Technetium-99m MAG_3 Kit Formulation: Preliminary Results in Normal Volunteers and Patients with Renal Failure

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Previous studies have shown that [^{99m}Tc]mercaptoacetyltriglycine (MAG_3) purified by high performance liquid chromatography (HPLC) is a very promising new renal imaging agent which has characteristics very similar to [^{131}I]orthoiodohippurate. An easily prepared kit formulation has been developed and evaluated in ten normal volunteers and three patients on hemodialysis. The average radiochemical purity was 96.6%. There were no adverse reactions. In the volunteers, the relative uptake ±1 s.d. was 49.1% ± 2.6% for the right kidney and 50.9% ± 2.6% or the left kidney. Urine activity was 71.4% ± 6.4% of the injected dose at 30 min and 94.4% ± 2.2% at 180 min. The 60-min plasma clearance was 340.0 ± 79.0 ml/min and the volume of distribution was 5.15 ± 1.1 l. Approximately 0.5% of the injected dose was present in the gallbladder at 30–60 min postinjection. Gut activity was not present 30–60 min postinjection but reached 1% of the injected dose by 3 hr. In the hemodialysis patients, ~1% of the injected dose was present in the gallbladder and 0.5% in the gut at 30–60 min; gut activity increased to ~5% at 3 hr. In summary, results using the kit formulation compare favorably to previously published data using the HPLC purified material. Based on these preliminary results, the kit formulation is expected to have widespread clinical utility.

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Previous studies have shown that technetium-99m (^{99m}Tc) mercaptoacetyltriglycine (MAG_3) purified by high performance liquid chromatography (HPLC) is a highly promising ^{99m}Tc renal imaging agent with biologic characteristics very similar to [^{131}I]orthoiodohippurate (OIH) (*1–7*). In normal volunteers the 30-min urine excretion of HPLC purified MAG_3 and the time to peak height of the renogram curves were essentially identical to OIH (*3*). Furthermore, in a limited series of patients, the 30-min urine excretion was also the same and the time to peak height was actually shorter for MAG_3 (*4*). While the clearance of MAG_3 was slower than OIH in volunteers, no statistically significant difference was noted in a small series of 12 patients (*3,4*). As mentioned, however, all the studies up to the present have been performed using [^{99m}Tc]MAG_3 purified by HPLC. Labeling and HPLC purification required ~2 hr for each study and this degree of effort is clearly impractical for routine clinical use. An easily prepared kit formulation has now been prepared and tested in animals (Mallinckrodt Inc., St. Louis MO unpublished data). The purpose of this report is to present our results using this kit in normal volunteers and in three patients with renal failure.

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MATERIALS AND METHODS

The S-benzoyl protected mercaptoacetyltriglycine (Bz-MAG_3) was labeled according to the manufacturers instructions. Forty to sixty mCi of pertechnetate in 0.4 to 0.8 ml were eluted from a commercially available generator (Mallinckrodt, Inc.) and diluted with nonbacteriostatic normal

saline (Abbott Laboratories) to a total volume of 5 to 6 ml. The diluted radioactivity was added to the Bz-MAG_3 kit to dissolve all of the components and the vial was placed into a rolling water bath (95°C) within 5 min after the addition of the pertechnetate. The vial was heated for 5 min, removed from the water bath, shaken briefly then placed back into the water bath for an additional 5 min. After the 10-min boiling period was complete the vial was removed from the water bath and allowed to cool.

An isocratic HPLC system (Beckman Instruments) with a radioactivity detector (Canberra Instruments) connected to an integrator recorder (Hewlett Packard HP-3390A) was used to monitor the outflow tubing and check for soluble impurities. The mobile phase consisted of 1.7 g (0.005 mol) of tetrabutylammonium dihydrogen phosphate dissolved in 800 ml of high performance liquid chromatography (HPLC) grade water and 200 ml of ethanol with the pH adjusted to 6.3. The stationary phase was a 5-μ C-18 ODS (octadecasilyl) reverse phase 4.6 by 150 mm column. With a flow rate of 1 ml/min, the [^{99m}Tc] MAG_3 complex had a retention time of 14 min while free pertechnetate had a retention time of 8.3 min.

Insoluble forms of ^{99m}Tc were determined by thin layer chromatography utilizing a solvent of 60 ml of normal saline, 40 ml of methanol, and 1 ml of glacial acetic acid. The stationary phase was a DC-Fertigplatten RP-18 F_{254} s plate 5 × 20 cm (Merck, Rahway, NJ). The plate was spotted with the kit formulation and placed wet into the solvent tank. After the solvent had migrated 10 cm, the plate was removed from the solution and scraped. The origin (0 to 1 cm) was counted and the remaining portion (1 to 10 cm) of the plate was counted to determine the percent insoluble activity. The percent radiochemical purity was calculated by multiplying the percent of labeled MAG_3 (the percent in the 14-min peak from the HPLC integrator recorder) by the percent soluble material (from the TLC plates).

Volunteer and Patient Selection

Ten male volunteers over 21 yr of age were selected for the study. All volunteers were screened by a medical history, were in good health, had a weight within 15% of normal for their age, sex, and height and had not received any prescription medications within 14 days or any over the counter medication within 2 days prior to admission into the study. A 12-lead electrocardiogram was obtained 1 to 7 days prior to the study and evaluated by a cardiologist. Clinical laboratory studies on both blood and urine samples were performed between one and seven days prior to the study (admission baseline). These studies were reviewed by a physician and repeated immediately prior to administration of the MAG_3 and again one day after the study. Blood pressure, pulse rate, respiratory rate and body temperature were recorded immediately prior to administration of the MAG_3 and at ~90 min postinjection. An electrocardiogram was also obtained ~90 min postinjection and evaluated by a cardiologist.

With the exception of water, the volunteers were asked to fast following supper the night before the study and the volunteers were given ~8 oz of water at 1 and again at 2 hr after injection of the MAG_3.

Three male patients, two patients on hemodialysis three days per week and one on hemodialysis 2 days per week, were selected for further study. The volunteer and patient protocols were approved by the Institutional Review Board and the FDA with informed consent obtained from all volunteers and patients.

Experimental Protocol

All subjects were injected within 3 hr of preparation of the kit. The subjects were imaged supine with the camera placed posteriorly below the kidneys. After injection of 5–8 mCi of the kit formulation, analog images were obtained at 2-min intervals for 20 min using a general, all purpose ^{99m}Tc collimator with a 20% window centered over the 140 keV photopeak of ^{99m}Tc. The camera was also interfaced to a digital computer (Technicare 560 Computer) and images were recorded at 20-sec intervals for 20 min. Each subject was asked to void at 30 and 180 min postinjection and the urine was assayed to determine the amount excreted. Pre-void and postvoid anterior and posterior images were obtained with a geometric mean used to correct for postvoid bladder residual.

Whole kidney and cortical regions of interest were placed over each kidney and computer generated renogram curves were obtained. Cortical regions of interest were assigned from the whole kidney region by eliminating the areas of the collecting system. Time-to-peak height was determined for each background corrected renogram curve.

Whole-body images of the volunteers were obtained after the 30-min postvoid bladder image. The pelvis, liver, chest, head, and neck regions were imaged both anteriorly and posteriorly. This imaging protocol was repeated at 180 min postinjection for radiation dosimetry calculations and for evaluating the localization of radioactivity in other organs. Bladder activity determined at the 30-min voiding was correlated with the geometric mean of anterior and posterior counts (corrected for postvoid residual urine) and the geometric mean bladder counts were used to calibrate the activity in the other organs. Twenty-four-hour pelvis and liver images were obtained in six of the volunteers and one patient. The dosimetry calculations have been briefly described (*8*) and will be the subject of a more detailed report in the future.

Blood samples of 3 to 5 ml were obtained at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, and 180 min postinjection through an indwelling venous line. These samples were centrifuged at 2,100 *G* for 10 min. Plasma samples were obtained and counted along with a dose standard. Plasma clearance was determined by a biexponential curve fit (*9,10*). Plasma protein binding was determined from both the 20 and 60 min plasma samples. The plasma sample was centrifuged at 1,600 *G* for 10 min at room temperature in a micropartition membrane system (Centrifree Micropartition System, Amicon Corporation, Danvers, MA) (*11*).

RESULTS

The radiochemical purity was 96.7% ± 1.1% for the volunteers and 96.5% ± 0.3% for the three patients. Combined, the radiochemical purity was 96.6% with the 3.4% impurities representing insoluble forms of ^{99m}Tc (1.2%) and non-MAG_3 labeled material eluting from the HPLC column (2.2%). There was negligible (<0.1%) free pertechnetate found in all preparations.

The resulting studies are based upon eight normal volunteers and three patients with impaired renal func-

tion. Since infiltration of the dose occurred in two volunteers, results from these subjects were not included in the data analysis. Infiltration was documented by imaging over the injection site. Statistical analysis was based on the test for independent samples.

Normal Volunteers

A representative normal study is shown in Figure 1; computer enhanced images of the same volunteer at 30–45 min postinjection illustrate minimal blood pool, hepatic, and gallbladder activity (Fig. 2). The relative function of the right kidney based on the background corrected 1–3 min uptake ranged from 45% to 54% with a mean value ±1 s.d. of 49.1 ± 2.6%; the range for the left kidney was 46% to 55% with a mean value of 50.9 ± 2.6%. The time to peak height of the renogram curves for the whole kidney and cortex, 30-min bladder corrected urine excretion and 60-min plasma clearance are listed in Table 1. When corrected for body surface area, the plasma clearance was 288 ± 53 ml/min and the volune of distribution (Vd) was 4.4 ± 0.8 l. The protein binding was 89.6% ± 1.1% at 20 min versus 89.2% ± 1.4% at 60 min. There were no adverse reactions and no significant changes in blood chemistry, urinalysis, electrocardiogram, or vital signs from baseline values.

Patient Studies

The 0–2-min images in the three patients on hemodialysis are illustrated in Figure 3. Two of the hemodialysis patients were unable to void at 30 min; the third excreted 10.5% of the injected dose. The mean cumulative 180-min urine excretion for the three patients was 26.1% ± 7.0% (range of 19.6% to 33.6%). Abdominal, chest and pelvic 30–45 min and 180-210 min digital images for one of the patients are illustrated in Figure 4. The mean 60-min clearance and volume of distribution corrected for body surface areas were 53.6 ± 4.1 ml/min and 4.8 ± 0.6 l, respectively. Protein binding was 75.9% ± 4.8% at 20 min and 80.3% ± 2.9% at 60 min.

Biodistribution and Dosimetry

Approximately 0.5% of the injected dose was present in the gallbladder of normal volunteers by 30–60 min postinjection and this value was essentially unchanged at 180 min; no gallbladder activity was seen at 24 hr (Table 2). Gut activity was not present at 30–60 min but increased to ~1% at 3 hr postinjection and remained at ~1% at 24 hr. Liver activity was 2.6% of the injected dose at 30–60 min but fell to ~0.5% at 3 hr. By 3 hr postinjection, 94.4% of the dose was in the bladder, 2% in the liver, gallbladder, and gut, and 2%

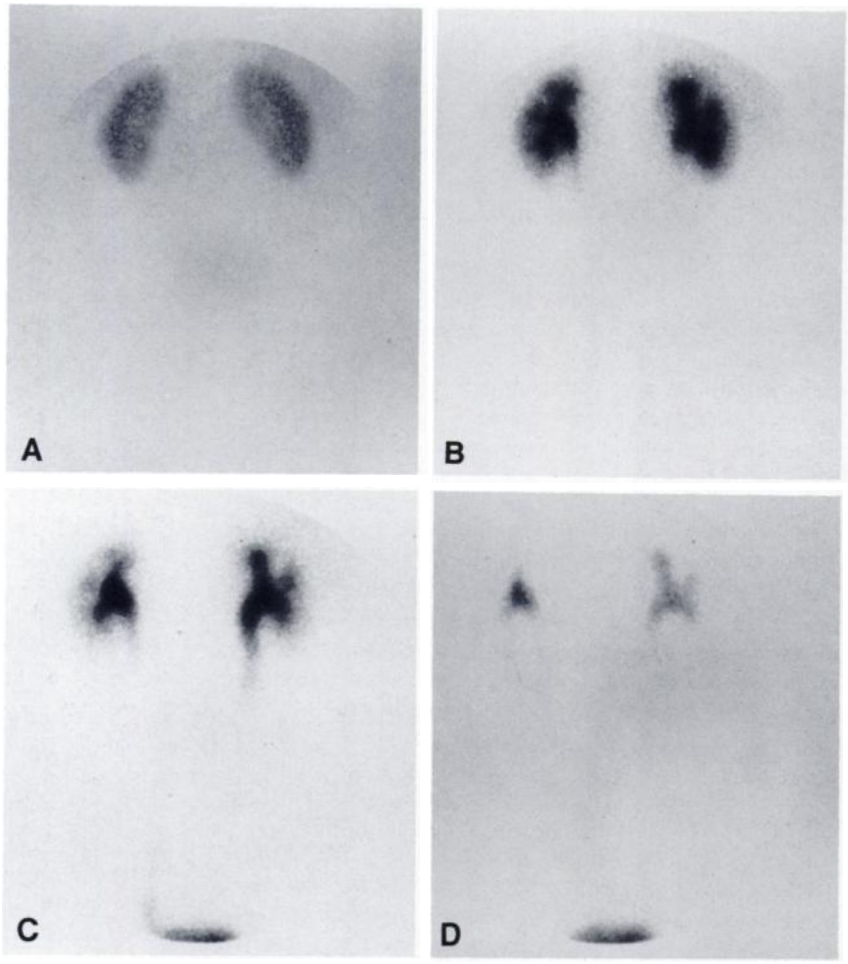

FIGURE 1
Technetium-99m MAG_3 analog images of the kidneys of a normal volunteer obtained at 0–2 min (upper left), 4–6 min (upper right), 12–14 min (lower left), and 26–28 min (lower right).

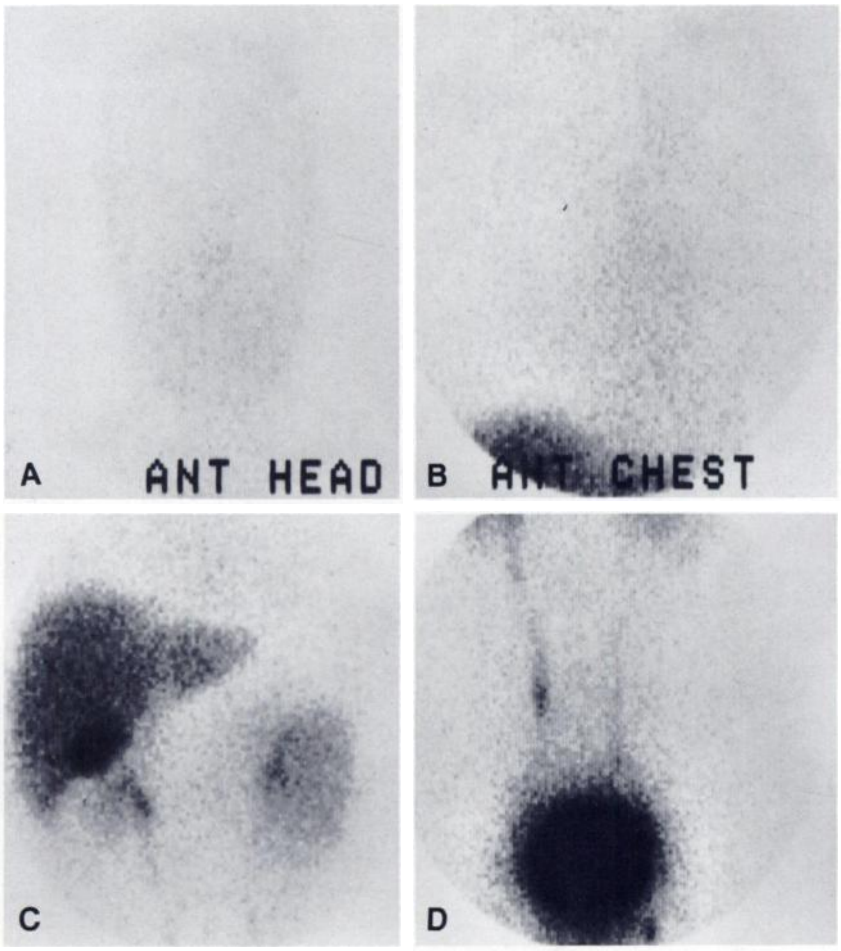

FIGURE 2
Head (upper left), chest (upper right), abdominal (lower left), and pelvic (lower right) images of a normal volunteer obtained 30 min postinjection. It is important to note that the intensity has been markedly enhanced on the computer. The change in intensity can be appreciated by comparing the posterior abdominal image with the standard 26–28-min renal image (Fig. 1).

remained in the plasma pool. The percent remaining in the plasma pool was calculated by multiplying the percent injected dose per gram of plasma at 3 hr by the volume of distribution at 3 hr.

In the renal failure patients, there was little change in gallbladder activity (~1% of the injected dose) for 45 min to 3 hr but gut activity increased during this period from 0.5% to 5% of the injected dose. Abdominal, chest, and pelvic images for a patient on hemodialysis are illustrated in Figure 4.

The calculated dosimetry of MAG_3 is presented in Table 3. For a probable clinical dose of 5 to 10 mCi, the critical organ will be the bladder, 0.88 to 1.75 rad. This calculation assumes that the patient voids at 30 min at the conclusion of the study. The total-body dose for 5 to 10 mCi will be ~0.02 to 0.04 rad, well within acceptable ranges.

DISCUSSION

We previously compared clearances and volumes of distribution of OIH and HPLC purified MAG_3 following simultaneous and sequential administration and reported that MAG_3 administered one hour prior to OIH was cleared faster than MAG_3 administered simultaneously with OIH; the OIH clearances were essentially the same in both studies (*3*). We investigated a

TABLE 1
Comparison of HPLC and Kit Formulation of MAG_3 in Normal Volunteers

	Percent injected dose in the urine		60 min plasma clearance	Vd	Time to peak	
	30 min	180 min*	ml/min	(l)	Whole*	Cortex*
HPLC	73.1 ± 6.2	99.9 ± 4.3	420 ± 120	5.21	207 ± 80	145 ± 22
KIT	71.4 ± 6.4	94.4 ± 2.2	340 ± 79	5.15	291 ± 121	197 ± 34

*$p \leq .05$

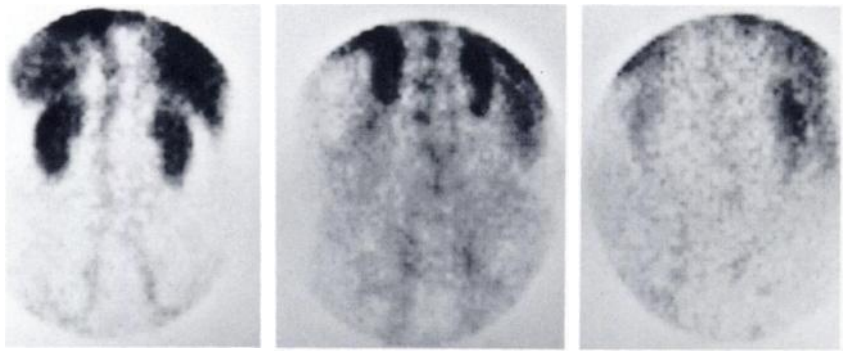

FIGURE 3
The 0–2-min computer acquired images for three patients on maintenance hemodialysis.

number of parameters but had no satisfactory explanations for our findings. We have continued to reevaluate our data and discovered an error in decay correcting the ^{99m}Tc for the MAG_3 sequential data. When this error was corrected, the 30-min blood clearances for the sequential studies and simultaneous studies were essentially the same, 0.73 ± 0.17 l/min versus 0.77 ± 0.14 l/min, respectively, versus 0.88 ± 0.19 l/min for OIH. Likewise, the volumes of distribution were also similar, 9.9 ± 2.9 l for the sequential MAG_3 studies versus 8.1 ± 1.0 l for the simultaneous studies compared to 14.5 ± 6.0 l for OIH. In our previous study using a simultaneous injection of OIH and HPLC purified MAG_3, we found that the 60-min clearance of OIH was 600 ml/min versus 420 ml/min for MAG_3 (*3*). Jafri et al. also obtained a slower plasma clearance for MAG_3 in patients (*12*).

There is a suggestion that the 60-min plasma clearance for the HPLC purified MAG_3 may be greater than that of the kit formulation, 420 ml/min versus 340 ml/min, but the difference was not significant at the 0.05 level. The volumes of distribution of the two formulations were essentially identical. The time to peak height for the whole kidney renogram curve was 291 sec for the kit MAG_3 versus 207 sec for the HPLC purified material ($p \leq 0.05$); when cortical regions of interest were assigned, the time to peak height was still longer for the kit compared to the HPLC purified material, 197 sec versus 145 sec ($p \leq 0.001$).

The reasons for the differences in clearances and times to peak height between the HPLC and kit preparations are not clear. Coveney, et al reported no differences between the kit MAG_3 and HPLC purified MAG_3 based on constant infusion renal clearances in rats (*13*).

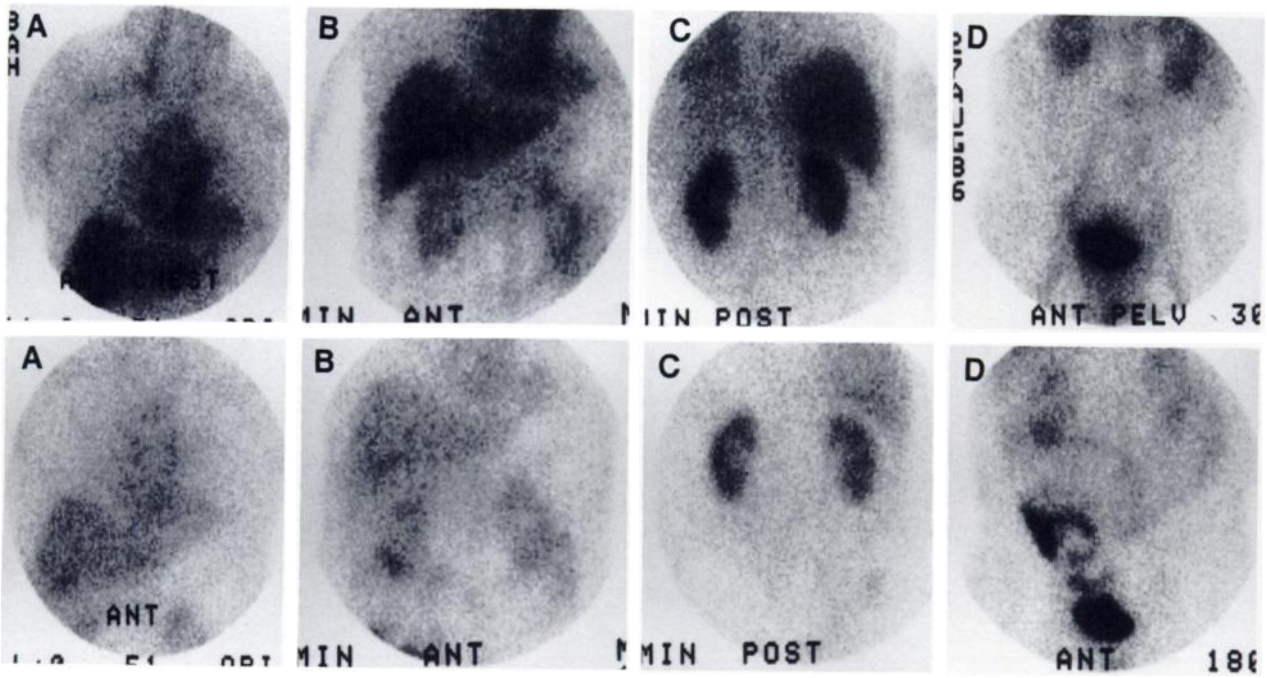

FIGURE 4
The images were obtained 30–45 and 180–210 min postinjection and were enhanced on the computer to visualize biodistribution. Upper panel, left to right, chest, anterior abdomen, posterior abdomen, and pelvis obtained at 30–45 min postinjection. Lower panel, left to right, chest, anterior abdomen, posterior abdomen, and pelvis obtained at 180–210 min postinjection.

TABLE 2
Biodistribution of [^{99m}Tc]MAG_3 in Normal Volunteers and Hemodialysis Patients

Time	Volunteers (N = 8)	Patients (N = 3)
Gallbladder		
30–60 min	0.49% ± 0.17%	1.0% ± 1.5%
180–210 min	0.58% ± 0.14%	0.77% ± 0.52%
Gastrointestinal tract		
30–60 min	0.01% ± 0.02%	0.4% ± 0.7%
180–210 min	0.98% ± 0.41%	5.3% ± 2.4%
24 hr (n = 6)	1.22% ± 0.61%	10.2 (n = 1)
Liver		
30–60 min	2.61% ± 1.34%	5.7% ± 1.0%
180–210 min	0.46% ± 0.3%	3.8% ± 0.5%

One explanation for our findings is the fact that OIH was not given as a control to the volunteers in the current study and these volunteers may also have had a slower OIH clearance and time to peak height than the volunteers studied a year earlier. There were no significant differences in the two volunteer populations in regard to sex, mean age, and weight. The 3.4% impurities could partially explain the observed differences. Further studies comparing OIH and the kit formulation will be needed to clarify these findings.

In the current study, all three patients given the kit formulation of MAG_3 had acceptable kidney definition in spite of very low plasma clearances (Fig. 3). Hepatobiliary excretion was increased but the gut activity was not excessive, approximating only 10% of the administered dose at 24 hr in one hemodialysis patient. Ten percent is similar to the amount of OIH and ^{99m}Tc MAG_3 in the gut 2 hr following intravenous administration to nephrectomized mice (*14*).

In summary, the kit formulation of MAG_3 has been shown to label efficiently and was rapidly cleared from the blood and excreted by the kidneys with minimal residual activity in other tissues. Based on these data and previously reported results, MAG_3 represents an important new first generation ^{99m}Tc renal radiopharmaceutical with characteristics similar to OIH and we expect that it will have widespread clinical applications in the future. Efforts are underway to attempt to develop an even better agent with less hepatobiliary excretion and more rapid plasma clearance (*2,15*).

TABLE 3
Absorbed Dose to Target Organ from 1.0 mCi of [^{99m}Tc] MAG_3

Target organ	rad
Bladder wall*	0.175
Lower large intestine	0.019
Upper large intestine	0.019
Kidneys	0.018
Gall bladder	0.015
Small intestine	0.013
Ovaries	0.012
Testes	0.006
Red marrow	0.005
Liver	0.005
Total body	0.004

* Assumes that the patient voids at 30 min after administration and thereafter at 4-hr intervals.

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