Pharmacokinetics of Technetium-99m-MAG_3 in Humans

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Technetium-99m-mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG_3) is introduced to replace o-iodohippurate (OIH) for renal function studies. For interpretation of clinical findings, extensive pharmacokinetic studies were performed on patients. These showed that ^{99m}Tc-MAG_3, compared with OIH, has a higher plasma-protein binding, an essentially higher intravascular concentration, a smaller volume of distribution and, with practically identical biologic half-lives, a correspondingly lower clearance. Simultaneous steady-state measurements resulted in a 1.5-fold higher clearance of OIH than of ^{99m}Tc-MAG_3 (n = 124). Competitive inhibition of the tubular transport system by p-aminohippurate (PAH) (20 patients) revealed a distinctly higher suppression of the ^{99m}Tc-MAG_3 clearance than of OIH which indicates a lower affinity of the ^{99m}Tc complex to the tubular cell. The plasma extraction efficiencies of both agents, measured during surgery (n = 5), did not indicate an extrarenal elimination of ^{99m}Tc-MAG_3. This new radiopharmaceutical is a pragmatic alternative to OIH and offers advantages not only for scintigraphic imaging but is also suited for quantitative renal function studies.

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Although o-iodohippuric acid (OIH) essentially fulfills the pharmacokinetical requirements for a renal function agent, the necessity of radioactive iodine labeling leads to distinct disadvantages for routine use: iodine-131 (^{131}I) labeled OIH has a poor imaging quality caused by the high energy of 364 keV and leads to a high radiation exposure particularly due to the beta component of the radioactive decay. The other frequently used label, ^{123}I, seems to solve these problems at first, but since it can only be produced in a cyclotron and has a half-life of 13 hr, logistic problems arise in the distribution of this very expensive iodine isotope and the radiopharmaceutical, respectively. Another disadvantage consists in a high photon energy of 530 keV which is released during the decay of ^{123}I. The resulting septum penetration, when employing a high-resolution low-energy collimator, is sufficient to reduce the image quality even if extremely pure ^{123}I is used. Considering all factors, a substance would be preferable which can be labeled with technetium-99m (^{99m}Tc) and is excreted by tubular secretion to a sufficient extent.

In this context, a series of diamide-dithiol-(N_2S_2) ligands was developed on the basis of the work by Davison et al. (*1,2*), revealing pharmacokinetic properties similar to OIH (*3–10*). The first clinical studies had shown the triamide-monothiol-(N_3S) complex Tc-99m-mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG_3) to be the most appropriate substance to replace OIH (*11,12*). Preliminary pharmacokinetic studies both in animals and in humans showed notable differences between this new radiopharmaceutical and OIH with respect to the clearance, biological distribution and plasma protein binding (*6,11,13–17*).

The aim of this paper is to present a systematic investigation of differences existing between ^{99m}Tc-MAG_3 and OIH in humans on the basis of various parameters. The results will be interpreted with respect to the reason for the lower clearance of ^{99m}Tc-MAG_3 as compared to hippuric acid derivatives, the affinity of the ^{99m}Tc complex to the tubular transport system, its presumed hepatobiliary excretion, and its renal elimination mechanism, in order to allow better comprehension of the quantitative results obtained from renal function studies using ^{99m}Tc-MAG_3.

MATERIALS AND METHODS

All patients, either with native or with transplant (Tx) kidneys, were referred for evaluation of the renal function and gave their written consent. This study was approved by the Ethical Commission of the University of Heidelberg and the Federal Board of Public Health (Bundesgesundheitsamt).

Radiopharmaceuticals

The precursor S-benzoyl-mercaptoacetyl-glycylglycylglycine was synthesized as previously described in another paper (*14*). All intermediates were characterized by NMR-, i.r.- and mass spectroscopy. The final product was additionally identified by elemental analysis.

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Technetium-99m-pertechnetate was obtained from a commercial generator system. Labeling was performed, with the kit procedure described elsewhere (*6*), in ~95% radiochemical yield and ^{99m}Tc-MAG$_3$ was purified by high-pressure liquid chromatography (HPLC) prior to application (*14*). The final solution contains ^{99m}Tc-MAG$_3$ with a specific activity of >7.4 GBq/mg (200 mCi/mg).

Iodine-131-OIH was prepared according to the method published by Sinn et al. (*18*) with a specific activity of ~37 MBq/mg (1 mCi/mg). The radiochemical purity of both agents was >99%.

Binding to Red Blood Cells and Plasma Proteins

Within 10 min following the withdrawal, the whole blood was centrifuged using a swing-out rotor at 700 g for 20 min. The count rates of each 1 ml whole blood and 1 ml plasma were measured in a well-type counter. Physical decay correction and, for simultaneous measurements, a correction for cross-talk of ^{131}I into the ^{99m}Tc channel (16.5%) were performed. For the calculation of the binding to the red blood cells (RBCs), the hematocrit (HC) value was determined with a Sysmax system CC 780 (Digitana, Hamburg, West Germany). In this instrument, the whole blood is diluted to a defined volume; the number and size of RBCs are recorded by electric resistance detection passing a transducer aperture. Plasma trapping between RBCs cannot influence the result.

For the determination of the plasma protein binding, the plasma was ultrafiltered in an Amicon cell through a Diaflow membrane PM 30 (exclusion limit 30,000 Dalton; 5 bar N_2-pressure). For the calculation of the protein bound fraction, the protein content of the plasma was assumed to be 7.2 g/100 ml.

p-Aminohippuric acid (PAH) was measured according to the procedure developed by Bratton and Marshall (*19*) and modified by Deetjen and Sonnenberg (*20*).

Compartment Analysis

According to Sapirstein et al. (*21*), the time-activity curves of the blood and the plasma, respectively, can be described by two components to be calculated by the following equation:

$$y = Ae^{-b_1 t} + Be^{-b_2 t}.$$

From the counted activity concentrations y at time t, the compartment coefficients b_1 (fast component) and b_2 (slow component) and the biologic half-lives T^A and T^B, respectively, as well as the distribution volumes V_1 and V_2, and the slope clearances can be calculated (*21,22*). In order to determine these parameters, 14 patients received 7.4 MBq [^{131}I]OIH and 7.4 MBq ^{99m}Tc-MAG$_3$ simultaneously. Blood (3 ml) was drawn 2, 5, 10, 17, 25 and 30 min postinjection.

Steady-State Clearance

Forty minutes after i.v. application of 11.1 MBq [^{131}I]OIH and 18.5 MBq ^{99m}Tc-MAG$_3$, simultaneous clearance determinations under steady-state conditions were performed in 124 patients (48 patients with transplant (Tx) kidneys) by a special feedback-controlled infusion pump device (*23*). The calculation was based on the activity dose required for maintenance of the steady state (not the activity eliminated with the urine!) and the plasma concentration in this condition. The patients' serum creatinine values ranged between 0.3 and 5.3 mg/100 ml (mean = 1.5) on the day they were examined.

PAH Loading

Twenty patients with native kidneys received an infusion of 80 mg PAH/kg body weight (50 ml volume) within 5 min, immediately after basic clearance measurement in steady state. Maximum plasma concentrations of 30–40 mg PAH/100 ml were reached. Serum creatinine amounted to values between 0.7 and 2.2 mg/100 ml (mean = 1.1). Three different time intervals were selected for the clearance calculation: second to fifth minute during the PAH infusion, first to tenth minute after the infusion and eleventh to twentieth minute following PAH application.

Lipophilicity

In order to determine the octanol-water partition as a measure for the lipophilicity of the two radiopharmaceuticals, 1g of 0.1 N citrate buffer (for pH = 4.0) and 1g of 0.1 N phosphate buffer (for pH = 6, 6.5, 7.0, 7.4, respectively) were mixed together with 1g octan-1-ol. After adding 10μl ^{99m}Tc-MAG$_3$ and [^{131}I]OIH (~4 MBq each), the samples were vortexed for 3 min, centrifuged at 2,500g for 10 min and then separated. Two-hundred mg of the water and the octanol phase, respectively, were measured in a well-type counter. The calculation (see Table 3) was performed according to the following equation:

$$\text{Lipophilicity [\%]} = \text{cpm(octanol)} \times 100 / [\text{cpm(octanol)} + \text{cpm}(H_2O)].$$

Renal Arterio-Venous Difference (Extraction Efficiency)

In these patients (n = 5) a unilateral nephrectomy due to a malignant renal tumor was to be performed. Steady-state clearances of ^{99m}Tc-MAG$_3$ and [^{131}I]OIH were determined during the week prior to surgery. [^{131}I]OIH (4 MBq) and ^{99m}Tc-MAG$_3$ (11 MBq) were administered intraoperatively after uncovering the diseased kidney. Five to 10 min afterwards, blood samples were taken from the renal artery and renal vene simultaneously, and the activity concentrations were then measured according to the method described above. The serum creatinine value examined during the operation ranged between 0.6 and 1.2 mg/100 ml. The intraoperative systolic blood pressure differed from preoperative clearance measurements by 15% at most.

Statistical Models

For examination of differences, the Wilcoxon test for pair differences was used, as well as the U-test to compare two independent random samples. The significance was defined to be $p \leq 0.05$ and all regression analyses were made for $p \leq 0.05$ (95%-confidence intervals).

RESULTS

The binding of OIH to red blood cells amounted to 15.3% ± 4.1 s.d. (n = 32; HC(mean) = 37.3%) and of ^{99m}Tc-MAG$_3$ to 5.1% ± 3.3 s.d. (n = 49; HC(mean) = 38.2%).

The plasma protein bindings of PAH, OIH, and ^{99m}Tc-MAG$_3$ revealed highly significant differences

TABLE 1
Plasma Protein Binding (% ± s.d.)

	p-amino-hippurate	o-iodo-hippurate	Tc-MAG_3
Maher and Tauxe (*24*)	18.3 ± 6.1 (n = 6)	66.8 ± 1.9 (n = 5)	—
Taylor et al. (*11*)	—	66.2 ± 6.9 (n = 8)	87.5 ± 2.6 (n = 8)
Taylor et al. (*25*)	—	53.1 ± 8.7 (n = 12)	78.6 ± 7.9 (n = 12)
Our results	26.1 ± 6.7 (n = 20)	70.7 ± 5.0 (n = 32)	90.1 ± 2.8 (n = 50)

($p \leq 0.01$). For PAH, it averaged 26.1%, for OIH 70.7% and for ^{99m}Tc-MAG_3 90.1% (Table 1). Since only the non-protein bound fraction of a substance can be filtered glomerularly (*6,24*), not more than about 2% of ^{99m}Tc-MAG_3 are ultrafiltered, assuming a normal filtration fraction (FF) of the human kidney of 20%. Thus, the clearance of ^{99m}Tc-MAG_3 corresponds approximately to the "tubular extraction rate" (TER) of this substance. [Definition: The TER corresponds to the virtual plasma volume per minute from which a substance (related to its plasma concentration in the renal artery) is completely removed by tubular extraction]. Therefore, the clearance of ^{99m}Tc-MAG_3 shall be designated as TER(MAG_3) in the following.

In 10 of 14 patients, the compartment analysis (*21*) was not only performed on the basis of plasma, but also of whole blood samples (Table 2). No significant differences of the biologic half-lives T^A and T^B and the compartment coefficients b_1 and b_2, respectively, between ^{99m}Tc-MAG_3 and OIH ($p > 0.1$) were revealed.

The differences between the two radiopharmaceuticals with respect to V_1 and V_2, based on the plasma values, were highly significant ($p \leq 0.01$). The total volume of distribution ($V_1 + V_2$) of OIH and ^{99m}Tc-MAG_3 amounted to 16.6% and 10.8% of the body weight (BW), respectively. The clearance (Cl) ratio ^{99m}Tc-MAG_3/OIH amounted to 0.66 (±0.11 s.d.) in the plasma and 0.74 (±0.13 s.d.) in the whole blood (Table 2).

After compartment distribution in vivo, the ratio of the plasma concentrations ^{99m}Tc-MAG_3/OIH (related to the injected dose) revealed no significant dependence on the time of withdrawal (intra-individual standard deviation between the tenth and the thirtieth min postinjection of max. 5%) and ranged from 1.06 to 2.12 (mean = 1.57). Figure 1 presents the mean (relative) time-activity concentrations and half-life exponentials in plasma (semilogarithmic) of all 14 patients during the first 30 min postinjection, thus clearly pointing out the difference between the two radiopharmaceuticals in this regard. The respective ratio in whole blood was 1.36 (1.01 – 1.76).

In simultaneous clearance measurements under steady-state conditions (n = 124), the OIH clearance ranged between 35 and 654 ml/min (mean = 299), whereas the TER(MAG_3) lay between 4 and 441 ml/min (mean = 203). Figure 2 shows the correlation of these values with the corresponding confidence intervals ($r = 0.94$; $p \leq 0.05$); the ratio of TER(MAG_3)/OIH clearance averaged 0.67.

PAH loading was performed in 20 patients following the basic clearance measurement, revealing a significant decrease in the ^{99m}Tc-MAG_3 clearance already in the course of the infusion of PAH. Within the first 10-min

TABLE 2
Compartment Analysis (mean ± s.d.)

		Plasma (n = 14)		Whole blood (n = 10)	
		o-I-hippurate	Tc-MAG_3	o-I-hippurate	Tc-MAG_3
Cl*	[ml/min]	412 ± 169	265 ± 98	639 ± 348	449 ± 228
b_1	[min^{-1}]	0.345 ± 0.165	0.353 ± 0.166	0.460 ± 0.300	0.476 ± 0.356
b_2	[min^{-1}]	0.033 ± 0.012	0.033 ± 0.012	0.033 ± 0.010	0.032 ± 0.013
T^A	[min]	2.50 ± 1.28	2.37 ± 1.03	2.21 ± 1.34	2.31 ± 1.62
T^B	[min]	26.0 ± 18.6	25.0 ± 13.5	22.6 ± 6.6	24.7 ± 9.5
V_1*	[l]	5.34 ± 1.83	3.67 ± 1.08	7.66 ± 4.40	5.55 ± 2.99
V_2*	[l]	5.54 ± 2.76	3.38 ± 1.22	7.77 ± 1.72	6.01 ± 1.22
V_1* + V_2*	[l]	10.88 ± 3.75	7.05 ± 1.58	15.43 ± 4.62	11.56 ± 3.12
$V_1 + V_2$	[% BW]	16.6 ± 5.6	10.8 ± 2.3	23.1 ± 7.0	17.3 ± 4.9

* Related to 1.73 m^2 body surface

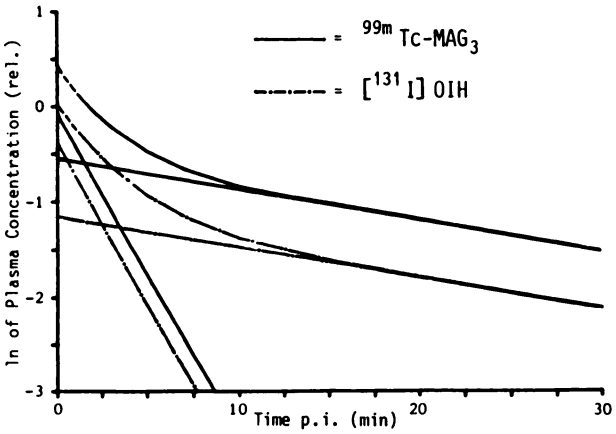

FIGURE 1
Relative time-activity concentrations and half-life exponentials (two-compartment-model) of ^{99m}Tc-MAG$_3$ and [^{131}I]OIH in plasma (mean of 14 patients, semilogarithmic graph).

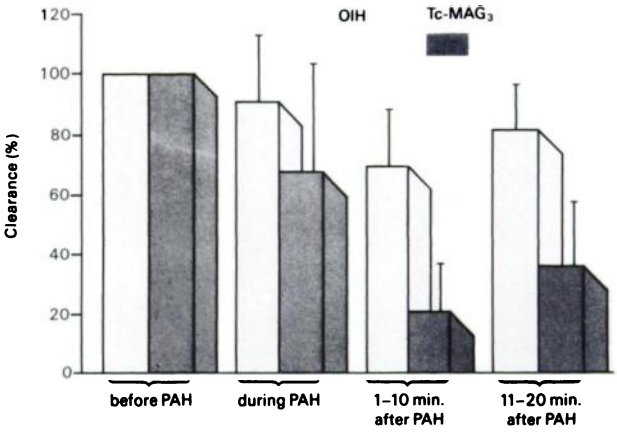

FIGURE 3
Changes in the OIH and ^{99m}Tc-MAG$_3$ clearances, respectively, during various intervals under PAH loading (n = 20).

interval post-PAH, the clearance of OIH was 69% and that of ^{99m}Tc-MAG$_3$ 21%, as compared to the basic values. During the second period (eleventh to twentieth minute post-PAH), the clearance of OIH and ^{99m}Tc-MAG$_3$ increased to 82% and 36%, respectively, due to the continuous reduction of the competitive inhibition by PAH (Fig. 3); all these changes were significant ($p \leq 0.05$).

The octanol-water partition only reached a high lipophilicity for OIH at pH 4.0 (~82%). The remaining OIH values and all ^{99m}Tc-MAG$_3$ values at pH 4.0–7.4 were clearly below 10% (Table 3).

Table 4 demonstrates the extraction ratios ^{99m}Tc-MAG$_3$/OIH in the patients during the operation, determined by measurement of the renal arterio-venous difference (extraction efficiency) as well as in the preoperative phase, calculated with the aid of clearance values in steady state. The ratios of the extraction efficiencies lay between 0.56 and 0.78, and the clearance ratios between 0.66 and 0.74. The differences observed in the individual patients were not significant ($p > 0.1$).

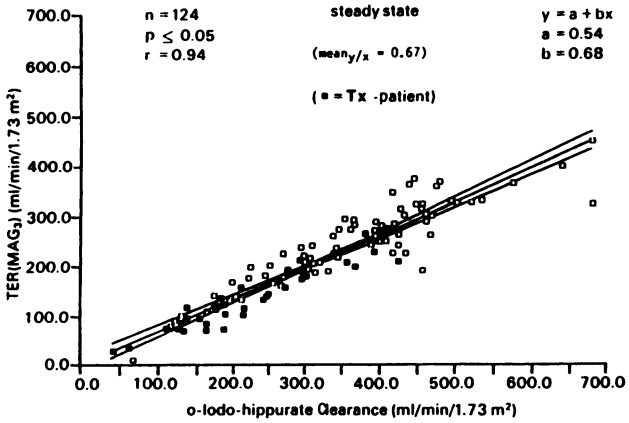

FIGURE 2
Correlation of the simultaneously determined TER(MAG$_3$) with the OIH clearance under steady-state conditions.

DISCUSSION

In agreement with the results obtained by other investigators (*24,25*), the plasma protein binding of ^{99m}Tc-MAG$_3$ amounting to 90%, was distinctly higher than that of OIH, which reached 71% (Table 1). The discrepancy appearing in values found by Taylor et al. in 1986 (*11*) and 1987 (*25*) was not explained by the authors. Taylor and Eshima (*13*) reported of a reduction of the protein binding of OIH (by 68%) and ^{99m}Tc-MAG$_3$ (by 49%) in rats under mannitol diuresis, as compared to (other) control animals in normal diuresis. Our own in vitro studies showed no change of the protein bound fraction of ^{99m}Tc-MAG$_3$ after a 1:10 (n = 4) or 1:100 (n = 3) dilution with 0.9% sodium chloride (unpublished results).

The higher protein binding of ^{99m}Tc-MAG$_3$ in comparison to OIH may be the main reason for the higher intravascular concentration of ^{99m}Tc-MAG$_3$, which correlates with the distinctly lower volume of distribution of ^{99m}Tc-MAG$_3$ than of OIH (Table 2). The volume-of-distribution ratios ^{99m}Tc-MAG$_3$/OIH coincided with other investigators (*25,26–28*). The contradicting results obtained by Taylor et al. (*11*) are now explained by the authors (*28*) and are no longer opposed to our results.

In agreement with Taylor et al. (*11*), the compartment coefficients (b_1 and b_2) and the half-lives in both

TABLE 3
Lipophilicity [%]
(Octanol/Water Partition)

	pH 4.0	pH 6.0	pH 6.5	pH 7.0	pH 7.4
OIH	82.39	3.98	2.60	1.69	1.61
Tc-MAG$_3$	6.05	0.20	0.10	0.08	0.07

TABLE 4
Extraction Ratios

	Pre-operative: steady-state clearance	During operation: extraction efficiency
Patient	^{99m}Tc-MAG$_3$ / OIH	^{99m}Tc-MAG$_3$ / OIH
OP 1	0.72	0.56
OP 3	0.66	0.76
OP 4	0.72	0.76
OP 5	0.74	0.58
OP 6	0.72	0.78
mean ± s.d.	0.71 ± 0.03	0.69 ± 0.11

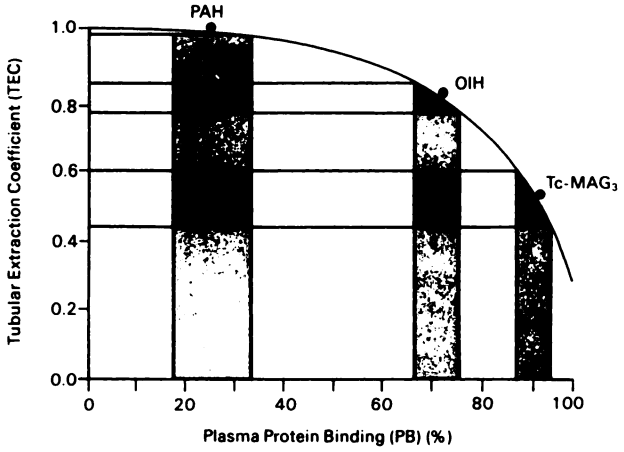

FIGURE 4
The TEC as a hypothetical function of the degree of plasma protein binding ($y = 1.0 - 0.0031\ e^{0.056x}$).

compartments, respectively, reveal no significant differences with respect to the two radiopharmaceuticals (Table 2). This means that the elimination half-lives are nearly identical for both substances and also correspond to urine measurements (*11*), in which ~70% of the administered dose of both radiopharmaceuticals have been excreted within 30 min postinjection.

With identical elimination half-lives for ^{99m}Tc-MAG$_3$ and OIH, and a plasma concentration of ^{99m}Tc-MAG$_3$ that is constantly higher by a factor of 1.57 (Fig. 1), the plasma clearance (in slope) for OIH is theoretically expected to be higher than that for ^{99m}Tc-MAG$_3$ by this factor and was calculated to be 1.52 (Table 2). In agreement with other authors (*11,29*) the average whole-blood-clearance ratio ^{99m}Tc-MAG$_3$/OIH reaching 0.74 was found to be notably higher ($p \leq 0.05$) than the respective plasma-clearance ratio (0.66). This is explained by the higher binding of OIH to RBCs as compared to ^{99m}Tc-MAG$_3$ (15% versus 5%), since the fraction of the substance bound to RBCs is not available to the tubular cell for extraction (*30*). This means that, in contrast to the supposition of Taylor and Eshima (*13*), the plasma clearance and not the whole blood clearance is clinically relevant.

We agree with Kim et al. (*31*) that the clearance of a substance does not depend upon the volume of distribution, which, in turn, is mainly determined by the degree of protein binding, but on the renal extraction efficiency, which may also be influenced by the plasma protein binding (Fig. 4). The supposition by Jafri et al. (*26*) that the smaller volume of distribution of ^{99m}Tc-MAG$_3$ is the result of a smaller fraction bound to RBCs and of a weaker protein binding, does not apply and is merely hypothetical, respectively, because this difference also exists when the calculation is based on plasma values and there were, until now, no data relating to the strength of the protein binding. Furthermore, the molecular sizes of both radiopharmaceuticals do not essentially differ from each other, following that this is an unlikely explanation for a slower diffusion of ^{99m}Tc-MAG$_3$ from the plasma than of OIH.

Clearance measurements in slope are generally disputed, especially in pathologic conditions, and only represent a compromise between accuracy and practicability (*32–34*). To determine accurate and reproducible clearance values, a steady state is required. We carried out steady-state-clearance measurements simultaneously with ^{99m}Tc-MAG$_3$ and [^{131}I]OIH (n = 124; Fig. 2), which resulted in an average clearance ratio ^{99m}Tc-MAG$_3$/OIH of 0.67 with a high correlation coefficient (r = 0.94), thus confirming the theoretical expectations (see above). In contrast to Jafri et al. (*35*), who calculated the same slope "b" in addition to a component "a" of 40 ml/min for the linear regression equation "y = a + bx", we found a negligible value of 0.6 ml/min for "a." The reasons for this difference are most probably that they only examined very few cases (n = 12) and used the slope technique.

The "tubular extraction rate" of an agent, which is renally secreted but not reabsorbed, can be calculated on the basis of its clearance and its protein-bound fraction; for ^{99m}Tc-MAG$_3$ it nearly corresponds to its clearance due to its very high protein binding in humans, designated as TER(MAG$_3$) (see Results). Knowing that the clearance of PAH is higher than the clearance of OIH by a factor of 1.2 (mean value from 7 references in (*36*)) and that the OIH clearance, as shown, is higher than the ^{99m}Tc-MAG$_3$ clearance by the factor 1.5, a "tubular extraction coefficient" (TEC) can be calculated for these agents, which indicates the portion of the substance extracted from the peritubular capillary blood and plasma, respectively. This parameter is required to compare the extent of tubular secretion of agents with different glomerular filtration fractions. Since PAH is extracted completely in the cortical nephrons from the plasma by the tubular transport system, its TEC amounts to 1.0. The TECs for OIH and ^{99m}Tc-MAG$_3$ were calculated to be 0.83 and 0.55, respectively (*37*).

As demonstrated in Figure 4, there could be a connection between plasma protein binding and TEC due

to the fact that in substances which are bound to plasma proteins to a high degree, like OIH and ^{99m}Tc-MAG$_3$, the peritubular transit time is too short for complete dissociation of the respective agent from the plasma protein, so as to be available for tubular secretion following subsequent diffusion into the extracellular fluid. This would also explain the observation made by Harth et al. (*38*), stating that an increase in renal perfusion leads to a decrease in PAH extraction, which they supposed was caused by the altered form and velocity of the blood flow. The reports of other authors (*11,13,39,40*) which point out that the clearance differences between OIH and ^{99m}Tc-MAG$_3$ are distinctly smaller in rats than in humans, also stated that the protein binding of both agents in rats is essentially lower. This supports the theory that the level of TEC depends on the protein binding (Fig. 4).

According to this hypothesis, the extent of protein binding of PAH within ± 1 s.d. (vertically shaded area in Fig. 4) would only have minimal influence on the TEC of PAH, whereas OIH would already reach values ranging between 0.79 and 0.88 in this area. The protein binding of ^{99m}Tc-MAG$_3$ in the range between 87% and 93% (i.e., ±1 s.d.) even leads to variations of TEC of between 0.45 and 0.6. These deviations of the respective radiopharmaceutical from the mean TEC value might be the cause for the infrequently observed but distinct deviation of the ratios TER(MAG$_3$)/OIH clearance (Fig. 5), which is nearly independent of the renal function. It will have to be examined in the future to what extent a drug therapy might influence the plasma protein binding of ^{99m}Tc-MAG$_3$ and consequently the TER(MAG$_3$) in these patients.

The PAH loading, which was introduced by Lum Winkel (*41*), has shown that the clearance of ^{99m}Tc-MAG$_3$ can be influenced to a higher degree than the clearance of OIH (Fig. 3; the slightly higher glomerularly filtered fraction of OIH is of negligible influence). These observations coincide with animal experiments (*13*) and lead to the conclusion that ^{99m}Tc-MAG$_3$ has a clearly lower affinity to the tubular transport proteins than OIH.

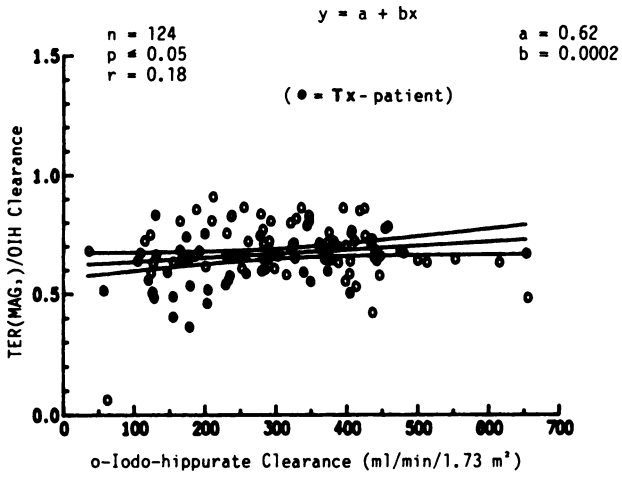

FIGURE 5
The ratio [TER(MAG$_3$)/OIH clearance] shows only a slight dependence of the renal function (= OIH clearance), but distinct interindividual deviations.

Further investigations have to demonstrate whether a drug treatment might influence the TER(MAG$_3$) in this respect. However, simultaneous examinations of transplant patients have shown that the uptake of ^{99m}Tc-MAG$_3$ in the renal parenchyma is similar to OIH even in cases of severely impaired renal function and this leads to diagnostically useful renograms (*12,42*).

Blaufox (*43*) discussed the "non-ionic diffusion" (tubular reabsorption of lipophilic molecules) as a possible reason for the difference between the clearances of PAH and OIH. Theoretically, this might also represent one cause for the lower clearance of ^{99m}Tc-MAG$_3$ against OIH. The determination of lipophilicity by means of the octanol-water partition revealed far less pH-dependent changes for ^{99m}Tc-MAG$_3$ than for OIH in the range between pH 7.4 (normal blood pH) and pH 4.0 (extremely low urine pH) (Table 3). In agreement with electrophoretic measurements (*44*), demonstrating that ^{99m}Tc-MAG$_3$ is negatively charged even at pH 4.0, a non-ionic diffusion of ^{99m}Tc-MAG$_3$ can be excluded. Additionally, a tubular retransport of ^{99m}Tc-MAG$_3$ during water reabsorption, a "solvent drag," cannot be responsible for the clearance differences because the TER(MAG$_3$) would then have to increase under accelerated diuresis; this was observed neither under furosemide nor under hydrochlorothiazide diuresis (*37*).

The intraoperative measurements revealed that the ratios of the plasma-clearance values determined preoperatively do not differ significantly from the ratios of the extraction efficiencies (EEs) measured during surgery (Table 4). In case of an additional hepatobiliary excretion of ^{99m}Tc-MAG$_3$, as reported by several authors using kit preparations (*26,28*), these ratios would consequently have to differ from each other if our clearance technique is used (see Materials and Methods).

Taylor and Eshima (*13*), who found a lower clearance of ^{99m}Tc-MAG$_3$ than of OIH in rats, calculated a higher extraction efficiency of ^{99m}Tc-MAG$_3$ in relation to OIH "in spite of" the higher protein binding of ^{99m}Tc-MAG$_3$. The discrepancy of the EE to the clearance values as well as the higher protein binding are not really contradictory, because the authors based the EE calculation on whole blood and not on plasma. Since the fraction of OIH bound to RBCs is higher than that of ^{99m}Tc-MAG$_3$, the EE is mathematically shifted in favor of ^{99m}Tc-MAG$_3$. However, this is not admissible, since the RBC-bound fractions of both agents do not participate in the process of tubular secretion because the diffusion of the radiopharmaceuticals out of the RBCs into the plasma is very slow (*30*). A respective correction (*45*)

is not necessary and would falsify the results. The reported extraction efficiencies for ^{99m}Tc-MAG$_3$ (*13*, *45*) do not correspond, as assumed (*13*), and cannot be compared because one value was determined from plasma (*45*) and the other from whole blood (*13*).

CONCLUSION

Technetium-99m-MAG$_3$ demonstrates renal uptake and elimination characteristics which are analogous to OIH, even in cases of severely impaired renal function (*12*,*25*,*26*,*28*,*42*). Therefore, it can replace radioiodinated OIH in qualitative renal function diagnostics with all the advantages originating from the Tc-99m label (Fig. 6), including the additional evaluation of the renal perfusion during the first pass.

Technetium-99m-MAG$_3$ has a distinctly lower affinity to the transport proteins of the tubular cell than OIH and possibly a higher range of deviation regarding the extent of the tubular extraction due to the high plasma protein binding. It would have to be studied in special patient groups, whether these pharmacokinetic differences are useful concerning differential diagnostics or whether they impair the accuracy of quantitative measurements. A strict correlation between the TER(MAG$_3$) and the OIH clearance exists in the majority of cases, so that quantitative clearance measurements in clinical routine appear to be justified. A simple slope-clearance method, developed by Tauxe et al. for OIH (*46*,*47*), was modified and validated for ^{99m}Tc-MAG$_3$ with the aid of steady-state-clearance measurements (*37*,*48*). Similarly, Russell et al. (*49*) modified the Tauxe algorithms using an 8-point-slope-clearance calculation.

The 1.4-fold higher intravascular concentration of ^{99m}Tc-MAG$_3$ than of OIH may result in a slightly higher radiation exposure of the kidneys by ^{99m}Tc-MAG$_3$ than by [^{123}I]OIH (*50*), although the elimination half-lives of both radiopharmaceuticals are nearly identical. In cases of impaired renal function, however, the shorter physical half-life of ^{99m}Tc versus ^{123}I can lead to a lower specific radiation dose using ^{99m}Tc-MAG$_3$.

The fact that neither hepatobiliary excretion (*16*) nor metabolization (*51*) of HPLC-purified ^{99m}Tc-MAG$_3$ has been observed in patients, confirms the presumption that the accumulation in the liver and the scintigraphic visualization of the gallbladder are due to by-products formed during the complex synthesis (*14*). For this reason and with regard to the accuracy of quantitative clearance measurements, the requirement for commercially available kit preparations, that ^{99m}Tc-MAG$_3$ will be delivered in reproducible radiochemical yields of ≥95%, must be fulfilled.

ADDENDUM

Müller-Suur and Müller-Suur (*52*) have most recently reported about excretion characteristics of ^{99m}Tc-MAG$_3$ in rats. In the context of the hypothetical dependence of the ^{99m}Tc-MAG$_3$ clearance on the high protein binding, they stated that "other substances predominantly protein bound have not shown an increased clearance when their protein binding is reduced," which, according to them, was reported by Ochwadt and Pitts (*53*). In contrast to this statement, these authors (*53*), investigating tubularly secreted agents in dogs, observed that "increasing the percentage of free phenol red by lowering plasma proteins leads to a specific increase in phenol red secretion." Furthermore they assumed that "other factors, perhaps connected with the different accumulation of such

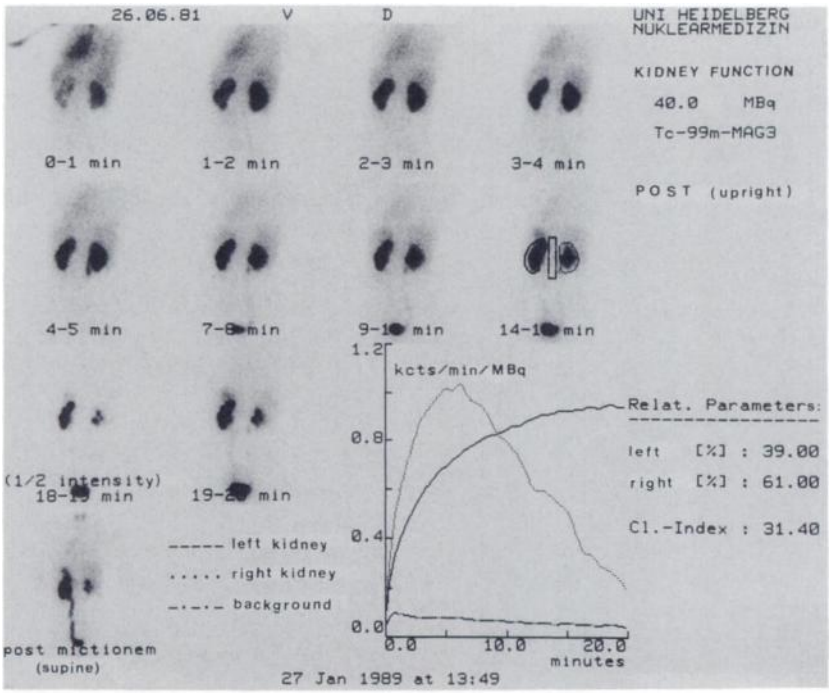

FIGURE 6
Left kidney: Relative reduced tubulosecretory function and outflow obstruction with accumulation of ^{99m}Tc-MAG$_3$ in the pelvicalyceal system (favourable demarcation with half-intensity imaging) in a young patient with left-sided megaureter (excellent visualization post-void in supine position). Right kidney: Normal tubular function with slight activity retention in the renal pelvis without pathological significance. The clearance index as shown, represents the average renal uptake (cps) between the 60th and 100th sec postinjection of both kidneys (used for split clearance determination) related to the injected dose, attenuation corrected (*54*), and adjusted for 1.73 m^2 body surface. This is of special significance for the intraindividual follow-up (e.g., pre- and postoperative) of infants and young children since quantitative clearance measurements are not possible for methodical reasons.

substances in the tubular cell must be involved." The latter could again be explained by different affinities to the tubular transport system, as has been shown for OIH and ^{99m}Tc-MAG_3 in the present paper.

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REFERENCES

1. Davison A, Sohn M, Orvig C, Jones AG, LaTegola MR. A tetradentate ligand designed specifically to coordinate technetium [Abstract]. *J Nucl Med* 1979; 20:641
2. Davison A, Jones A, Orvig C, Sohn M. A new class of oxotechnetium (5+) chelate complexes containing a $TcON_2S_2$ core. *Inorg Chem* 1981; 20:1629–1632
3. Fritzberg AR, Klingensmith WC III, Whitney WP, Kuni CC. Chemical and biological studies of Tc-99m N,N′-bis(mercaptoacetamido)ethylenediamine: a potential replacement for I-131-iodohippurate. *J Nucl Med* 1981; 22:258–263
4. Fritzberg AR, Kuni CC, Klingensmith WC III, Stevens J, Whitney WP. Synthesis and biological evaluation of Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate: A potential replacement for [^{131}I]o-iodohippurate. *J Nucl Med* 1982; 23:592–598
5. Fritzberg AR, Kasina S, Johnson DL, Eshima D. Triamide mercaptide (N_3S) ligands for Tc-99m as potential Tc-99m renal function agents [Abstract]. *J Nucl Med* 1985; 26:P19
6. Fritzberg AR, Kasina S, Eshima D, Johnson DL. Synthesis and biological evaluation of technetium-99m MAG_3 as a hippuran replacement. *J Nucl Med* 1986; 27:111–116
7. Klingensmith WC III, Gerhold JP, Fritzberg AR, et al. Clinical comparison of Tc-99m N,N′-bis(mercaptoacetamido)ethylenediamine and [^{131}I]ortho-iodohippurate for evaluation of renal tubular function: concise communication. *J Nucl Med* 1982; 23:377–380.
8. Klingensmith WC III, Fritzberg AR, Spitzer VM, et al. Clinical evaluation of Tc-99m N,N′-bis(mercaptoacetyl)-2,3-diaminopropanoate as a replacement for I-131 hippurate: concise communication. *J Nucl Med* 1984; 25:42–48.
9. Schneider RE, Subramanian G, Feld TA, et al. N,N′-bis(S-benzoylmercaptoacetamido) ethylenediamine and propylenediamine ligands as renal function imaging agents. I. Alternate synthetic methods. *J Nucl Med* 1984; 25:223–229.
10. Brandau W. *Physikalisch-chemische und biologische Eigenschaften von Technetium-99m-Amin-Komplexen.* Doctoral Dissertation, Medizinische Fakultät der Universität Heidelberg, 1985.
11. Taylor Jr A, Eshima D, Fritzberg AR, Christian PE, Kasina S. Comparison of Iodine-131 OIH and Technetium-99m MAG_3 renal imaging in volunteers. *J Nucl Med* 1986; 27:795–803.
12. Bubeck B, Brandau W, Dreikorn K, et al. Clinical comparison of I-131 o-iodohippurate with Tc-99m CO_2-DADS-A and Tc-99m MAG_3 by simultaneous double tracer measurement. *Nuc Compact* 1986; 17:135–138.
13. Taylor Jr A, Eshima D. Effects of altered physiologic states on clearance and biodistribution of technetium-99mMAG_3, iodine-131-OIH, and iodine-125-iothalamate. *J Nucl Med* 1988; 29:669–675.
14. Brandau W, Bubeck B, Eisenhut M, Taylor DM. Technetium-99m labeled renal function and imaging agents: III. Synthesis of ^{99m}Tc MAG_3 and biodistribution of by-products. *Int J Radiat Appl Instrum—Appl Radiat Isot* 1988; 39:121–129.
15. Brandau W, Bubeck B, Eisenhut M, Taylor DM. Biodistribution of complex impurities and steady state clearance of Tc-99m-MAG_3. In: Schmidt HAE, Csernay L, eds. *New trends and possibilities in Nuclear Medicine.* Stuttgart-New York: Schattauer; 1988:748–752.
16. Bubeck B, Brandau W, Eisenhut M, Weidenhammer K, Georgi P. The tubular extraction rate (TER) of Tc-99m MAG_3: a new quantitative parameter of renal function. *Nuc Compact* 1987; 18:260–267.
17. Bubeck B, Eisenhut M, Brandau W, Kälble T, Georgi P. Pharmacokinetics and metabolism of Tc-99m-MAG_3 [Abstract]. *J Nucl Med* 1988; 29:906.
18. Sinn HJ, Schrenk HH, Maier-Borst W. A new radioiodine exchange labeling technique. *Int J Radiat Appl Instrum—Appl Radiat Isot* 1986; 37:17–21.
19. Bratton AC, Marshall EK. A new coupling component for sulfanilamide determination. *J Biol Chem* 1939; 128:537–550.
20. Deetjen P, Sonnenberg H. Der tubuläre Transport von p-Aminohippursäure. Mikroperfusionsversuche am Einzelnephron der Rattenniere in situ. *Pflügers Arch ges Physiol* 1965; 285:35–44.
21. Sapirstein LA, Vidt DG, Mandel MJ, Hanusek G. Volumes of distribution and clearances of intravenously injected creatinine in the dog. *Am J Physiol* 1955; 181:330–336.
22. Blaufox MD, Potchen EJ, Merrill JP. Measurement of effective renal plasma flow in man by external counting methods. *J Nucl Med* 1967; 8:77–85.
23. Bubeck B, Brandau W, Reinbold F, et al. Technetium-99m labeled renal function and imaging agents: I. Clinical evaluation of ^{99m}Tc CO_2-DADS-A (^{99m}Tc N,N′-bis-(mercaptoacetyl)-2,3-diaminopropanoate). *Int J Radiat Appl Instrum—Nucl Med Biol* 1988; 15:99–108.
24. Maher FT, Tauxe WN. Renal clearance in man of pharmaceuticals containing radioactive iodine. Influence of plasma binding. *JAMA* 1969; 207:97–104.
25. Taylor Jr A, Eshima D, Christian PE, Milton W. Evaluation of Tc-99m mercaptoacetyltriglycine in patients with impaired renal function. *Radiology* 1987; 162:365–370.
26. Jafri RA, Britton KE, Nimmon CC, et al. Technetium-99m MAG_3, a comparison with iodine-123 and iodine-131 orthoiodohippurate, in patients with renal disorders. *J Nucl Med* 1988; 29:147–158.
27. Taylor Jr A, Eshima D, Fritzberg AR, Kasina S, Christian PE. Preliminary evaluation of ^{99m}Tc mercaptoacetyltriglycine as a replacement for ^{131}I OIH. In: Bischof-Delaloye A, Blaufox MD, eds. *Radionuclides in nephrology.* Basel: Karger; 1987:38–42.
28. Taylor Jr A, Eshima D, Christian PE, Wooten WW, Hansen L, McElvany K. Technetium-99m-MAG_3 kit formulation: preliminary results in normal volunteers and patients with renal failure. *J Nucl Med* 1988; 29:616–622.
29. Jong de R, Nielsen J, van Steenbergen J, Isaacs H, van den Brom W. Results of ERBF and ERPF measurements in healthy dogs with two new radiopharmaceutical principles. In: Bischof-Delaloye A, Blaufox MD, eds. *Radionuclides in nephrology.* Basel: Karger; 1987:49–52.
30. Burbank MK, Tauxe WN, Maher FT, Hunt JC. Evaluation of radioiodinated hippuran for the estimation of renal plasma flow. *Mayo Clin Proc* 1961; 36:372–386.
31. Kim CK, Stritzke P, Goldsmith SJ. Comparison of the clearance of technetium-99m MAG_3 and iodine-131 OIH [Letter]. *J Nucl Med* 1988; 29:1878–1879.
32. Lerso G, Delwaide PA, Lejeune G, Rorive G, Merchie G. The value of two simplified methods for the measurement of renal plasma flow by ^{131}I-hippuran. In: Blaufox MD, Funck-Brentano JL, eds. *Radionuclides in nephrology.* New York:

Grune & Stratton; 1972:241–247.
33. Hierholzer K. Remarks of the chairman. In: Lum Winkel K, Blaufox MD, Funck-Brentano JL, eds. *Radionuclides in nephrology*. Stuttgart: Georg Thieme; 1975:282–283.
34. Kuni H, Naber K, Pongratz W, Graul EH. Zwei systematische Fehler der "Ganzkörper"-Clearance bei abfallendem Blutspiegel und ihre gegenseitige Beeinflussung. In: Pabst HW, Oeff K, eds. *Verhandlungen d Ges f Nuclearmedizin 1972*. Berlin: Medico-Informationsdienste; 1975:276–280.
35. Jafri RA, Nimmon CC, Britton KE, et al. Tc-99m MAG_3, a clinical trial in nephrological disorders. In: Schmidt HAE, Csernay L, eds. *New trends and possibilities in nuclear medicine*. Stuttgart-New York: Schattauer; 1988:459–462.
36. Hör G, Kretschko J, Heidenreich P, Pabst HW, Kempken K, Schwarzendorfer A. Bestimmung der renalen Filtrationsfraktion durch simultane Doppelradionuklid-clearancetechnik. *Fortschr Röntgenstr* 1974; 120:322–330.
37. Bubeck B. *Technetium-99m-MAG_3 für die nuklearmedizinische Nierenfunktionsdiagnostik*. Pharmakokinetik und klinische Anwendung im Vergleich mit radiojodierter Hippursäure. Bern-Stuttgart-Toronto: Hans Huber; 1990.
38. Harth O, Kreienberg W, Lutz J. Der Einfluß von Hydrämie und Nierendurchblutung auf die renale Extraktion von p-Amino-hippursäure. *Pflügers Arch Physiol* 1959; 270:174.
39. Coveney JR, Robbins MS. Comparison of technetium-99m-MAG_3 kit with HPLC-purified technetium-99m-MAG_3 and OIH in rats. *J Nucl Med* 1987; 28:1881–1887.
40. Lee HB, Blaufox MD. Technetium-99m MAG-3 clearances after captopril in experimental renovascular hypertension. *J Nucl Med* 1989; 30:666–671.
41. Zum Winkel K. *Nierendiagnostik mit Radioisotopen*. Funktiionelle und morphologische Ergebnisse in Experiment und Klinik. Glauner L, ed. Stuttgart: Georg Thieme; 1964.
42. Bubeck B, Brandau W, Steinbächer M, et al. Technetium-99m labeled renal function and imaging agents: II. Clinical evaluation of ^{99m}Tc-MAG_3 (^{99m}Tc mercaptoacetylglycylglycylglycine). *Int J Radiat Appl Instrum—Nucl Med Biol* 1988; 15:109–118.
43. Blaufox MD. The current status of renal radiopharmaceuticals. In: Bischof-Delaloye A, Blaufox MD, eds. *Radionuclides in nephrology*. Basel: Karger; 1987:31–37.
44. Brandau W, Bubeck B, Schober O, Weber E, Taylor DM. ^{99m}Tc-MAG_3: chemistry and biokinetics of by-products. In: Blaufox MD, Hollenberg NK, Raynaud C, eds. *Radionuclides in nephro-urology*. Basel: Karger; 1990:11–16.
45. Müller-Suur R, Müller-Suur C. Renal and extrarenal handling of a new imaging compound (99m-Tc-MAG-3) in the rat. *Eur J Nucl Med* 1986; 12:438–442.
46. Tauxe WN, Maher FT, Taylor WF. Effective renal plasma flow: Estimation from theoretical volumes of distribution of intravenously injected ^{131}I orthoiodohippurate. *Mayo Clin Proc* 1971; 46:524–531.
47. Tauxe WN, Dubovsky EV, Kidd Jr T, Diaz F, Smith LR. New formulas for the calculation of effective renal plasma flow. *Eur J Nucl Med* 1982; 7:51–54.
48. Bubeck B, Brandau W, Weber E, Pomer S, Georgi P. Renal function studies using Tc-99m-MAG_3: pharmacokinetics and slope clearance determination. In: Blaufox MD, Hollenberg NK, Raynaud C, eds. *Radionuclides in nephro-urology*. Basel: Karger; 1990:72–73.
49. Russell CD, Taylor A, Eshima D. Estimation of technetium-99m-MAG_3 plasma clearance in adults from one or two blood samples. *J Nucl Med* 1989; 30:1955–1959.
50. Bubeck B. Radiation exposure by technetium-99m-MAG_3 [Letter]. *J Nucl Med* 1989; 30:720–721.
51. Brandau W, Bubeck B, Berger J, Georgi P. Excretion characteristics of Tc-99m-MAG_3 in patients. In: Schmidt HAE, Buraggi GL, eds. *Trends and possibilities in nuclear medicine*. Stuttgart-New York: Schattauer; 1989:409–412.
52. Müller-Suur R, Müller-Suur C. Glomerular filtration and tubular secretion of MAG-3 in the rat kidney. *J Nucl Med* 1989; 30:1986–1991.
53. Ochwadt BK, Pitts RF. Disparity between phenol red and diodrast clearances in the dog. *Am J Physiol* 1956; 187:318–322.
54. Gates GF. Glomerular Filtration Rate: Estimation from fractional renal accumulation of Tc-99m-DTPA (stannous). *Am J Roentgenol* 1982; 138:565–570.