Animal Evaluation of Technetium-99m Triamide Mercaptide Complexes as Potential Renal Imaging Agents

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Technetium-99m mercaptoacetylglycylglycylglycine (MAG_3), a [^{99m}Tc]triamide mercaptide (N_3S) compound has been synthesized in an attempt to obviate the stereochemistry problems associated with the diamide dimercaptide (N_2S_2) ligands. Because initial studies have been promising, the terminal glycine on the MAG_3 compound has been varied to create a new series of N_3S compounds. Twelve new N_3S complexes were initially screened in mice and the more promising complexes, ^{99m}Tc mercaptoacetylglycylglycyl-glycine ([^{99m}Tc]MAG_3), ^{99m}Tc mercaptoacetylglycylglycyl-L-alanine ([^{99m}Tc]MAG_2-Ala), and both complexes of ^{99m}Tc mercaptoeacetylglycylglycyl-L-asparagine ([^{99m}Tc]MAG_2-Asn) and ^{99m}Tc mercaptoacetylglycylglycyl-L-glutamine ([^{99m}Tc]MAG_2-Gln), were further evaluated in rats utilizing constant infusion blood clearances, extraction efficiencies and protein binding assays. The renal excretion of all these complexes compared favorably with simultaneously administered [^{131}I]OIH and [^{125}I]iothalamate. The triamide mercaptide complexes represent a new ligand class for ^{99m}Tc, which may provide a variety of complexes for the evaluation of renal tubular function.

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Twenty-one diamide dimercaptide (N_2S_2) ligands have been synthesized and evaluated in an attempt to develop a technetium-99m (^{99m}Tc) replacement for iodine-131 hippurate [^{131}I]OIH (*1–6*). Previous studies with these groups of ligands suggest that the optimal rate and specificity for renal excretion occurs when the core ligand system has a carboxylate group in a preferred stereochemical relationship (*3*). Addition of this functional group, however, has resulted in the formation of stereoisomers with different rates and specificities for renal clearance. Furthermore, high performance liquid chromatography (HPLC) separation of the stereoisomers is required to obtain the desired complex.

A means of obviating the stereochemical problem was to change the core donor atoms from a N_2S_2 (diamide dimercaptide) to a N_3S (triamide mercaptide) set of donor atoms. Placement of the carboxylate group on the third amide nitrogen results in a single radiochemical product as long as no additional groups are present that introduce a second asymmetric center. The simplest ligand which meets these requirements is mercaptoacetylglycylglycylglycine (MAG_3) (*6*). Because [^{99m}Tc]MAG_3 was found to have comparable performance to [^{131}I]OIH in animal and human studies (*6–8*), a series of N_3S compounds to chelate with ^{99m}Tc have subsequently been synthesized by varying the terminal amino acid (*9*). The purpose of the study was to evaluate the effect of different terminal amino acids on the renal clearance of these complexes and to assess their potential as ^{99m}Tc tubular function agents which might replace [^{131}I]hippurate. This paper presents the results of animal studies evaluating this new group of N_3S complexes.

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MATERIALS AND METHODS

The synthesis of mercaptoacetyltriglycine has been previously reported (*6*). The synthesis of the other ligands is the subject of a separate report. Briefly, the general procedure for the preparation of the different ligands involved two steps. The first step involved the preparation of the key intermediate benzoylmercaptoacetylglycylglycine. The second step utilized this key intermediate for coupling with the desired terminal amino acid in either its tertiary butyl ester form or as the free amino acid. The tertiary butyl ester was hydrolyzed in trifluoroacetic acid to yield the final product. Elemental analysis was obtained commercially* and was carried out either on the tertiary butyl esters or their corresponding acids. Proton magnetic resonance spectra were obtained on a 90 MHz instrument.†

General Method for Radiolabeling with ^{99m}Tc

One-half to 1.0 ml of [^{99m}Tc]sodium pertechnetate in generator saline was added to 1 mg of the benzoate protected ligand which had been previously dissolved in 100 μl of 1 N NaOH. Twenty microliters of freshly dissolved sodium dithionite (50 mg/ml) was then added and the mixture was heated for 5 min at 85°C to 95°C for [^{99m}Tc]mercaptoacetylglycylglycyl-L-asparagine (MAG_2-Asn), 15 min for [^{99m}Tc]mercaptoacetylglycylglycyl-L-alanine (MAG_2-Ala) and 10 min for the remaining ligands. The solution was then neutralized to a pH between 6 and 8 by the addition of HPLC buffer. The resulting mixture was purified on a 5-micron 4.6 × 250 mm octadecylsilyl HPLC column utilizing an ethanol/phosphate buffer at a flow rate of 1.0 ml/min (Table 1) with a sodium iodide detector connected to the outflow.

The major peak which eluted from the HPLC column was collected and diluted to the appropriate concentration with normal saline. All of the [^{99m}Tc]mercaptoacetylglycylglycylglycylamino acids ([^{99m}Tc]MAG_2-AA) were first radiolabeled with ^{99m}Tc and then purified by HPLC before administration. In all cases where two peaks were found, the first component which eluted from the C-18 column was designated as A, while the second peak was designated as the B component.

Iodine-125 Iothalamate and [^{131}I]Hippurate

Iodine-131 hippurate‡ (OIH) was obtained commercially and was simultaneously administered in all studies; the radiochemical purity in the commercial OIH ranged from 96.5% to 98.5% by paper chromatography. Iothalamate was radiolabeled with ^{125}I by the method of Kato et al. (*10*) and was used as a glomerular filtration rate marker in the renal clearance studies (*11,12*), HPLC analysis showed <1% free iodine.

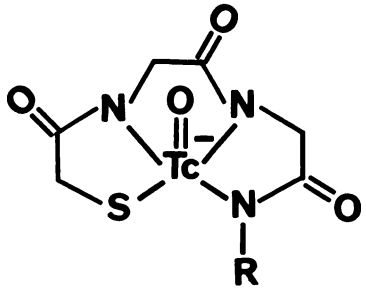
ANIMAL STUDIES

Mouse Biodistribution Studies

The time course of organ distribution was evaluated in a female Swiss-Webster outbred strain of albino mice in groups of five at each time period. Each animal was injected intravenously through a tail vein with 0.1 ml of the preparation which contained 1 μCi of the [^{99m}Tc]MAG_2-AA and 0.3 μCi of [^{131}I]OIH.

TABLE 1

Proposed Structure of the Triamide Mercaptide (N_3S) Complexes with ^{99m}Tc and Their HPLC Retention Volumes (mls)



R		HPLC Retention	HPLC Volume	% Labeling yields
$-CH_2COOH$	[^{99m}Tc]MAG_3	4.20¶		85–90%
$-CH_2CONHCH_2COOH$	[^{99m}Tc]MAG_4	5.34¶		95%
$-CH(CH_3)COOH$	[^{99m}Tc]MAG_2-Ala	3.76¶		75–85%
$-CH(CH_2C_6H_5)COOH$	[^{99m}Tc]MAG_2-Phe	14.69	16.19**	93%
$-CH(COOH)CH_2COOH$	[^{99m}Tc]MAG_2-Asp	4.44	6.27*	78%
$-CH(COOH)CH_2CONH_2$	[^{99m}Tc]MAG_2-Asn	4.50	9.96§	70%
$-CH(COOH)CH_2CH_2COOH$	[^{99m}Tc]MAG_2-Glu	2.74	3.62†	55%
$-CH(COOH)CH_2CH_2CONH_2$	[^{99m}Tc]MAG_2-Gln	5.78	7.36‡	95%

* 5-micron ODS column, 100% phosphate 0.10*M*, pH 7.
† 5-micron ODS column, 1% EtOH/99% phosphate 0.01*M*, pH 7.
‡ 5-micron ODS column, 2% EtOH/98% phosphate 0.01*M*, pH 7.
§ 5-micron ODS column, 2% EtOH/98% phosphate 0.01*M*, pH 6.1.
¶ 5-micron ODS column, 5% EtOH/95% phosphate 0.01*M*, pH 7.
** 5-micron ODS column, 10% EtOH/90% phosphate 0.01*M*, pH 7.

After injection the mice were placed in metabolic cages for the collection of excreted urine. Ten or 120 minutes postinjection the urethra was ligated and the mice were killed. The organs were then removed and counted in a dual channel well counter with corrections made for ^{131}I scatter into the ^{99m}Tc channel.

Rat Studies

The renal blood clearance, extraction efficiency, and protein binding determinations were all carried out in rats. Male Sprague-Dawley rats weighing between 250 and 350 g were used in all experiments. For each study, the animal was anesthetized with an intraperitoneal injection of ketamine hydrochloride 100 mg/kg. Following tracheostomy, one jugular vein was cannulated for infusion of the radiopharmaceuticals ([^{99m}Tc]MAG_2-AA, [^{131}I]OIH and [^{125}I]iothalamate), one carotid artery was cannulated for blood sampling and the bladder was cannulated, with a heat flared PE 50 tubing, for urine collections. A second cannula was placed into the jugular vein for the infusion of normal saline to replace fluid lost due to blood sampling and urine excretion.

The blood clearances of [^{125}I]iothalamate, [^{131}I]OIH and the ^{99m}Tc radiopharmaceuticals were calculated from three clearance periods once steady-state blood levels were reached.

Normal saline containing 10 μCi/ml of the HPLC purified [^{99m}Tc]MAG_2-AA, 2 μCi/ml of [^{125}I]iothalamate and 1 μCi/ml of [^{131}I]OIH were infused with a constant infusion pump at a flow rate of 20 μl/min. After an equilibrium period of 45 min and intravenous replacement of intraoperative fluid losses with normal saline, urine was collected under oil for three 10-min collection periods. Blood from the carotid artery was obtained at the midpoint of each clearance period. After the clearance measurements were complete, a 0.5 ml blood sample was withdrawn from the renal vein and a 3 ml blood sample was obtained from the carotid artery. One-half milliliter of the arterial blood was saved for radioactivity determinations and the rest was centrifuged to obtain a plasma sample. The plasma protein binding was determined by an ultrafiltration membrane system[§] (*13*). The micropartition system was centrifuged in a sealed container at 1,050 *g* in a swinging bucket centrifuge for 10 min. Samples of the plasma and the ultrafiltrate were counted for radioactivity. All samples were counted in a well counter with correction for ^{131}I scatter into the ^{99m}Tc window. The samples were recounted once ^{99m}Tc had been allowed to decay to background, and corrections for ^{131}I scatter into the ^{125}I channel were then made. Percent protein binding was calculated by the following formula: [1.0 − (cpm/g ultrafiltrate divided by cpm/g plasma)] × 100. Nonspecific membrane binding was evaluated by centrifuging the complexes in normal saline and none showed any significant (0.2% or greater) membrane binding.

Radioactivity in the urine was analyzed by HPLC to determine if the complexes had been metabolized or undergone degradation. The rat was surgically prepared as previously described, except the carotid artery was not cannulated. Approximately 1–2 mCi of the [^{99m}Tc]MAG_2-AA were collected from the HPLC column and injected intravenously as a bolus dose into the jugular vein of the animals. Urine was obtained at 60 min postinjection and assayed for total radioactivity. A sample was diluted 1:1 with HPLC grade water and injected onto the HPLC column. A second urine sample was diluted 1:1 with a known standard of the same [^{99m}Tc]MAG_2-AA complex and also injected onto the HPLC column.

RESULTS

Table 1 lists the different radiolabeled triamide mercaptide ligands, their corresponding HPLC solvent systems, retention volumes and percent labeling efficiency. The simplest complex of the series, mercaptoacetylglycylglycyl-glycine (MAG_3), was found to form a single ^{99m}Tc product. The addition of a fourth glycine group gave ^{99m}Tc mercaptoacetylglycylglycyl-glycylglycine ([^{99m}Tc]MAG_4) which also formed a single product by reverse phase HPLC. The addition of a second asymmetric center typically yielded two components separable by reverse phase HPLC. Technetium-99m mercaptoacetylglycylglycyl-L-phenylalanine ([^{99m}Tc]-MAG_2-Phe), ^{99m}Tc mercaptoacetylglycylglycyl-L-aspartic acid ([^{99m}Tc]MAG_2-Asp), ^{99m}Tc mercaptoacetylglycylglycyl-L-asparagine ([^{99m}Tc]MAG_2-Asn), ^{99m}Tc mercaptoacetylglycylglycyl-L-glutamic acid ([^{99m}Tc]-MAG_2-Glu) and ^{99m}Tc mercaptoacetylglycylglycyl-L-glutamine ([^{99m}Tc]MAG_2-Gln) all had two components. Technetium-99m mercaptoacetylglycylglycyl-L-alanine ([^{99m}Tc]MAG_2-Ala) has a second asymmetric center, but its components were unresolved by reverse phase or anion exchange HPLC.

Biodistribution Results

The biodistribution of the [^{99m}Tc]MAG_2-AA complexes were evaluated in mice at 10 min and 120 min postinjection (Table 2). Rapid renal excretion was apparent at the 10-min time period for [^{99m}Tc]MAG_3, [^{99m}Tc]MAG_2-Ala, [^{99m}Tc]MAG_2-Asn-A and for both complexes of [^{99m}Tc]MAG_2-Gln. At 10 min, the urine excretion of these complexes was 107%, 106%, 98%, 99%, and 97% that of [^{131}I]OIH, respectively. By 120 min postinjection [^{99m}Tc]MAG_3, [^{99m}Tc]MAG_2-Ala, [^{99m}Tc]MAG_2-Asn-A and both complexes of [^{99m}Tc]-MAG_2-Gln showed high specificity for renal elimination with almost quantitative excretion into the urine.

Compared with the previous compounds, the remaining N_3S derivatives had either a decreased 10- or 120-min urinary excretion compared with [^{131}I]OIH or a loss in renal specificity. The trianionic complexes, [^{99m}Tc]MAG_2-Asp and [^{99m}Tc]MAG_2-Glu all had diminished urine activity 10 min postinjection. Technetium-99m MAG_4 and [^{99m}Tc]MAG_2-Asn-B had slightly diminished 10-min urine activity as well as increased hepatobiliary elimination. The addition of L-phenylalanine ([^{99m}Tc]MAG_2-Phe) was found to give two components by HPLC with a significant amount of hepatobiliary excretion for both complexes as well as a substantial decrease in the 10- and 120-min renal excretion. The B complex was found to have less renal

TABLE 2
Biodistribution of [^{99m}Tc]N_3S Complexes and [^{131}I]OIH in Mice (% Injected Dose)*

[^{99m}Tc]MAG_2-AA	Time (min)	Blood	Liver	Kidney	Intestines	Urine	Percent of OIH in urine†
Glycine	10	2.63 ± 0.15	2.93 ± 0.08	3.53 ± 0.27	1.10 ± 0.05	79.89 ± 0.86	107.3 ± 0.3
	120	0.03 ± 0.00	0.08 ± 0.02	0.06 ± 0.03	1.20 ± 0.11	98.46 ± 0.72	102.6 ± 0.5
Glycylglycine	10	2.71 ± 0.24	9.92 ± 0.91	3.33 ± 0.27	10.26 ± 0.76	58.43 ± 2.69	86.7 ± 1.8
	120	0.04 ± 0.00	1.08 ± 0.37	0.25 ± 0.10	15.97 ± 0.63	82.54 ± 0.77	87.6 ± 1.0
Alanine	10	2.63 ± 0.27	2.56 ± 0.28	5.21 ± 1.04	1.58 ± 0.19	75.09 ± 1.01	106.4 ± 0.7
	120	0.16 ± 0.00	0.13 ± 0.01	0.27 ± 0.09	2.20 ± 0.22	95.94 ± 0.73	102.2 ± 0.4
Phenylalanine-A	10	9.70 ± 0.30	22.16 ± 1.28	7.59 ± 0.48	7.69 ± 0.80	32.27 ± 1.25	43.9 ± 1.0
	120	0.24 ± 0.05	2.31 ± 0.62	0.24 ± 0.10	26.13 ± 2.33	69.49 ± 2.11	73.5 ± 2.3
Phenylalanine-B	10	19.40 ± 0.26	14.73 ± 0.68	5.26 ± 0.45	14.88 ± 0.47	16.19 ± 0.95	21.9 ± 0.7
	120	0.80 ± 0.19	2.96 ± 0.65	0.37 ± 0.08	41.66 ± 1.76	48.61 ± 1.61	52.3 ± 1.3
Aspartic acid-A	10	6.70 ± 0.66	4.77 ± 0.36	5.28 ± 0.35	1.00 ± 0.15	56.58 ± 3.48	69.4 ± 2.0
	120	0.19 ± 0.01	1.60 ± 0.06	0.16 ± 0.01	1.38 ± 0.10	88.83 ± 4.07	96.7 ± 0.7
Aspartic acid-B	10	6.28 ± 0.38	4.92 ± 0.13	8.29 ± 0.48	1.27 ± 0.13	60.60 ± 1.48	75.7 ± 2.4
	120	0.34 ± 0.05	1.39 ± 0.09	0.49 ± 0.10	2.31 ± 0.43	92.26 ± 2.92	95.3 ± 1.0
Asparagine-A	10	3.21 ± 0.25	4.47 ± 0.19	4.17 ± 0.34	0.94 ± 0.06	70.66 ± 1.60	97.6 ± 1.0
	120	0.04 ± 0.00	0.75 ± 0.09	0.08 ± 0.02	0.97 ± 0.07	95.87 ± 0.44	103.4 ± 0.7
Asparagine-B	10	2.76 ± 0.14	8.69 ± 0.69	4.50 ± 0.67	17.41 ± 0.65	63.17 ± 3.28	81.4 ± 0.8
	120	0.07 ± 0.02	1.96 ± 0.35	0.33 ± 0.22	19.46 ± 1.97	81.66 ± 1.15	81.3 ± 1.2
Glutamic acid-A	10	7.49 ± 0.47	4.79 ± 0.45	5.23 ± 0.55	1.79 ± 0.14	50.15 ± 2.59	73.0 ± 2.0
	120	0.19 ± 0.01	1.66 ± 0.16	0.13 ± 0.20	1.11 ± 0.10	94.22 ± 0.71	99.7 ± 0.6
Glutamic acid-B	10	4.16 ± 0.12	3.59 ± 0.14	6.46 ± 0.44	1.09 ± 0.02	65.10 ± 0.58	87.5 ± 0.5
	120	0.19 ± 0.02	0.87 ± 0.07	0.14 ± 0.05	2.40 ± 0.32	94.29 ± 0.24	98.8 ± 0.7
Glutamine-A	10	2.62 ± 0.28	5.29 ± 0.24	4.06 ± 0.38	0.92 ± 0.06	74.13 ± 2.25	98.9 ± 0.6
	120	0.04 ± 0.00	0.62 ± 0.14	0.04 ± 0.00	2.17 ± 0.46	94.49 ± 0.80	102.2 ± 0.3
Glutamine-B	10	2.60 ± 0.37	6.30 ± 0.76	4.22 ± 0.20	0.88 ± 0.09	73.64 ± 2.83	97.1 ± 1.2
	120	0.03 ± 0.00	0.35 ± 0.11	0.04 ± 0.00	1.83 ± 0.15	96.68 ± 0.54	103.5 ± 0.4
[^{131}I]OIH‡	10	4.06 ± 0.12	2.07 ± 0.07	2.63 ± 0.17	1.12 ± 0.03	74.12 ± 0.79	
	120	0.18 ± 0.01	0.13 ± 0.02	0.10 ± 0.02	0.34 ± 0.02	94.67 ± 0.58	

* Data are mean ± s.e.m. percent dose per organ in five mice.
† OIH was injected simultaneously in all studies.
‡ Data are mean ± s.e.m. for 65 mice.

excretion and more biliary excretion than its corresponding A complex.

Rat Studies

The rat blood clearance, extraction efficiency and protein binding studies were performed on HPLC purified [^{99m}Tc]MAG_3, [^{99m}Tc]MAG_2-Ala, and both complexes of [^{99m}Tc]MAG_2-Asn and [^{99m}Tc]MAG_2-Gln. All of these complexes had renal clearances which compared favorably to [^{131}I]OIH (Table 3). Constant infusion whole blood clearances were obtained rather than the modified plasma clearances as previously reported (*6*) to avoid the necessity of correcting for hippuran uptake by the red blood cells (*14*).

Whole blood extraction efficiencies are also listed in Table 3 and range from 58% for [^{99m}Tc]MAG_2-Gln-B to 86% for [^{99m}Tc]MAG_3 versus 35% for [^{125}I]iothalamate and 62% for [^{131}I]OIH. The degree of protein binding of the derivatives varied between 24% and 80% (Table 3). Protein binding was 35% for [^{131}I]OIH and 9% for [^{125}I]iothalamate.

Single-dose urine collection of the [^{99m}Tc]MAG_2-AA resulted in 60%–90% of the dose recovered within 60 min. HPLC analysis of the urinary radioactivity obtained following intravenous administration of the complexes indicated a single peak which contained 96% or more of the injected radioactivity but had a slightly longer retention time than the purified preparation due to the presence of nonradioactive constituents in the urine. When the urine was diluted with an equivalent amount of activity of the known starting material, only one peak was found to elute from the HPLC column. These results indicate that the complexes are excreted into the urine without change.

DISCUSSION

Previous work with the [^{99m}Tc]diamide dimercaptide complexes (N_2S_2) in animals (*2–5*) and humans (*15–17*) has established that a ^{99m}Tc complex can be prepared that is secreted by the renal tubular cells without retention. The optimal complex in the N_2S_2 series, however, resulted in chelate ring stereoisomers that required HPLC purification. HPLC purification makes it unlikely that these agents will be used routinely for the replacement of [^{131}I]OIH or [^{99m}Tc]DTPA.

Continued synthetic work to develop an easily pre-

TABLE 3

[^{99m}Tc]MAG_2-AA, [^{131}I]OIH and [^{125}I]Iothalamate, Whole Blood Clearance, Extraction Efficiency and Protein Binding in Rats*

Agent	Clearance (ml/min/100 g)	Clearance‡ (% of OIH)	Extraction efficiency (%)	Extraction‡ efficiency (% of OIH)	Protein binding (%)
[^{131}I]OIH†	3.36 ± 0.60		62% ± 7%		35% ± 5%
[^{99m}Tc]MAG_3	4.73 ± 0.12	118% ± 8%	86% ± 2%	124% ± 10%	77% ± 2%
[^{99m}Tc]MAG_2-Ala	4.32 ± 0.68	122% ± 9%	84% ± 7%	128% ± 10%	78% ± 9%
[^{99m}Tc]MAG_2-Asn-A	3.11 ± 0.62	97% ± 11%	69% ± 5%	107% ± 2%	24% ± 9%
[^{99m}Tc]MAG_2-Asn-B	3.53 ± 0.65	83% ± 9%	61% ± 7%	90% ± 10%	80% ± 1%
[^{99m}Tc]MAG_2-Gln-A	3.26 ± 0.24	108% ± 13%	67% ± 7%	114% ± 15%	36% ± 2%
[^{99m}Tc]MAG_2-Gln-B	2.67 ± 0.14	86% ± 4%	58% ± 3%	101% ± 14%	52% ± 9%
[^{125}I]Iothalamate†	1.80 ± 0.27		35% ± 5%	9% ± 4%	

* Values are mean ± s.d. for five rats.

† Values are mean ± s.d. for 30 rats.

‡ These values are based on the simultaneously infused OIH and [^{99m}Tc]MAG_2-AA.

pared renal agent which did not require HPLC purification led to the synthesis of the N_3S complexes of which [^{99m}Tc]MAG_3 was the first (*6*). Technetium-99m MAG_3 was found to give a single ^{99m}Tc complex when analyzed by HPLC and it maintained a high renal specificity and rate of renal excretion comparable with [^{131}I]OIH in mice, rats and normal volunteers (*6–8*). Additional N_3S ligands for ^{99m}Tc were subsequently synthesized to evaluate the effect of different terminal amino acids and the form of the anionic group on the renal elimination of the complexes.

Reverse phase HPLC studies with the [^{99m}Tc]MAG_2-AA complexes typically gave high yields and excellent separations of the complexes when utilizing a 0.01*M* phosphate buffer at a pH of 7.0 and various concentrations of ethanol. However, [^{99m}Tc]MAG_2-Asn, [^{99m}Tc]-MAG_2-Asp, and [^{99m}Tc]MAG_2-Ala were more difficult to separate and required different mobile phase conditions. Technetium-99m MAG_2-Asn yielded two resolved peaks when the pH of the buffer was changed from 7.0 to 6.1. In the case of [^{99m}Tc]MAG_2-Asp two complexes were observed when ethanol was eliminated from the mobile phase and the phosphate buffer concentration was increased to 0.1*M*. Previous reports (*18*) have reported the separation of the two diastereomers of [^{99m}Tc]MAG_2-Ala; however, similar studies in our laboratory could not confirm these results. Utilizing a mobile phase of 8% ethanol/0.05*M* phosphate at a pH of 6.0, a late eluting peak (13 ml retention volume) was found, but typically the yields of this complex were only 1%–10%. Continued efforts with reverse phase HPLC appeared to allow us to separate the major peak into two unresolved components by varying the ethanol concentration, pH and the flow rate of the mobile phase, however the results were inconsistent. Clearly more work with [^{99m}Tc]MAG_2-Ala is needed to clarify the optimal HPLC conditions required for separation of this complex.

The biodistribution and urine excretion of [^{99m}Tc]-MAG_3 and several of the other dianionic ^{99m}Tc complexes were comparable with [^{131}I]OIH in mice (Table 2). The rapid excretion of these ^{99m}Tc dianionic groups [^{99m}Tc]MAG_2-Ala, [^{99m}Tc]MAG_2-Asn-A and both complexes of [^{99m}Tc]MAG_2-Gln) suggests that renal excretion is not rigidly dependent on the terminal amino acid. Technetium-99m MAG_2-Asn showed similar characteristics to the N_2S_2 group of ligands in the biological behavior of the A and the B components. The A component was found to be excreted more rapidly and maintained a high renal specificity compared with the B component. The trianionic complexes, [^{99m}Tc]-MAG_2-Asp and [^{99m}Tc]MAG_2-Glu, were excreted more slowly than [^{131}I]OIH and these results were similar to those obtained with the trianionic compounds of the N_2S_2 chelates (*5*). Changing the terminal amino acid to a more lipophilic group ([^{99m}Tc]MAG_2-Phe) was found to decrease the rate of renal excretion while increasing hepatobiliary excretion. This trend was also similar to that observed with the more lipophilic N_2S_2 ligands (*5*). The addition of another glycine ([^{99m}Tc]MAG_4) was again found to increase hepatobiliary excretion and this same trend occurred within the N_2S_2 group of complexes (*5*). Thus, the relative effect of different substituent groups for both the N_2S_2 and the N_3S core donor groups remains similar.

In the rat studies, the blood clearance and urinary excretion of the [^{99m}Tc]MAG_3, MAG_2-Ala, and both complexes of MAG_2-Asn and MAG_2-Gln were comparable with the values obtained from the simultaneously administered [^{131}I]OIH (Table 3). In the case of [^{99m}Tc]-MAG_2-Asn both complexes were evaluated more extensively, since both components would be present in any kit formulation. Interestingly, in the case of [^{99m}Tc]MAG_2-Asn both complexes were found to have rapid renal clearances. In all cases, the clearance of these compounds significantly exceeded the glomer-

ular filtration rate (iothalamate clearance) indicating a high degree of tubular secretion; whether or not these compounds share the same pathway of secretion remains to be determined.

Protein binding measured by the ultrafiltration technique provides a comparison of the protein binding affinities of the different complexes used in this study but its physiological significance is uncertain. In the current study, the ^{99m}Tc agents with the greatest protein binding (MAG_3, MAG_2-Asn-B, and MAG_2-Ala) had the highest blood clearances. Levy has recently proposed theoretical equations describing the relationship between plasma protein binding, GFR, and tubular clearance (*19*). When a drug is cleared primarily by filtration, renal clearance is dependent on the unbound fraction; when tubular clearance is high, renal clearance will be relatively independent of the unbound fraction. These complexes did exhibit different degrees of plasma protein binding, therefore, the percentage of their clearance attributable to glomerular filtration may be somewhat different for each complex. Clearly, however plasma protein binding was not an important factor in affecting tubular transport. In a recent study comparing OIH and [^{99m}Tc]MAG_3 in normal volunteers, the increased protein binding of [^{99m}Tc]MAG_3 was associated with a smaller volume of distribution (*7*). In this study the plasma clearance of [^{99m}Tc]MAG_3 was found to be slower than OIH, although equivalent amounts of both agents were found in the urine at 30 min postinjection; this observation may be related to [^{99m}Tc]MAG_3's smaller volume of distribution.

Technetium-99m MAG_2-Ala has kinetic parameters comparable with [^{99m}Tc]MAG_3 in animals and may also exhibit similar behavior in disease states. The protein binding, blood clearance and extraction efficiencies of [^{99m}Tc]MAG_2-Asn-A and [^{99m}Tc]MAG_2-Gln-A were similar to [^{131}I]OIH and these two complexes may behave more similarly to [^{131}I]OIH under altered physiological or disease conditions. While these possibilities will require further evaluation, the N_3S group of ligands do provide a number of very promising ^{99m}Tc complexes for the evaluation of renal tubular function and more extensive evaluation in animals and humans is warranted.

NOTES

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§ Centrifree Micropartition System, Amicon Corp., Danvers, MA.

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