrogen to avoid oxidation of the phenol rings. Although the ligand to tin ratio ranged from 10:1 to 16:1, this variation did not affect the extent of complexation of the reduced technetium with the ligand II.

In both instances, the exact nature of the technetium complexes ($^{99m}$Tc-I and $^{99m}$Tc-II) was uncertain. The presence of a thiol and a carboxyl group in I and the carboxyl, secondary amine, and hydroxyl groups in II were necessary for the formation of stable chelates. The high molecular weight (mol. wt. 518) of ligand II and the presence of two bromophenolic rings rendered the molecule susceptible to extensive biliary excretion. In contrast, ligand I had a lower molecular weight (mol. wt. 163) and lacked lipophilic groups. Therefore, it was expected to be less suitable than II for hepatobiliary excretion.

The sequential scintigraphic images (Fig. 1) of the liver and gallbladder of a beagle indicated that both $^{99m}$Tc-I and $^{99m}$Tc-II had a similar distribution pattern. Specifically, $^{99m}$Tc-I started accumulating in the liver 5 min following intravenous administration. During the same period, the gallbladder became visible, and it was delineated clearly 30 min postinjection. When $^{99m}$Tc-II was administered, the gallbladder was clearly delineated in the first 30 min postinjection, and the quality of the scintigraphic images was the same as that received with $^{99m}$Tc-I. Upon intravenous administration of 50 μg of cerulein, the gallbladder in both cases contracted and enabled visualization of the common bile duct as the radioactivity was excreted into the intestines. A slight kidney uptake was noted with $^{99m}$Tc-I.

The scintigraphic images taken following intravenous administration of $^{99m}$Tc-I in rabbits (Fig. 1) indicated rapid accumulation of the radioactivity in the liver 5–15 min postinjection. During the same period, the gallbladder became slightly visible but never was clearly delineated

[8] Logic model 111, Abbott Laboratories, North Chicago, Ill.
[9] Picker Spectro Scaler-4, North Haven, Conn.
[10] Compound II was obtained by the described synthetic process.

**Table VI—Target to Nontarget Ratios for $^{99m}$Tc-I and $^{99m}$Tc-II in Rabbits**

| | 0.25 hr | 0.50 hr | 1.00 hr | 2.00 hr | 3.00 hr | 5.00 hr | 24.00 hr |
|---|---|---|---|---|---|---|---|
| | | | Gallbladder to Liver Ratio[a] | | | | |
| $^{99m}$Tc-II | 1.8 | 1.7 | 7.2 | 7.8 | 7.1 | 9.6 | 0.0 |
| $^{99m}$Tc-I | 1.4 | 1.8 | 4.5 | 5.3 | 3.1 | 1.5 | 0.0 |
| | | | Gallbladder to Blood Ratio[a] | | | | |
| $^{99m}$Tc-II | 1.1 | 1.0 | 4.1 | 3.0 | 2.6 | 3.2 | 0.0 |
| $^{99m}$Tc-I | 0.1 | 0.2 | 0.5 | 0.7 | 0.9 | 0.4 | 0.0 |

[a] Ratio of percent of dose per gram of tissue to percent of dose per gram of tissue.

during the experiment. The visualization of the kidneys, urinary bladder, and small intestine indicated that part of the radioactivity was shunted through the urinary system and part was excreted through the intestines. The scintigraphic images (Fig. 1) taken when $^{99m}$Tc-II was administered indicated a similar distribution pattern, except that the gallbladder was clearly visible and delineated 30 min postinjection and remained as such throughout the experiment.

The distribution of the radioactivity as a function of time in rabbits (Tables II and III) indicated that $^{99m}$Tc-II cleared from the blood more rapidly than $^{99m}$Tc-I and was accumulated rapidly in the liver. For instance, at 30 min postinjection, the radioactivity levels of $^{99m}$Tc-I in the liver and blood were 0.08 and 0.69% of the dose/g, respectively; the corresponding levels for $^{99m}$Tc-II were 0.18 and 0.30%, respectively. On the other hand, the urinary excretion, as denoted by the kidney, urine, and bladder levels, was higher for $^{99m}$Tc-I than for $^{99m}$Tc-II during the same period. In contrast, the levels of the gallbladder and intestines were higher for $^{99m}$Tc-II than for $^{99m}$Tc-I throughout the testing period. Furthermore, the target organ to nontarget organ ratio, which is the parameter for assessing the imaging capability of any diagnostic radiopharmaceutical, was higher for $^{99m}$Tc-II than for $^{99m}$Tc-I (Table VI). In particular, the gallbladder to blood ratio of $^{99m}$Tc-II was five to 10 times higher than that of $^{99m}$Tc-I during the first 30 or 60 min postinjection. The gallbladder to liver ratio of $^{99m}$Tc-II was higher than that of $^{99m}$Tc-I and remained as such up to 5 hr postinjection.

The distribution of $^{99m}$Tc-I and $^{99m}$Tc-II in rats (Tables IV and V) indicated that a good portion of $^{99m}$Tc-I was excreted through the kidneys while the urinary excretion of $^{99m}$Tc-II was lower. The high urinary excretion component of $^{99m}$Tc-I was in agreement with observations made by previous investigators that low molecular weight mercaptans are retained in the kidneys and excreted through the urine in rats (9). Liver uptake was appreciable for $^{99m}$Tc-II.

The scintigraphic images in dogs (Fig. 1) of both $^{99m}$Tc-I and $^{99m}$Tc-II were of equivalent quality. In both instances, the liver and gallbladder were delineated clearly and rapidly. The bile collection during the first 75 min postinjection did not reveal appreciable differences in the bile excretion of the two compounds (Table I). The tissue levels in rats revealed certain differences in the distribution of both $^{99m}$Tc-I and $^{99m}$Tc-II; however, the tissue levels in rabbits revealed gross differences, as was corroborated by the target to nontarget ratios (Table VI) and the differences in the quality of scintigraphic images (Fig. 1).

The *in vivo* distribution data agree with certain observations of previous investigators concerning the hepatobiliary excretion of substances as affected by the species of the experimental animal and the molecular characteristics of the compound. According to one investigator (10), hepatobiliary excretion in both dogs and rats is extensive. These species are classified as good hepatobiliary excretors, while rabbits, humans, and primates are classified relative to dogs and rats as poor hepatobiliary excretors. Furthermore, high lipophilicity and high molecular weight are factors that favor hepatobiliary excretion, while low molecular weight hydrophilic substances are shunted through the urinary pathway of excretion (10).

The exact reason for the higher biliary excretion of $^{99m}$Tc-I in dogs and rats is not known. It may be the result of increased lipophilicity and increased molecular weight due to the formation of $^{99m}$Tc-I or due to the formation of a complex with two ligands ($^{99m}$Tc-$I_2$). Further studies are in progress to investigate the complex. In rabbits, which like humans and primates are poor biliary excretors relative to dogs and rats (10), $^{99m}$Tc-II, due to its favorable molecular characteristics, was a better hepatobiliary scintigraphic agent than $^{99m}$Tc-I. Finally, in light of the presented data, it is concluded that rabbits are a better model than dogs or rats for evaluating hepatobiliary scintigraphic agents for use in humans.

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