Technetium-99m-L,L-Ethylenedicysteine: A Renal Imaging Agent. I. Labeling and Evaluation in Animals

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L,L-ethylenedicysteine (L,L-EC) can be labeled efficiently with ^{99m}Tc at pH 12 to obtain a highly pure and very stable tracer agent (^{99m}Tc-L,L-EC). The biological behavior of ^{99m}Tc-L,L-EC was studied in mice and a baboon. In mice, ^{99m}Tc-L,L-EC demonstrated a more rapid urinary excretion and less retention in the kidneys, the liver, the intestines, and the blood than did ^{99m}Tc-MAG_3 at 10 and 60 min p.i. Urinary excretion decreased in probenecid pretreated mice, which indicates active tubular transport. In the baboon, the renograms for ^{99m}Tc-MAG_3 and ^{99m}Tc-L,L-EC were comparable. Plasma-protein binding of ^{99m}Tc-L,L-EC was lower than that of ^{99m}Tc-MAG_3 while its distribution volume and 1-hr plasma clearance were clearly higher. The promising results of the animal experiments suggest that ^{99m}Tc-L,L-ethylenedicysteine may be a useful alternative to ^{99m}Tc-MAG_3 for renal function studies in humans.

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Over the last ten years, several chelates labeled with ^{99m}Tc have been proposed as alternatives to radioiodinated 2-iodohippurate (Hippuran, OIH) for renal function studies. The newly developed compounds belong to different complex forming ligand classes: dimercaptodiamides (N_2S_2), mercaptotriamides (N_3S), and substances bearing an iminodiacetic acid (IDA) moiety. Within these ^{99m}Tc-labeled compounds, evidence for efficient tubular secretion was first observed with ^{99m}Tc-N,N′-bis-(mercaptoacetyl)ethylenediamine (^{99m}Tc-DADS), but the pronounced hepatobiliary clearance of ^{99m}Tc-DADS was a major obstacle for its clinical use (*1–3*). A large number of derivatives of ^{99m}Tc-DADS was then evaluated (*4–6*) of which ^{99m}Tc-N,N′-bis-(mercaptoacetyl)-2,3-diaminopropanoate (^{99m}Tc-CO_2DADS) showed the most favorable renal excretion characteristics (*7–9*). However, labeling of CO_2DADS with ^{99m}Tc always results in a mixture of stereo-isomers with clearly different biological properties (*10*). The need for an HPLC purification step to separate the most suitable isomer has precluded its introduction into clinical practice. A completely different approach to a ^{99m}Tc-labeled renal function agent was the development of PAHIDA, a derivative of para-aminohippuric acid with a side chain containing an IDA group to bind technetium. In mice, ^{99m}Tc-PAHIDA shows a rapid and high urinary excretion, but in dogs its plasma clearance resembles that of ^{99m}Tc-DTPA, so ^{99m}Tc-PAHIDA does not seem to be a suitable substitute for hippuran (*11–13*).

The most appropriate and successful ^{99m}Tc-labeled chelate for renal function studies up to the present is ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG_3). It is based on a N_3S donor ligand system and does not suffer from the problem of isomers with different biological behavior (*14*). In humans, ^{99m}Tc-MAG_3 is extracted efficiently from the plasma by the kidneys and excreted rapidly in the urine, which results in renograms very similar to those obtained with OIH (*15–18*).

Technetium-99m-MAG_3 has now been approved by the Food and Drug Administration as a radiopharmaceutical for renal function studies. In Europe, it has already been in clinical use for several years. The superior physical characteristics of the ^{99m}Tc label and its attractive biological properties make ^{99m}Tc-MAG_3 the agent of choice at present, especially for the evaluation of transplant kidney and tubular necrosis and kidney function in general.

Nevertheless, ^{99m}Tc-MAG_3 is still not the ideal replacement for OIH, and improvements are still possible. The plasma-protein binding of ^{99m}Tc-MAG_3 is very high (*19, 20*), and its plasma clearance in humans is no higher than about 60%–65% of the OIH value. Therefore, accurate determination of the effective renal plasma flow (ERPF) is rather difficult using ^{99m}Tc-MAG_3, although formulae have been proposed to calculate ERPF from the acquired data (*20,21*). For these reasons, the development of a ^{99m}Tc-labeled renal function agent that approaches hippuran more closely than ^{99m}Tc-MAG_3 would be welcome.

During a study of the biodistribution of the metabolites of the brain agent ^{99m}Tc-L,L-ethylenedicysteine diethyles-

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ter (^{99m}Tc-L,L-ECD) in mice (*22*), we found that the most polar metabolite, i.e., ^{99m}Tc-L,L-ethylenedicysteine (^{99m}Tc-L,L-EC, Fig. 1) is excreted rapidly and efficiently in the urine. Other authors have also reported that after intravenous injection of ^{99m}Tc-L,L-ECD in humans significant amounts of radioactivity are excreted in the urine due to the in vivo formation of more polar de-esterified metabolites including the di-acid (*23–25*). These observations have prompted us to investigate the potential usefulness of ^{99m}Tc-L,L-EC as a renal function agent that may be a more adequate substitute for OIH than ^{99m}Tc-MAG_3.

The optimal conditions for labeling L,L-EC with ^{99m}Tc and the biological evaluation of the new tracer agent in animals are reported in this paper.

MATERIALS AND METHODS

Ligand Synthesis and Labeling with ^{99m}Tc

L,L-ethylenedicysteine was synthesized following a published procedure (*26*) and the structure was confirmed by ^{1}H-NMR spectroscopy. Labeling with ^{99m}Tc was studied by a direct labeling method at room temperature. To a 10-ml vial were added successively 1 ml of an aqueous solution of L,L-EC (0.1–5 mg/ml) at pH 6–13, 5–100 μg $SnCl_2 \cdot 2H_2O$ dissolved in 25 μl HCl 0.05 N and 2–8 ml eluate from a commercial ^{99}Mo/^{99m}Tc generator (Ultratechnekow FM™, Mallinckrodt Diagnostica, Holland) containing 370 MBq–3,700 MBq [^{99m}Tc]pertechnetate. After an incubation period (10 sec–10 min), the pH was adjusted to 7 ± 0.5 by addition of the required volume of a 0.5 *M* phosphate buffer pH 5. Technetium-99m-MAG_3 and ^{99m}Tc-DTPA were prepared from commercially available labeling kits (Mallinckrodt Diagnostica, Holland) following the manufacturer's instructions.

Analysis of ^{99m}Tc-L,L-EC

Thin-Layer Chromatography (TLC). TLC of the reaction product after labeling was performed on two ITLC-SG™ sheets (Gelman Sciences, Ann Arbor, MI) eluted with, respectively, acetone (system A) and 0.5 *M* acetic acid (system B). The R_f values of ^{99m}Tc-L,L-EC, $^{99m}TcO_2$, and $^{99m}TcO_4^-$ are, respectively, 0, 0, and 1 in system A and 1, 0, and 1 in system B. A quantitative analysis of the chromatograms was performed by cutting the strips at half the migration distance and counting the radioactivity on each part.

High-Performance Liquid Chromatography (HPLC). The HPLC system for radiochemical analysis of the reaction mixtures and preparative separation of highly pure ^{99m}Tc-MAG_3 and ^{99m}Tc-L,L-EC consisted of a Merck-Hitachi L6200 ternary gradient pump, a Rheodyne 9125 injector, and a 250 mm × 4.6 mm (i.d.) column filled with Hypersil ODS 5 μm (Shandon Scientific Limited, England). The solvent mixtures and gradient profile used for the preparative separation of ^{99m}Tc-MAG_3 have been described elsewhere (*27*). In the case of ^{99m}Tc-L,L-EC, the column was eluted at a flow rate of 1 ml/min with a gradient mixture of 0.0125 *M* phosphate buffer, pH 2.5, and ethanol (0–6 min: gradient from 0 to 9% ethanol; 6–20 min: 9% ethanol). Radioactivity in the effluent was monitored with a 2-inch NaI(Tl) detector coupled to a single channel analyzer and integrated by a Ramona-4 software program (Raytest, Isotopenmeßgerate GmbH, Germany) installed on a personal computer.

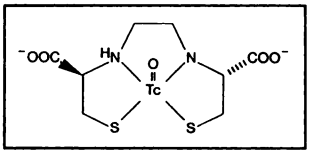

FIGURE 1. Proposed structure of ^{99m}Tc-L,L-EC.

Biodistribution in Mice

Isolated HPLC peaks of ^{99m}Tc-DTPA, ^{99m}Tc-MAG_3, and ^{99m}Tc-L,L-EC were diluted to a concentration of 144 kBq/ml with saline. Iodine-131-hippuran (Mallinckrodt Diagnostica, Holland) was added to a concentration of 14.4 kBq/ml as an internal biological standard. Male NMRI mice (body mass 20–30 g) were first sedated by i.m. injection of 0.02 ml Hypnorm^R (Janssen Pharmaceutica, Belgium) diluted with water to a concentration of 2.5 mg fluanisone and 0.05 mg fentanyl per ml. 0.1 ml of the tracer solution, containing 148 kBq of the ^{99m}Tc agent and 14.8 kBq of ^{131}I-OIH, was injected via a tail vein. The mice were killed by decapitation at respectively 10 and 30 min p.i. and the organs as well as other body parts were dissected. Activity in all organs was counted in a 3-inch NaI(Tl) well crystal coupled to a dual-channel analyzer and scaler, and was expressed as a percentage of injected activity equal to the sum of the activity in all organs. Blood was assumed to be 7% of the total body mass. Corrections were made for background radiation, physical decay during counting, and ^{131}I crossover into the ^{99m}Tc channel. Similar experiments were performed on mice pretreated with 25 mg/kg probenecid 10 min prior to the tracer injection in order to study the ability of the new tracer agent to compete with other compounds for the hippurate anion transport system in the renal tubules.

Evaluation in a Baboon

A male baboon (body mass approximately 23 kg) was anesthetized by intramuscular injection of 180 mg ketamine (Imalgène^R, Rhône Mérieux, France). Anesthesia was sustained by intravenous injection of 15 mg sodium pentobarbital (Nembutal^R, Sanofi, Belgium) at 10-min intervals. The ^{99m}Tc-labeled agent (18.5 MBq), purified by HPLC, was coinjected with 1.85 MBq of ^{131}I-OIH via a limb vein.

The camera was positioned over the kidney region of the baboon in supine position. A dynamic series of 30-sec frames was collected for 30 min after injection using a gamma camera (Pho/Gamma IV, Searle) equipped with a diverging low-energy collimator. Data were acquired and stored in a HP 2100 computer in order to generate time-activity curves. Scintiphotos (30 kcts) were taken at regular intervals.

To construct the plasma disappearance curve, 2-ml blood samples were collected at 2, 4, 6, 8, 10, 15, 20, 30, 45, and 60 min in heparinized tubes. After centrifugation for 10 min at 1,000 g, 500-μl plasma samples were pipetted, weighed and counted in a 3-inch NaI(Tl) well crystal connected to a dual channel analyzer and scaler. Corrections were made for physical decay of ^{99m}Tc, ^{131}I crossover into the ^{99m}Tc channel, and residual activity in the syringe. The data were expressed as a percentage of the injected dose (%ID). From this curve, the 1-hr plasma clearance of both tracer agents was calculated by computer using a double exponential fitting method based on a two-compartmental system (*28*). Plasma-protein binding was determined by ultrafiltration (Ultrafree-PF filter units, type UFP1 LGC, Millipore).

RESULTS

Labeling and Analysis

HPLC chromatograms of the reaction mixtures after labeling of 1 mg L,L-ethylenedicysteine with ^{99m}Tc at different pH values (pH 6–pH 13) are compared in Figure 2. The labeling yields of these experiments are given in Table 1. Labeling at neutral pH does not result in the formation of the same ^{99m}Tc-complex that is obtained after hydrolysis of both ester groups in ^{99m}Tc-L,L-ECD but yields mainly a mixture of peaks with shorter retention times (Rt) on the reversed-phase (RP) HPLC system. TLC demonstrates that these peaks are different from pertechnetate. At pH<6, the preparation becomes turbid, probably due to the reduced solubility of the ligand. As the pH during labeling is higher, the relative amount of the peak with Rt = 12 min, which corresponds to the di-acid metabolite of ^{99m}Tc-L,L-ECD, increases. This compound is formed predominantly as a single radiochemical species at pH 12–pH 13. In these conditions, labeling is very rapid and a radiochemical purity over 98% is obtained almost instantaneously, as is shown by the TLC and HPLC results. The composition of such a preparation reconstituted at high pH is not altered by subsequent neutralization to pH 7.0–7.5. The stability of the preparation is not affected by this manipulation and no changes in the radiochemical purity were observed up to 24 hr after the neutralization step. As an illustration, Figure 3 shows the HPLC chromatograms of a reaction mixture (a) immediately after labeling at pH 12, (b) after neutralization to pH 7.3, and (c) after storage of the neutralized preparation for 24 hr.

The results of the labeling experiments at pH 12 with varying amounts of [^{99m}Tc]pertechnetate (370–3700 MBq), L,L-EC (0.1–5 mg) and stannous chloride dihydrate (5–100 μg) and analyzed at different time intervals after reconstitution are summarized in Table 1. It appears that identical labeling yields are obtained with amounts of the ligand between 0.1 and 10 mg. A very small quantity of stannous chloride dihydrate (5 μg) is sufficient for efficient labeling, while higher amounts do not affect the labeling yield. In all the conditions mentioned above, the complexation of ^{99m}Tc by the tetraligand occurs almost immediately. Radiochemical purity is not adversely affected by increasing the added radioactivity up to 3700 MBq.

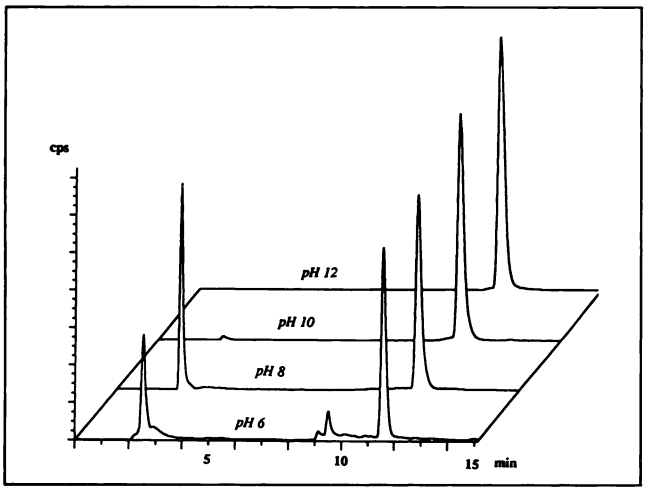

FIGURE 2. Radio-HPLC chromatograms of labeling reaction mixtures showing the influence of the pH on the labeling yield of ^{99m}Tc-L,L-EC.

TABLE 1
Influence of Incubation Time (A), Amount of Ligand (B), Amount of Reductant (C), and pH (D) on the Radiochemical Purity (RCP) of ^{99m}Tc-L,L-EC After Labeling

A*	Time after reconstitution (min)	RCP (%)
	0.75	55.4
	1.33	84.7
	2.00	98.9
	5.00	>99
	15.0	>99
B†	Amount of L,L-EC (mg)	RCP (%)
	0.10	>98
	0.25	>99
	0.50	>98
	1.00	>99
	2.00	>99
	5.00	>99
C‡	Amount of $SnCl_2 \cdot 2H_2O$ (μg)	RCP (%)
	5	>95
	10	>99
	25	>99
	50	>99
	100	>99
D[q]	pH	RCP (%)
	6	42.7
	7	47.6
	8	61.9
	9	69.1
	10	97.1
	11	98.3
	12	99.6
	13	98.8
E§	Amount of $^{99m}TcO_4^-$ (MBq)	RCP (%)
	370	>99
	3700	>97

* Labeling at pH 12, 1 mg L,L-EC, 100 μg $SnCl_2 \cdot 2H_2O$, 740 MBq ^{99m}Tc.

† Labeling at pH 12, 100 μg $SnCl_2 \cdot 2H_2O$, 740 MBq ^{99m}Tc, analysis after 5 min.

‡ Labeling at pH 12, 1 mg L,L-EC, 740 MBq ^{99m}Tc, analysis after 5 min.

[q] Labeling with 1 mg L,L-EC, 100 μg $SnCl_2 \cdot 2H_2O$, 740 MBq ^{99m}Tc, analysis after 5 min.

§ Labeling at pH 12, 1 mg L,L-EC, 100 μg $SnCl_2 \cdot 2H_2O$, analysis after 5 min.

Animal Studies

Experiments in Mice. The results of the biodistribution study of ^{99m}Tc-L,L-EC in mice at 10 min and 30 min p.i. are compared with the values obtained for ^{99m}Tc-MAG_3,

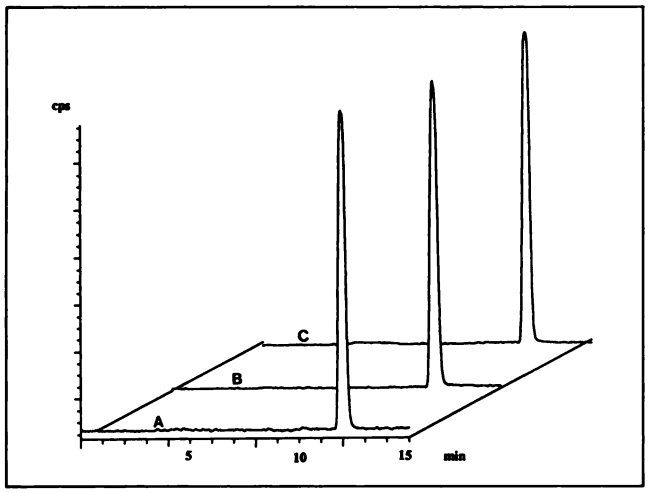

FIGURE 3. Radio-HPLC chromatograms of ^{99m}Tc-L,L-EC: (A) labeled at pH 12, (B) labeled at pH 12 and adjusted to pH 7.4 with 0.2 ml of a 0.5 *M* phosphate buffer pH 5, (C) preparation B after 12 hr at room temperature.

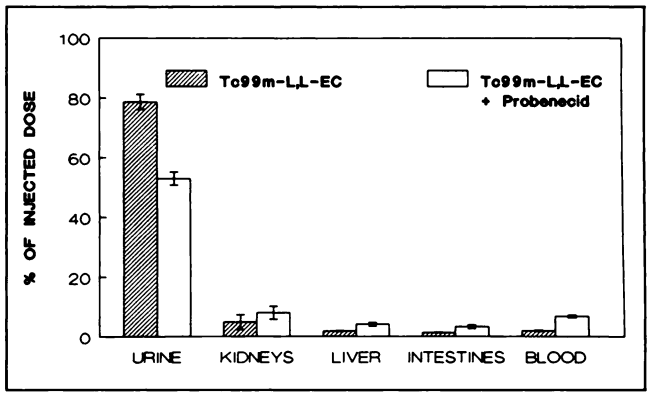

FIGURE 4. Effect of probenecid (25 mg/kg) on the biodistribution of ^{99m}Tc-L,L-EC in mice (n=5) at 10 min p.i.

^{99m}Tc-DTPA, and commercial ^{131}I-OIH in Table 2. The biodistribution in mice pretreated with probenecid at 10 min p.i. is shown graphically in Figure 4 and is compared with the biodistribution profile of untreated animals. Technetium-99m-L,L-EC is characterized by rapid clearance from the plasma, rapid and high excretion to urine with minimal retention in the kidneys, and negligible uptake in the liver, the intestines and other organs or body parts. Compared to ^{99m}Tc-MAG$_3$, ^{99m}Tc-L,L-EC has a slightly higher urinary excretion at 10 and 30 min p.i., while the uptake of ^{99m}Tc-L,L-EC in the hepatobiliary system is markedly lower at both time intervals. It is apparent that ^{99m}Tc-L,L-EC approaches hippuran more closely than does ^{99m}Tc-MAG$_3$. Pretreatment of the animals with probenecid impairs the renal excretion characteristics as shown at 10 min p.i. by a reduction of about 25% in urinary excretion (53.11% ± 2.16% versus 78.71% ± 2.53%), a more pronounced handling by the hepatobiliary system (4.11% ± 0.58% versus 1.86% ± 0.19%), and a slower clearance from the blood (6.79% ± 0.48% versus 1.97% ± 0.24%) (Fig. 4). Both these characteristics and the higher urinary excretion of ^{99m}Tc-L,L-EC as compared with that of ^{99m}Tc-DTPA indicate that ^{99m}Tc-L,L-EC is eliminated at least partially by active tubular transport.

Baboon Study. Figure 5 shows the left kidney renogram obtained for ^{99m}Tc-L,L-EC and ^{99m}Tc-MAG$_3$ in the same baboon with a 7-day time interval. The time to reach the renal maximum (T_{max}) is somewhat shorter for ^{99m}Tc-L,L-EC and its renal maximum (R_{max}) attains 93% of the value of ^{99m}Tc-MAG$_3$ (Table 3). Images of the lower abdomen at 5 min and 10 min p.i. are represented in Figure 6. Activity in the surrounding tissues is less visible in the case of ^{99m}Tc-L,L-EC. The 1-hr plasma clearance, distribution volume, and plasma-protein binding are given for both ^{99m}Tc agents in Table 3. Protein binding of ^{99m}Tc-L,L-EC is only about a third of that of ^{99m}Tc-MAG$_3$. However, the distribution volume of ^{99m}Tc-L,L-EC is nearly twice that of ^{99m}Tc-MAG$_3$. The 1-hr plasma clearance of the new tracer agent exceeds the plasma clearance of ^{99m}Tc-MAG$_3$ by a factor of 1.5.

DISCUSSION

Bis-aminoethanethiol (BAT) tetradentate ligands, also called diaminodithiol (DADT) compounds, are known to form very stable Tc(V)O-complexes on the basis of efficient binding of the oxotechnetium group to two thiol-

TABLE 2
Biodistribution in Mice (n = 10) of ^{131}I-Hippuran, ^{99m}Tc-MAG$_3$, ^{99m}Tc-DTPA, and ^{99m}Tc-L,L-EC at 10 and 30 Minutes Postinjection

	Time (p.i.)	Blood	Liver	Intestines	Kidneys	Urine
	min	% Injected Dose ± s.d.				
^{131}I-Hippuran	10	2.83 ± 0.49	1.63 ± 0.42	1.39 ± 0.26	2.73 ± 1.11	81.11 ± 2.68
	30	1.38 ± 0.60	0.76 ± 0.28	1.03 ± 0.28	0.62 ± 0.25	90.65 ± 2.35
^{99m}Tc-MAG$_3$	10	1.67 ± 0.45	3.40 ± 1.49	2.07 ± 0.94	6.76 ± 2.28	77.26 ± 2.22
	30	0.47 ± 0.25	1.32 ± 0.62	4.49 ± 0.82	1.77 ± 0.99	88.99 ± 3.27
^{99m}Tc-DTPA	10	9.27 ± 1.57	1.71 ± 0.25	3.39 ± 0.14	5.12 ± 0.79	53.46 ± 4.92
	30	2.87 ± 0.76	1.00 ± 0.20	1.58 ± 0.28	2.13 ± 0.61	81.82 ± 5.29
^{99m}Tc-L,L-EC	10	1.97 ± 0.24	1.86 ± 0.19	1.35 ± 0.17	4.87 ± 2.48	78.71 ± 2.53
	30	0.31 ± 0.09	1.04 ± 0.38	1.05 ± 0.25	0.68 ± 0.16	93.99 ± 1.35

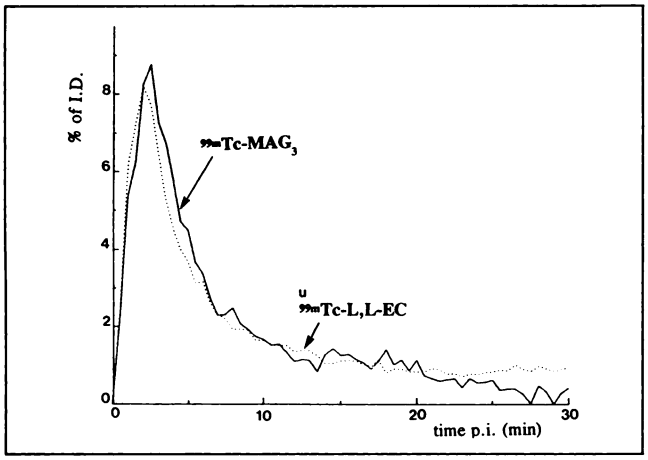

FIGURE 5. Computer-generated renograms (left kidney) of ^{99m}Tc-MAG$_3$ and ^{99m}Tc-L,L-EC in the same baboon from 0–30 min p.i.

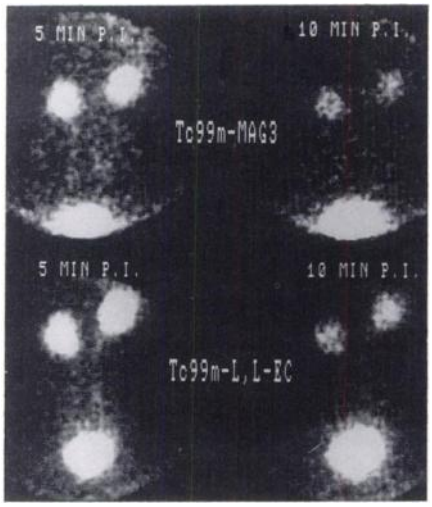

FIGURE 6. Images of the lower abdomen at 5 and 10 min after injection of ^{99m}Tc-MAG$_3$ and ^{99m}Tc-L,L-EC in the same baboon.

sulphur and two amine-nitrogen atoms. This class of N_2S_2 chelates has been developed in the search for neutral lipid-soluble complexes with ^{99m}Tc displaying high in vivo brain uptake and retention (*29,30*). Technetium-99m-L,L-ECD is the most recent and successful example of this series. The strong complexing properties of the BAT backbone are now also being explored for stable, rapid, and efficient incorporation of ^{99m}Tc in proteins after attachment of an N_2S_2-derived moiety (*31–33*).

The general experience is that BAT-compounds can easily be labeled with ^{99m}Tc in a high yield at pH 7 by both direct and exchange labeling. The preparation of ^{99m}Tc-L,L-ECD with almost a 100% yield from a Neurolite™ labeling kit (E.I. du Pont-Merck, North Billerica, MA) is a practical application of this. Therefore, it is surprising that direct labeling of L,L-ethylenedicysteine, the di-acid derivate of L,L-ECD, with ^{99m}Tc at pH 7 yields no more than about 50% of the expected ^{99m}Tc-L,L-EC, which can also be formed by alkaline hydrolysis of the di-ester brain agent (Fig. 7) (*22*). Labeling of L,L-ethylenedicysteine at pH 10 or higher is required to form the desired complex with a radiochemical purity over 95%, and, ideally, the pH should be 12. From a chemical point of view L,L-ethylenedicysteine is, in fact, a bis aminoacid. Consequently, the amino groups are probably partially ionized up to high pH values due to zwitterion formation. It is likely that the protons on the ionized amines compete with Tc for binding to the nitrogen atoms and thus prevent adequate complexation of ^{99m}Tc by the tetraligand. The main impurity formed upon labeling of L,L-EC below pH 10 has a very short retention time on RP-HPLC (Fig. 2). This same complex is formed in a high yield during direct

TABLE 3
Renogram Parameters, One-Hour Plasma Clearance, Distribution Volume, and Plasma-Protein Binding of ^{99m}Tc-MAG$_3$ and ^{99m}Tc-L,L-EC in a Baboon

	^{99m}Tc-MAG$_3$	^{99m}Tc-L,L-EC
T_{max} (min)*	2.5	2.0
R_{max} (as % of ID)†	8.8	8.2
THAP (min)‡	2.2	1.7
Clearance (ml/min/1.73 m^2)	320	541
Clearance (as % of coinj. OIH)	51.7	75.4
Distribution volume (ml)	789	1964
Distribution volume (as % of coinj. OIH)	58.9	96.3
Plasma protein binding (%)	92	28
Plasma protein binding (as % of coinj. OIH)	153	46

* T_{max} = time after injection to reach the maximum in the kidneys.
† R_{max} = renal maximum as % ID.
‡ THAP = time to reach half of the maximum after the peak.

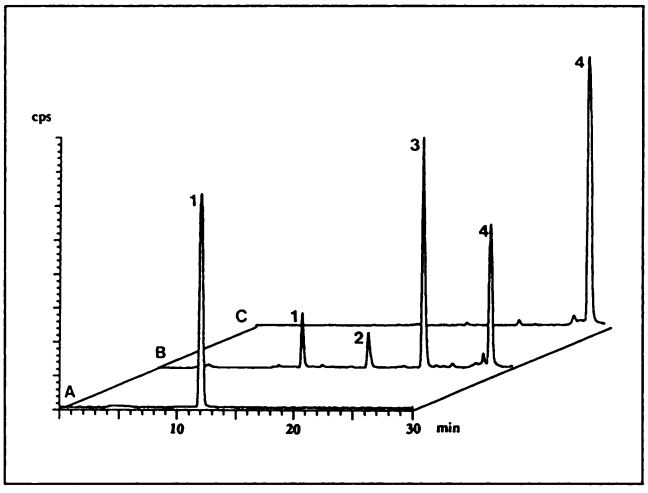

FIGURE 7. Comparison of the radio-HPLC chromatograms of (A) ^{99m}Tc-L,L-EC, (B) ^{99m}Tc-L,L-ECD after alkaline hydrolysis (1 = di-acid, 2 and 3 = diastereomeric monoesters, 4 = di-ester) and (C) ^{99m}Tc-L,L-ECD. HPLC conditions: stationary phase 250 mm × 4.6 mm (ID) column filled with Hypersil ODS 5 μm (Shandon Scientific Limited, England); mobile phase A = 0.0125 *M* phosphate buffer pH 2.5, B = 30% ethanol in A and C = ethanol p.a. Gradient profile: 0 to 20 min: 0 to 100% B, C = 0%; 20.1 to 30 min: B = 57%, A = 0%.

labeling of L,L-EC in 0.5 *M* HCl. TLC excluded the presence of pertechnetate in this reaction mixture. On the basis of the short retention time, a high polarity can be attributed to this complex. However, biodistribution in mice showed a high degree of hepatobiliary excretion. So far the structure of this very polar complex has not yet been elucidated.

The need for labeling L,L-ethylenedicysteine at pH 12 does not compromise the simplicity and ease of preparation of ^{99m}Tc-L,L-EC and its practical usefulness in daily routine. On the basis of the results of the labeling experiments, we developed a labeling kit that can make its use in clinical practice more attractive. This kit formulation consists of a 10-ml vial containing the lyophylisate of 1 mg L,L-EC and 100 μg $SnCl_2 \cdot 2H_2O$ dissolved in 1 ml of a 0.05 *M* phosphate buffer pH 12. Labeling can easily be performed by addition of up to 3.7 GBq [^{99m}Tc]pertechnetate (2–8 ml generator eluate) followed by neutralization with 0.2 ml of a 0.5 *M* phosphate buffer pH 5. The radiochemical purity of 30 preparations reconstituted in this way was always over 98%. Despite the absence of an antioxidant, the preparation remains stable for at least 8 hr. In view of the possible future application of ^{99m}Tc-L,L-EC, a stable labeling kit is essential. One year of experience with the described lyophylized kit allows us to conclude that the quality and the properties of the kit remain unaltered.

The results of the biodistribution study in mice and a baboon reveal that the renal excretion characteristics of ^{99m}Tc-L,L-EC are superior to those of ^{99m}Tc-MAG_3. At 10 min p.i., the difference in organ uptake in mice between ^{131}I-OIH and ^{99m}Tc-L,L-EC on the one side and between ^{99m}Tc-MAG_3 and ^{99m}Tc-L,L-EC on the other side is not significant for the blood, the kidneys and the urine (unpaired, two-tailed Student t-test, $p < 0.05$). However, the accumulation in the liver and the intestines is significantly lower for ^{131}I-OIH as compared to ^{99m}Tc-L,L-EC and for ^{99m}Tc-L,L-EC as compared to ^{99m}Tc-MAG_3. At 30 min p.i., the concentration in the blood and the amount excreted in the urine is significantly different between ^{131}I-OIH and ^{99m}Tc-L,L-EC in favor of the ^{99m}Tc-agent. Compared to ^{99m}Tc-MAG_3, ^{99m}Tc-L,L-EC shows at this moment a significantly higher excretion to the urine, a lower renal retention and a lower excretion to the intestines.

The 1-hr plasma clearance of ^{99m}Tc-L,L-EC exceeds the clearance of ^{99m}Tc-MAG_3 in the same baboon by 50% and the image quality of the study with ^{99m}Tc-L,L-EC is superior due to a lower activity in surrounding tissues. The higher plasma clearance value is mainly the consequence of the larger distribution volume, which in turn can be attributed to the markedly lower plasma protein binding. However, neither of the ^{99m}Tc-labeled tracer agents can be considered as a true substitute for hippuran, which remains the "golden" standard in terms of biological behavior and clearance properties. However, ^{99m}Tc-L,L-EC does approach hippuran more closely, and, on the basis of the results of the animal experiments, the new tracer agent could be more appropriate and convenient than ^{99m}Tc-MAG_3 for accurate determination of the effective renal plasma flow.

The impaired renal handling of ^{99m}Tc-L,L-EC in mice pretreated with probenecid demonstrates that renal excretion of the new agent is principally by active tubular transport, probably by the same carrier proteins that are responsible for the renal extraction and transport of hippurate anions. This is also reflected in the shape of the baboon renograms, which are nearly superimposable for both ^{99m}Tc labeled compounds.

Unlike CO_2DADS and MAG_3, L,L-EC does not contain a carbonylglycine moiety (—CO—NH—CH_2—COOH, —CO—G) which reconstitutes the side chain of hippuran. This structural entity is generally believed to be essential in these compounds for an efficacious fit with the receptor proteins of the tubular transport system according to Despopoulos' theory (*34*). However, ^{99m}Tc-L,L-EC contains twice an oxotechnetium-glycine sequence (TcO—NH—CH_2—COOH, TcO—G), which structurally resembles the —CO—G side chain of hippuran. Therefore, the TcO—G moieties are probably at the origin of the efficient handling of the new agent by the postulated tubular receptor protein. As a result, it is possible that in ^{99m}Tc-MAG_3 and ^{99m}Tc-CO_2DADS, it might be the same TcO—G sequence that accounts for the interaction at the tubular cells and not the —CO—G moiety. From the superior renal handling of ^{99m}Tc-CO_2DADS-A, the diastereomer with the oxotechnetium and carboxylate group in cis position, it has been assumed that both electron-rich groups are simultaneously involved in the contact of this compound with the renal receptor (*10,35*). The terminal carboxylate group of ^{99m}Tc-MAG_3 can freely rotate and is thus able to take a cis-orientation with respect to the oxotechnetium core. In ^{99m}Tc-L,L-EC, one of the carboxyl groups is orientated in the same direction as the TcO-core (cis) and the other one in the opposite direction (trans) (Fig. 1). This allows these three renal function agents to meet the proposed requirements for a simultaneous interaction of the carboxyl and oxo-group with the same receptor protein.

On the basis of our first experience with ^{99m}Tc-L,L-EC, it can be concluded that this agent has some very attractive properties that can make it more appropriate for renal function studies than ^{99m}Tc-MAG_3. It can be labeled very easily and efficiently at room temperature starting from a labeling kit with long shelf-life resulting in a preparation with excellent radiochemical purity and stability. It would thus constitute a very practical and reliable radiopharmaceutical that can be available in a few minutes without a boiling step. Another major advantage is the fact that it matches hippuran more closely than does ^{99m}Tc-MAG_3 with respect to the plasma clearance. The information obtained from scintigraphic images and renograms is of the same clinical value for both ^{99m}Tc-MAG_3 and ^{99m}Tc-L,L-EC.

For these reasons, ^{99m}Tc-L,L-EC deserves further investigation in humans to elucidate its clinical usefulness as a practical substitute for both hippuran and ^{99m}Tc-MAG$_3$ in radioisotopic renal function studies.

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