Comparison of Technetium-99m MAG_3 Kit with HPLC-Purified Technetium-99m MAG_3 and OIH in Rats

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Technetium-99m (^{99m}Tc) mercaptoacetylglycylglycylglycine (MAG_3) in high (≥95%) radiochemical purity is prepared from lyophilized kits containing benzoylMAG_3, sodium tartrate, lactose, and stannous chloride by adding sodium [^{99m}Tc]pertechnetate and heating the contents briefly. Constant-infusion renal whole-blood clearance obtained with [^{99m}Tc]MAG_3 kits was compared with that obtained with high performance liquid chromatography (HPLC) pure [^{99m}Tc]MAG_3 and with co-infused iodine-131 (^{131}I) iodohippurate (OIH) in anesthetized rats. Average renal whole-blood clearance of [^{99m}Tc]MAG_3 from kits was 3.9 ± 0.4 ml/min/100 g body weight (mean ± s.e.m. n = 5) and that for HPLC-pure [^{99m}Tc]MAG_3 was 4.6 ± 0.3 (n = 3). Renal whole-blood clearance ratios for [^{99m}Tc]MAG_3 to co-infused iodine-131 (^{131}I) OIH were greater than unity for both kit formulation (1.7 ± 0.1) and HPLC-pure [^{99m}Tc]MAG_3 (1.9 ± 0.2). Differences in these two measures were not significant. Plasma binding (determined from blood drawn at the end of the infusion) of [^{99m}Tc]MAG_3 prepared from both kits (75 ± 2%, n = 4) and HPLC-separation (76 ± 4%) were greater than that of [^{131}I]OIH in corresponding plasma samples (31 ± 1% and 32 ± 2%, respectively). Renograms performed in anesthetized rats revealed no statistically significant differences between kit-prepared [^{99m}Tc]MAG_3 and [^{131}I]OIH in terms of time-to-peak renal activity (5.0 ± 1.7 min, n = 6; and 2.2 ± 0.2 min, n = 3, mean ± s.e.m. for [^{99m}Tc]MAG_3 and [^{131}I]OIH, respectively), in terms of time to fall to half-maximal activity (15.3 ± 2.4 min and 9.6 ± 2.1 min, respectively), or in terms of fraction of peak radioactivity in right kidney (0.53 ± 0.01 for both substances). To assess possible interference from hepatobiliary uptake and excretion in renal failure, radioactivity in liver regions of interest was followed by gamma camera scintigraphy for 30 min after intravenous injection of [^{131}I]OIH and kit and HPLC-purified [^{99m}Tc]MAG_3 in anesthetized rats rendered anephric by ligating renal peduncles. Liver activity was 25% of total for both preparations of [^{99m}Tc]MAG_3 and was 22% of total for [^{131}I]OIH. There were no significant differences among the substances.

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Recently [technetium-99m] oxotechnetiummercaptoacetylglycylglycylglycine ([^{99m}Tc]MAG_3) has been disclosed as a novel renal imaging radiopharmaceutical (*1*, *2*). Technetium-99m MAG_3 appears to be handled by the kidneys in a manner like that of [iodine-131]*o*-iodohippurate ([^{131}I]OIH), i.e., by active secretion into the urine at tubular sites of the nephron in addition to passive filtration at the glomerulus. In comparison, the other ^{99m}Tc renal imaging agents are excreted by glomerular filtration alone ([^{99m}Tc]diethylenetriamine pentacetic acid (DTPA)) or are partially retained in the renal parenchyma ([^{99m}Tc]dimercaptosuccinic acid (DMSA) and [^{99m}Tc]glucoheptonate).

Previous studies by Fritzberg, Taylor, and their coworkers (*1*,*2*) produced [^{99m}Tc]MAG_3 in a strongly alkaline reaction medium, which had to be neutralized and further processed by high performance liquid chromatography (HPLC). The involved preparation and separation would be expected to inhibit clinical acceptance of this promising replacement for [^{131}I]OIH. In this report, the biologic evaluation of a kit formulation of [^{99m}Tc]MAG_3 is presented. The preparation is described briefly as follows. Contents of the nonradioactive benzoylMAG_3 kit are reconstituted with 20 to 200 mCi of ^{99m}Tc generator eluate and heated for 10 min in a boiling water bath. Resulting [^{99m}Tc]MAG_3 is

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≥95% radiochemically pure, and stable for at least 6 hr, enabling multiple examinations from each kit. Biologic evaluation of these kits included comparing constant-infusion renal clearance and renogram data obtained with kit-produced [^{99m}Tc]MAG_3, with HPLC-purified [^{99m}Tc]MAG_3 and with [^{131}I]OIH in the rat.

Although the radiopharmaceutical has excellent specificity for the kidney, some excretion of kit product does occur through the hepatobiliary route. In poor renal function, a significant amount of hepatobiliary excretion of [^{99m}Tc]MAG_3 might occur, making this product less desirable as an [^{131}I]OIH replacement for renal function studies. To test this, hepatic handling of [^{99m}Tc]MAG_3 was examined by gamma camera scintigraphy in rats rendered anephric by ligation of the renal peduncles. For comparison, the liver uptake of HPLC-purified [^{99m}Tc]MAG_3 and of [^{131}I]OIH was examined in additional rats with ligated renal peduncles.

Materials and Methods

General

Technetium-99m MAG_3 was prepared from lyophilized kits containing 1 mg benzoylMAG_3, 40 mg sodium tartrate dihydrate, 20 mg lactose monohydrate and 0.2 mg stannous chloride dihydrate. Vials were stored at room temperature and reconstituted with 4 ml sodium pertechnetate ^{99m}Tc injection, U.S.P., containing 20 to 100 mCi ^{99m}Tc. In order to oxidize excess stannous ions, vials were vented with a hypodermic needle immediately following reconstitution, and 2 cc of sterile air were introduced into the vial using a hypodermic syringe and an 0.22-μm Luer adaptor-fitted in-line filter. Vials were then heated for 10 min in a boiling water bath and assayed for radiochemical purity by HPLC (C-18 column eluted at 27 min with a gradient of 0-5% ethanol:0.01*M* sodium phosphate, pH 6.3, at 1.0 ml/min) and by ascending paper chromatography (60:40, acetonitrile:water, R_f = 0.65). Chromatography and bioassay demonstrate that [^{99m}Tc]MAG_3 produced from these kits is stable for at least 6 hr after reconstitution. HPLC-purified [^{99m}Tc]MAG_3 was prepared from kit-produced [^{99m}Tc]MAG_3 by collecting the appropriate fraction from reverse phase chromatography described above.

Iodohippurate sodium ^{131}I injection was purchased as a commercial clinical preparation and diluted as necessary for use (see below). It was assayed at ≥97% radiochemical purity by the manufacturer, and was used prior to expiry. Sprague-Dawley rats* were acclimated for at least 2 days prior to use.

Renal Clearance

Male rats (300–350 g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed supine over a heating pad. A femoral vein was cannulated with PE-50 tubing (filled with 100 U heparin/ml saline) for administration of radiopharmaceuticals. A lower laparotomy was performed and the bladder incised to permit cannulation of two lengths of PE-50 in parallel. One of the PE-50 bladder cannulas was affixed to a syringe containing saline for flushing the bladder at collection times. A rectal probe was inserted to monitor core body temperature. Adjustments of the heating pad were made to maintain rectal temperature as close to 38°C as practical. Temperatures ranged from 36.3 to 38.0°.

After surgical preparation, a 1-ml loading dose (containing ~200 μCi [^{99m}Tc]MAG_3 and 10 μCi [^{131}I]OIH in 3% mannitol, w/v, in saline) was injected through the venous cannula, and this was succeeded by a continuous administration of infusion solution (containing ~100 μCi [^{99m}Tc]MAG_3 and 5 μCi [^{131}I]OIH per ml of 3% mannitol, w/v, in saline) using a Harvard infusion/withdrawal pump set to deliver ~0.02 ml/min.

At 5 min after the beginning of infusion, the bladder was flushed and the contents discarded. Afterwards, urine collections were made every 10 min for a total of 40 min. The bladder was flushed at the end of each 10-min collection period. The entire contents of the urine collection were diluted to 20 ml with distilled water and an 0.1-ml aliquot was taken for assay of radioactivity.

At midpoints of the urine collection periods, blood was sampled from the tail tip into two tared heparinized 0.1-ml capillary tubes. Gross weight of the tubes was taken and the tubes with their contents were placed into gamma counting tubes for assay of radioactivity.

For several rats, a sample of blood was collected by cardiac puncture into a heparinized syringe at the end of the 45-min infusion period. This blood was centrifuged to recover plasma. An 0.1-ml aliquot of plasma was taken for radioactivity assay. An additional 1-ml plasma aliquot was placed in the reservoir of an ultrafiltration system† and centrifuged at ~1,500 rpm for 20 min. An 0.1-ml aliquot of the ultrafiltrate was taken for radioactivity assay.

Plasma binding was calculated for each radionuclide according to the following formula:

$$\% \text{ bound} = \left(1 - \frac{\text{cpm in ultrafiltrate}}{\text{cpm in plasma}}\right) \times 100.$$

Radioactivity in each sample was assayed for 1 min with reference to background radiation (blank tubes) at window settings of 100–180 keV (^{99m}Tc) and 300–400 keV (^{131}I). Correction of the ^{99m}Tc counts was made for crossover from the high-energy channel.

Renal blood clearance was calculated for each radionuclide for each of the four urine collection intervals according to the following formula:

$$Cl = \frac{\dfrac{\text{net cpm urine} \times 200}{10 \text{ min}}}{\dfrac{\text{net cpm blood}}{\text{net blood mass}}}$$

+ bodyweight (in hundreds of grams).

Clearance values (ml/min/100 g) calculated by this formula assume a blood density of 1.

The blood levels were stable for the final three collection periods; therefore, clearance values corresponding to these periods were used. These values were averaged for each rat.

Means and standard errors were calculated for clearance and for plasma binding data. Student's t-statistic at a probability level of 0.05 for Type I error was used to compare renal clearance data in terms of ml/min/100 g for [^{99m}Tc]MAG_3, and in terms of the ratio of [^{99m}Tc]MAG_3 clearance to clearance of co-administered [^{131}I]OIH.

Gamma Camera Scintigraphy

Female rats (179–269 g) were anesthetized with ketamine hydrochloride (100 mg of the base/kg, i.p.) and positioned ventral side facing the collimator. Kit-prepared [^{99m}Tc]MAG_3 or [^{131}I]OIH was injected i.v. (0.31–0.63 ml, 12.5–50 μCi), and imaging commenced immediately. Thirty consecutive 1-min images were acquired in a 128 × 128 pixel format with 256 maximal pixel count. The gamma camera was equipped with a 140 keV high-resolution collimator for imaging rats given [^{99m}Tc]MAG_3, and with a 410-keV parallel hole collimator for imaging rats given [^{131}I]OIH.

Renogram analysis was performed for each rat. Time-to-peak activity (T_{max}) and time to half-maximal activity ($T_{½\,max}$) values were averaged for left and right kidneys for each rat. In addition, in order to provide an index of hepatobiliary interference with kidney activity, the counts in the right kidney region of interest (ROI) were divided by the sum of counts in both right and left kidney ROIs to give fraction of activity in right kidney at peak activity. Means and standard errors for these data were calculated for [^{131}I]OIH and for [^{99m}Tc]MAG_3. These data were also subjected to Student's t-testing at a probability level of 0.05 for Type I error.

Renal Ligation

Female rats (190–246 g) were anesthetized with 45 mg sodium pentobarbital/kg, i.p. A laparotomy was performed and each renal peduncle was ligated with surgical silk thread. The incision was sutured and the rat was positioned for anterior gamma camera imaging. Three rats received kit preparation [^{99m}Tc]MAG_3, three received HPLC-purified [^{99m}Tc]MAG_3, and three received [^{131}I]OIH. Dose volumes were set to deliver ~70 μCi. All injections were made through a lateral tail vein. Immediately after injection, dynamic gamma scintigraphy was performed as described above for renogram evaluation.

A liver ROI was determined for each rat by summing the first five frames of image data. Care was taken to exclude cardiac blood pool. Background activity was determined from an ROI adjacent to the rat's body activity outline. The total count in each quadrant frame was also determined: the number of pixels in the quadrant frame's field of view was estimated by imaging a cesium-137 flood source for 10 min and an ROI circumscribing this flood source image was obtained. Net counts in the animal (NET_{TOT}) and liver (NET_{LIV}) were calculated according to the following formulas:

$$NET_{TOT} = C_{TOT} - \frac{C_{BKG}}{N_{BKG}} \times N_{TOT}$$

$$NET_{LIV} = C_{LIV} - \frac{C_{BKG}}{N_{BKG}} \times N_{LIV},$$

where C_{BKG}, C_{TOT}, and C_{LIV} are counts in the background ROI, frame, and liver ROI, and N_{BKG}, N_{TOT} and N_{LIV} are numbers of pixels in these regions. Net activity in the liver, expressed as a percent of net activity in the frame (animal) was calculated for each frame and subjected to nonparametric analysis of variance (*3*).

RESULTS

Renal Clearance

Data for renal clearance and plasma binding of [^{131}I]OIH and the two [^{99m}Tc]MAG_3 preparations are presented in Table 1. Radioactive clearance of [^{99m}Tc]MAG_3 prepared from the kit formulation and HPLC-pure preparation averaged 3.9 ml/min/100 g and 4.6 ml/min/100 g, respectively. This difference was not statistically significant. Renal clearance ratios of [^{99m}Tc]MAG_3 to [^{131}I]OIH were greater than 1 for both preparations (kit formulation: 1.7 ± 0.1 [average ± s.e.m.], HPLC-pure preparation: 1.9 ± 0.2). Although the ratio for the HPLC-pure preparation appeared somewhat higher than that for the kit formulation, the difference was not statistically significant. Plasma binding of [^{99m}Tc]MAG_3 after both preparations was in the 70–80% range, while that of [^{131}I]OIH was in the 30–40% range (Table 1).

Renograms

Results for renograms are summarized in Table 2. Average times to peak activity ranged from 2 to 5 min, and average times to half-maximal activity ranged from 10 to 15 min. Average fraction in right kidney was 0.53 for both radiopharmaceuticals. There were no significant differences between groups for any measure. Figure 1 shows scintigrams for representative cases of rats given [^{99m}Tc]MAG_3 and [^{131}I]OIH. Target-to-background activity ratios enabled good kidney delineation for both agents at their peak kidney uptake times.

Renal Ligation

Representative scintigrams for [^{99m}Tc]MAG_3 and [^{131}I]OIH in rats following renal ligation are shown in Figure 2. To avoid systematic differences in estimating

TABLE 1
Renal Whole Blood Clearance and Plasma Binding

Kit formulation			
Renal whole blood clearance (ml/min/100 g)		Plasma binding	
[^{99m}Tc]MAG_3	[^{131}I]OIH	[^{99m}Tc]MAG_3	[^{131}I]OIH
2.50	1.98	N.D.	N.D.
4.04	2.40	79.8	32.3
5.17	2.62	76.9	31.8
3.74	2.13	73.6	28.6
4.23	2.39	79.6	35.8
3.9 ± 0.4	2.3 ± 0.1	75 ± 2	31 ± 1
HPLC-pure preparation			
Renal whole blood clearance (ml/min/100 g)		Plasma binding	
[^{99m}Tc]MAG_3	[^{131}I]OIH	[^{99m}Tc]MAG_3	[^{131}I]OIH
4.11	2.44	80.2	30.7
4.65	2.12	68.2	28.8
5.04	2.72	81.0	36.8
4.6 ± 0.3	2.4 ± 0.2	76 ± 4	32 ± 2

Values are for individual rats.
Group averages and standard errors are indicated beneath each column of data.
N.D. = not determined.

TABLE 2
Renogram Data

T_{max} (min)	Fraction right kidney	$T_{½ max}$ (min)
[^{99m}Tc]MAG_3		
1.0	0.56	7.9
11.5	0.52	24.9
7.5	0.53	16.4
2.0	0.52	14.2
6.0	0.52	17.6
2.0	0.54	10.9
5.0 ± 1.7	0.53 ± 0.01	15.3 ± 2.4
[^{131}I]OIH		
2.5	0.53	11.8
2.0	0.51	5.4
2.0	0.54	11.6
2.2 ± 0.2	0.53 ± 0.01	9.6 ± 2.1

Time data are averages for left and right kidneys of individual rats. Group averages and standard errors are shown below bottom lines.

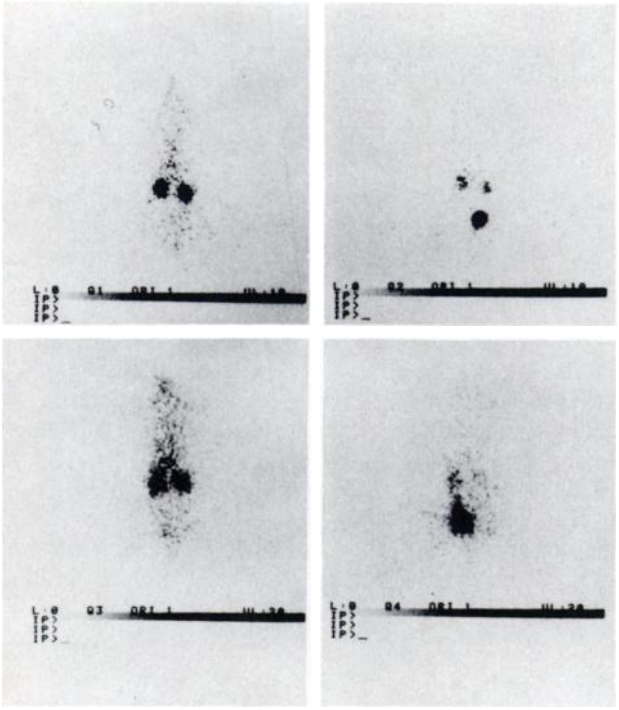


FIGURE 1
Abdominal scintiphotos in rats. Upper two scintiphotos are for a rat given [^{99m}Tc]MAG_3 (T_{max} 2.0 min, $T_{½ max}$ 14.2 min in Table 2), and the lower two are for a rat given [^{131}I]OIH (T_{max} 2.0 min and $T_{½ max}$ 11.6 min in Table 2). Images are displayed for minutes 2–3 and 19–20 after injection, corresponding to times of peak activity, and to the final image during a customary renogram procedure.

liver activity, ROIs drawn over the liver area for each animal were of comparable size (Table 3). Variation in ROIs both within and among groups amounted to only a few percent.

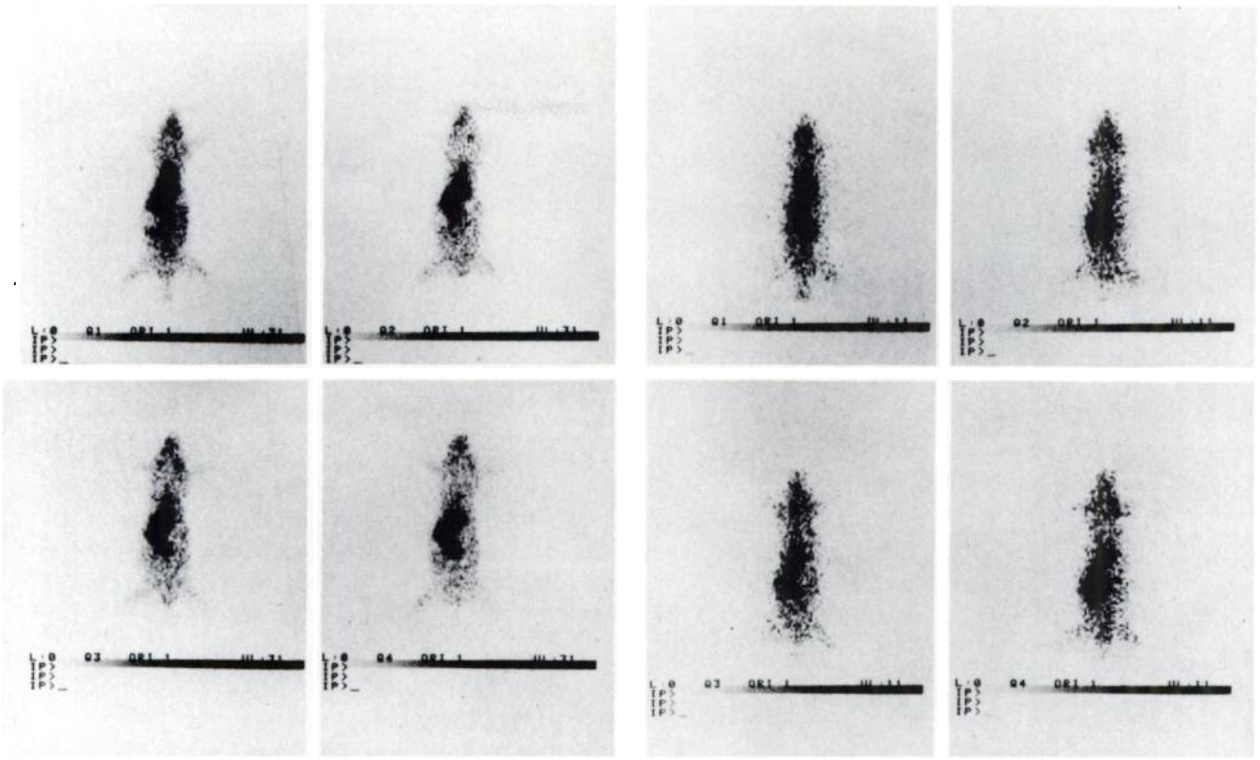
Percent activity in the liver, averaged across the 30 1-min frames, is shown in Table 3. Two of the three rats given [^{131}I]OIH had liver activity levels lower than any of the [^{99m}Tc]MAG_3 rats, but one had levels higher than any of the latter. Average liver activity for the HPLC preparation of [^{99m}Tc]MAG_3 was the same as that for the kit formulation, each at 25% of the total activity. There were no statistically significant differences among the groups. There was, however, a small but significant ($p < 0.05$) time-varying change in liver activity (Fig. 3). Over the first 10 min, percent activity in the liver ROI declined from initial values, then gradually returned to initial levels by the end of the observation period.

DISCUSSION

The purpose of these studies was to assess the renal handling of a kit formulation of [^{99m}Tc]MAG_3, and to compare it to [^{131}I]OIH and HPLC-pure [^{99m}Tc]MAG_3 in rats. Previous clinical work with this technetium renal functional agent (*2,4*) employed at-site chemical synthesis and HPLC purification, steps that would make routine clinical application of this radiopharmaceutical logistically unacceptable.

Recently, there has been a report of a [^{99m}Tc]MAG_3 kit whose biologic handling in the rat was poorer than that of OIH, and of HPLC-purified [^{99m}Tc]MAG_3 (*5*). The formulation described in the present report was independently developed with differences in ingredients, and with an aeration step to consume residual stannous ion—a step providing for enhanced stability of the reconstituted kit. The kit used in the present studies has ≥95% radiochemical purity for at least 6 hr postreconstitution, whereas the initial radiochemical purity and postreconstitution stability of the [^{99m}Tc]MAG_3 prepared by the Müller-Suurs were unknown (*5*).

Previous biologic evaluation of [^{99m}Tc]MAG_3, using HPLC-pure material, indicated a renal clearance of [^{99m}Tc]MAG_3 in the rat that was greater than that of [^{131}I]OIH by the constant-infusion technique (*1*). This was despite a higher plasma binding of the technetium radiopharmaceutical in this species—a phenomenon expected to decrease glomerular contribution to total renal excretion. Similar findings are presented here with kit-formulated [^{99m}Tc]MAG_3 as well as with the HPLC-purified radiopharmaceutical. However, Müller-Suur and Müller-Suur (*5*) found an HPLC-purified preparation of [^{99m}Tc]MAG_3 to have only comparable renal plasma clearance to [^{125}I]OIH in the rat. The finding that [^{99m}Tc]MAG_3 has a higher clearance than that of [^{131}I]OIH previously (*1*), and in this report, probably



A B

FIGURE 2
Scintiphotos of anephric rats given kit [^{99m}Tc]MAG_3 (Fig. 2A) and [^{131}I]OIH (Fig. 2B). Scintiphotos are those taken 0–1, 9–10, 19–20 and 29–30 min after injection. Rats chosen were those with median values of liver activities in their respective treatment groups as displayed in Table 3.

stems from the assay of whole blood rather than plasma for renal clearance determinations. OIH is known to enter erythrocytes, and the kidney does not appear to clear that fraction of total blood OIH contained in red blood cells (*6*). The smaller volume of distribution for [^{99m}Tc]MAG_3 (*2*) indicates that it is more difficult effort for this radiopharmaceutical to traverse biologic boundaries, including erythrocyte membranes: in rat blood, 31% of [^{125}I]OIH is associated with blood cells, while only 11% of HPLC-purified [^{99m}Tc]MAG_3 is so associated (*5*). That is, more of [^{99m}Tc]MAG_3 in blood might be available in plasma for filtration and active tubular excretion than is the case for OIH. The portion of tracer in blood available for excretion would be 1 minus the fraction in blood cells. When conversion is made from renal plasma clearance to renal whole-blood clearance (dividing by plasmacrit) and when differential blood cell content of tracer is taken into account, the Müller-Suur results for HPLC-purified [^{99m}Tc]MAG_3 and OIH, expressed in terms of whole blood clearance, are similar to those respective values found here.

TABLE 3
Liver ROI Size and Activity

	ROI*		
	[^{99m}Tc]MAG_3 (Kit)	[^{99m}Tc]MAG_3 (HPLC)	[^{131}I]OIH
	195	198	201
	195	187	200
	199	196	191
	Liver activities†		
	[^{99m}Tc]MAG_3 (Kit)	[^{99m}Tc]MAG_3 (HPLC)	[^{131}I]OIH
	21.9	22.9	18.5
	27.5	26.7	18.9
	26.1	24.0	28.6
Average	25	25	22

* Values are number of pixels in region of interest drawn about liver radioactivity for individual rats.

† Values are percent of total activity for individual rats. Averages for each treatment group are given below.

Evaluation of renograms from 30-min anterior dynamic scintigraphy of rats did not disclose any significant differences between kit-formulated [^{99m}Tc]MAG_3 and [^{131}I]OIH, although at equal radioactive doses the quality of the image can be expected to be much improved with the technetium-based radiopharmaceutical due to its superior physical imaging properties. In anterior images, designed to maximize appearance of liver uptake, maximum counts in right kidney were not grossly greater than that of left in renogram analysis.

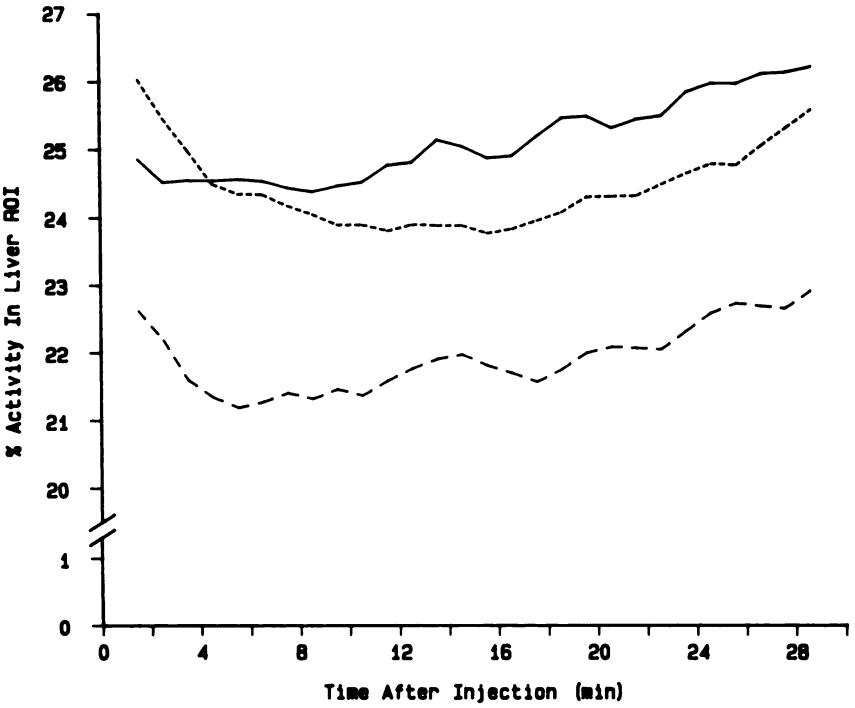


FIGURE 3
Time course of liver activity. Average liver activity (as a percent of total body activity) is presented for groups of three albino rats given kit-formulated [^{99m}Tc]MAG_3 (solid line), HPLC-purified [^{99m}Tc]MAG_3 (short dashes) or [^{131}I]OIH (long dashes). To reduce minute-to-minute variability, three-point moving averages are plotted. Note that the y-axis is offset to 20%.

Thus, liver uptake and excretion should not be a major detriment in the use of [^{99m}Tc]MAG_3 for renal scintigraphy. This is emphasized in the model of maximal renal impairment used here. Liver activity levels of renal function agents in this model averaged 22–25% of total. This level corresponds to hepatic blood pool for the rat, estimated at 23% (see Appendix I). Iodine-131 OIH and [^{99m}Tc]MAG_3 were similar in this model. This suggests that [^{99m}Tc]MAG_3 would not result in excessive liver activity under conditions of renal impairment, a detriment some renal function agents possess. There was also no difference between [^{99m}Tc] MAG_3 kit formulation and that purified by HPLC, suggesting that whatever impurities might exist in the kit reaction product do not contribute disproportionately to activity in the hepatic ROI.

The small initial decline in liver ROI activity might reflect distribution of activity from the blood pool (a major early contribution in liver ROI) into the peripheral tissues. A similar distribution into the extravascular organ space of the liver undoubtedly occurred, and this probably attenuated the decline in liver ROI activities. That a decline was seen at all in liver attests to the greater volume of blood per gram of liver compared with that of all other tissues except lung (*8*). The secondary rise in hepatic activity appears to be related to reaccumulation of activity by the liver and excretion into the gastrointestinal tract: a cinematic display of images for [^{99m}Tc]MAG_3 showed a concentrating of activity toward the central caudal aspect of the region of interest as would be expected from uptake and excretion by the liver. This same phenomenon was probably responsible for similar increases in liver region of activity after [^{131}I]OIH, but the poor imaging characteristics of the 364-keV gamma emission prevented the scintigraphic resolution necessary to see it in every case.

In summary, a kit formulation, after reconstitution with commercial generator eluate and simple preparative steps, provides [^{99m}Tc]MAG_3 with similar biologic properties to that of [^{131}I]OIH in the laboratory rat.

APPENDIX

Estimates of liver weight and blood volume as a percent of total body weight, and estimates of total blood volume in the liver are used to calculate the percent of total blood pool which is present in the liver. The former two estimates are found in W. O. Caster and co-workers (*7*); the latter are from N. B. Everett cited in (*8*).

Blood in the liver, as a fraction of total blood pool, may be estimated as =

$$\frac{M_{LIV}}{V_{TOT}} \times V_{LIV},$$

where M_{LIV} is the liver weight as a percent of body weight, V_{TOT} is the blood-pool volume as a percent of body weight, and V_{LIV} is the liver content of blood (ml/g). When the values are substituted,

$$\frac{4.15 \text{ g liver}/100 \text{ g b.w.}}{4.95 \text{ ml blood}/100 \text{ g b.w.}} \times 0.27 \text{ ml blood/g liver} = 0.226, \text{ or } 23\%$$

NOTES

* Sasco, Inc., O'Fallon, MO.

† Centrifree, Amicon Corporation, Danvers, MA.

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