Comparison of Renal Extraction Efficiencies for Radioactive Agents in the Normal Dog

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The renal extraction efficiencies for various radioactive agents were measured in normal anesthetized dogs during 1 hr after a single intravenous injection. Radioassays were made on serial blood samples drawn simultaneously from the aorta upstream from the renal arteries and from one renal vein. As a reference substance [^{131}I]*o*-iodohippurate was injected concurrently in all experiments. Blood clearances from serial venous samples and urinary excretion also were measured. Extraction efficiency from whole blood was calculated as (A − V) ÷ A, where A = aortic concentration and V = renal venous concentration. This ratio for commercial [^{131}I]*o*-iodohippurate fell steadily from 88% at 30 sec to 50% at 1 hr. For "purified" [^{131}I]*o*-iodohippurate the fall was less marked, to 61% at 1 hr. The EE ratios for all other agents were stable after the first minute. The Tc-99m complexes of DTPA, glucoheptonate, and acetylcysteine had ratios averaging 27–29%. The ratios of Tc-99m DMS and Hg-197 chlormerodrin had much lower average values of 8 and 14%, respectively. None of the newer agents approached the extraction efficiency of [^{131}I]*o*-iodohippurate.

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In the investigation of radioactive agents for the detection of renal disease or for evaluation of renal function, it is customary to measure and compare their relative blood and plasma clearances, urinary excretion, and organ distribution at various time intervals after intravenous injection. Renal clearance is also calculated under steady-state conditions from the total quantity excreted in the urine in a given time, divided by the average plasma level. Renal clearance, however, is not applicable to substances that are stored, synthesized, or metabolized by the kidney (*1*). This would include several radioactive agents that accumulate in the renal parenchyma.

On the other hand, the renal extraction efficiency (EE) is applicable to any agent and is widely used in the assessment of stable drugs. The "plasma extraction ratio" as defined by Smith (*2*) is that fraction of any substance removed from the plasma during a single circulation through the kidney and is expressed by (A − V)/A, where A is the arterial or systemic venous concentration and V is the renal venous concentration. At high urine flow rates, however, this expression must be modified for substances with a low EE (*3*). In this study, the EEs for various radioactive agents were measured, since no prior data were available except for [^{131}I]orthoiodohippurate (Hippuran). Mongrel dogs were used because renal venous catheterization of normal human subjects for this purpose did not appear justifiable.

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MATERIALS AND METHODS

Adult male mongrel dogs weighing 17–33 kg were anesthetized with 30–35 mg/kg of sodium pentobarbital intravenously. Arterial and venous catheters were placed by the percutaneous transfemoral Seldinger technique. The tip of the aortic catheter was positioned above the

TABLE 1. % EXTRACTION EFFICIENCY (EE) OF RADIOACTIVE AGENTS FROM THE BLOOD IN DOGS*

		0–30″	1′0″	1′30″	2′0″	2′30″	3′0″	3′30″	4′0″	4′30″	5′0″	10′	15′	20′	30′	45′	60′	Average EE 1′–60″
DTPA	x̄	34.4	25.7	29.1	28.9	31.3	28.8	28.3	30.7	28.6	28.8	28.8	28.5	27.6	24.7	25.1	25.3	26.7
AC	x̄	39.6	24.8	27.7	26.9	23.9	27.2	27.7	26.7	30.5	26.9	28.9	25.5	26.9	27.9	35.4	22.2	27.5
GHA	x̄	35.5	21.2	26.1	26.6	25.6	25.3	25.7	24.1	23.6	25.5	27.3	27.7	27.0	30.8	31.4	32.5	29.1
Hg-197 chlormerodrin	x̄	20.4	12.3	17.0	14.9	16.7	17.1	16.5	15.9	13.4	15.3	14.8	12.6	15.9	13.8	12.3	11.8	13.6
DMSA	x̄	7.6	11.5	9.8	11.8	9.4	10.1	12.8	10.5	6.2	9.5	10.2	9.3	6.7	7.0	5.3	6.8	7.7
MDP	x̄	26.2	26.4	27.4	27.3	28.0	29.1	28.4	29.0	29.4	28.8	29.6	29.5	32.1	31.3	32.3	30.4	30.4
PPi	x̄	13.8	27.6	24.0	24.2	24.5	22.6	24.5	25.7	24.6	25.6	25.8	26.6	31.0	27.0	24.1	21.5	24.9
HEDP	x̄	21.8	23.7	24.0	24.6	24.0	22.8	22.4	27.1	24.2	24.0	25.4	25.9	23.8	25.2	23.4	24.7	24.3
[131]I-o-hippurate	x̄	88.2	85.7	83.8	81.5	80.1	78.7	77.3	75.4	74.1	73.0	64.6	59.7	57.9	54.7	53.7	49.7	60.6
	s.d.	4.55	3.49	4.12	3.77	4.03	3.66	4.30	4.42	4.73	4.22	5.85	6.43	5.48	5.70	5.89	8.63	4.8

* Mean values, three animals in each group, except 33 animals for [131]I-o-iodohippurate

renal arteries. The tip of the renal venous catheter was placed 3 cm distal to its vena-caval orifice. The radiopharmaceuticals were injected intravenously through a 21 g × 1 in. butterfly. Peripheral venous blood samples were drawn through a 21 g × 1 in. butterfly placed in the contralateral forelimb. The "dead space" in the catheters was eliminated immediately before sampling by withdrawing and discarding 2 ml of blood. Heparinized blood samples were drawn simultaneously from the renal-vein and aortic catheters at 30-sec intervals for the first 5 min after injection, then at 10, 15, 20, 30, 45, and 60 min. Peripheral venous samples were collected at 2, 5, 10, 15, 20, 30, and 60 min. The urinary bladder was catheterized and emptied at 15-min intervals up to 1 hr. As recommended by Phillips et al. (*4*), 100 ml of physiological saline were instilled into the bladder just before each collection to recover all intravesical radioactivity. One-milliliter blood samples were pipetted for well counting. The EE was calculated as (aortic blood net cpm − renal venous blood net cpm) × 100/(aortic blood net CPM). Microhematocrits were routinely obtained for aortic, renal venous, and peripheral venous blood. These ratios were calculated for whole blood, and in some experiments the EE from plasma also was determined.

Seventy to 90 μCi of [131I]*o*-iodohippurate* were injected, together with 700–900 μCi of one of the Tc-99m complexes or 350–400 μCi of Hg-197 chlormerodrin. The commercial [131I]*o*-iodohippurate always contained less than 5% free radioiodide, as determined by paper chromatography. For comparison, radiochemically pure [131I]*o*-iodohippurate was prepared from 0.5-ml commercial samples by HPLC.† This radioiodide-free material was used immediately after purification. The Tc-99m complexes of DTPA,‡ dimercaptosuccinic acid (DMSA),‖ pyrophosphate (PPi),§ and ethane-1-hydroxy-1, 1-diphosphonate (HEDP)¶ were obtained commercially, and glucoheptonate (GHA) and methylene diphosphonate (MDP) were prepared locally. The Tc-99m complex of acetylcysteine (AC) was also evaluated because it has recently been tried as a renal imaging agent (*5*). Each radioactive agent was studied in at least three experiments.

The EEs of each of the radioactive agents at various time intervals up to 1 hr are listed in Table 1 and shown graphically in Fig. 1. In 33 experiments with commercial [131I]*o*-iodohippurate, the mean blood extraction ratio was 88% in the first 30 sec after injection, but fell progressively to a mean level of 50% at 1 hr. The mean blood EE, from 1 min to 1 hr, was 61%. In 22 experiments in which the right renal vein was catheterized, the mean blood EE was the same, (60.6 ± 4.8)%, as the mean values in 11 experiments that used the left renal vein, 60.9 ± 5.0)%. The fraction of the total blood radioactivity that diffused into the red-cell fraction remained constant during the first hour, averaging 16%. Samples of aortic and renal venous blood from the same animal nearly always had an identical hematocrit. The mean hematocrit for aortic and renal venous blood was 45%,

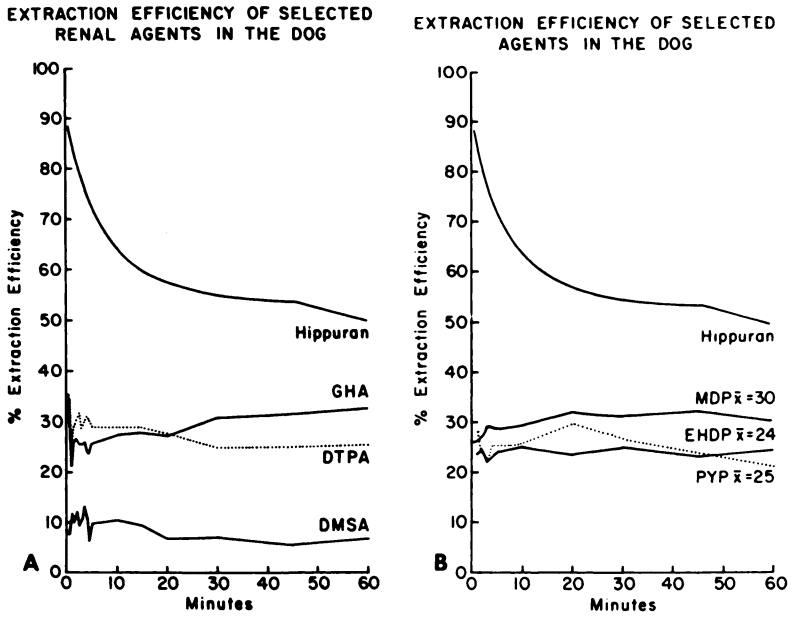

FIG. 1. Renal extraction efficiency for removal of selected agents from blood in a dog during first hour after single intravenous injection: (A) renal agents, (B) skeletal agents and *o*-iodohippurate.

slightly higher than the mean peripheral venous hematocrit of 42%.

Figure 2 shows the mean EE in three experiments with [^{131}I]*o*-iodohippurate immediately after purification by HPLC. This preparation did not contain the well-known impurities, orthoiodo-benzoic acid or free radioiodide. The decline in EE from the blood is less marked than that of commercial [^{131}I]*o*-iodohippurate (Fig. 1), reaching a level of 61% at 1 hr. Compared with whole blood, the EE from plasma was higher, but fell to 79% from an initial level of 90%. For the "purified" [^{131}I]*o*-iodohippurate the mean EE from whole blood during the first hour was 65%, and from plasma, 82%. This decline in EE of purified *o*-iodohippurate from whole blood could be quantified by the expression $0.25e^{-0.14t} + 0.64e^{-0.00074t}$, with a coefficient of correlation (r) of 0.99, and from plasma, by the expression $0.05e^{-0.18t} + 0.85e^{-0.0013t}$ (r = 0.99).

Unlike the falling ratios observed with [^{131}I]*o*-iodohippurate, the EEs of all other agents studied did not change significantly after the first minute. The ratios for the Tc-99m complexes of DTPA, GHA, and AC were similar, averaging 27-29%. The average EE of Tc-99m MDP was 30%, whereas the HEDP and PPi complexes averaged 24-25%. Tc-99m DMS and Hg-197 chlormerodrin had much lower average values of 8 and 14%, respectively. For several agents—including DTPA, AC, GHA, and Hg-197 chlormerodrin—the EE was much higher during the first 30-sec interval after injection than during the remainder of the 1-hr period of study. There was poor correlation between EE and the previously reported cumulative concentrations of the various agents in the renal parenchyma (*6*). There was, however, a high positive linear correlation (r = 0.94) between EE and the sum of the urinary excretion and the cumulative concentration in the renal parenchyma (Table 2).

The whole-blood clearances of the different agents are illustrated in Fig. 3. After the initial rapid loss of radioactivity from the bloodstream within the first 5 min or so, the blood levels for the different radioactive agents during the first hour are inversely related to EE. [^{131}I]*o*-iodohippurate has the highest EE and the fastest blood clearance, whereas Tc-99m DMSA has the lowest EE and slowest blood clearance, probably because it is more tightly bound to plasma proteins than are other Tc-99m agents. During the first hour about 75% of blood activity is protein-bound (*6*). Despite its low rate of extraction by the kidney, however, Tc-99m DMSA pro-

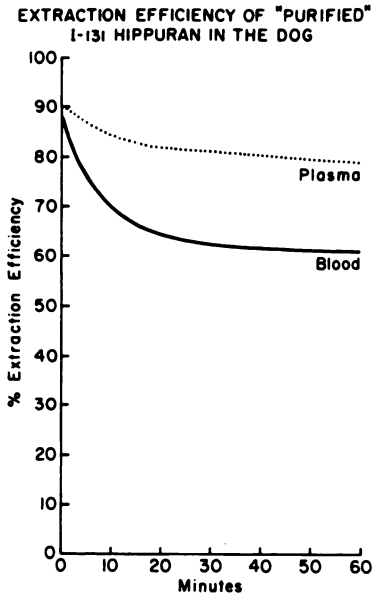

FIG. 2. Renal extraction efficiency (in dog) for removal of purified [^{131}I]*o*-iodohippurate from blood and plasma. Decline in EE is less marked for both plasma and blood than for "unpurified" [^{131}I]*o*-iodohippurate (Fig. 1).

TABLE 2. ONE-HOUR DISTRIBUTION IN DOGS

Agent	% Administered radioactivity: Urine	% Administered radioactivity: Sum of kidneys and urine
Tc-99m DTPA	43.3	45
Tc-99m GHA	34.2	44
Tc-99m AC	23.5	31
Tc-99m DMSA	6.1	22
Hg-197 chlormerodrin	6.5	32
Tc-99m MDP	30.5	—
Tc-99m HEDP	24.7	—
Tc-99m PPi	29.7	—
[^{131}I]*o*-iodohippurate	53.7 ± 12.5	54
[^{131}I]*o*-iodohippurate, "purified"	66.9 ± 3.10	67

gressively accumulates in the cortex, reaching cortical concentrations higher than those of other Tc-99m agents (*7*). Tc-99m MDP has a fast blood clearance (second only to [^{131}I]*o*-iodohippurate), and its EE is somewhat higher than for the HEDP or PPi complexes. Only about 30% of the plasma activities of the MDP and HEDP complexes are associated with plasma proteins, and about 50% of the PPi complex is protein-bound at 1 hr (*8*).

Although the GHA, AC, and DTPA complexes have similar blood clearance curves and similar EEs, their handling by the kidney differs. The DTPA complex is cleared into the urine by glomerular filtration, with only minimal cortical retention. In contrast, each kidney retains about 5% of the administered activity (*6*) of GHA intracellularly in the cortical tubular cells by 1 hr. During the first hour, only about 4% of the DTPA complexes is associated with plasma proteins (*9*), as opposed to 47–54% for the GHA complex (*6*). The renal handling of the AC complex is apparently similar to that of the GHA complex. Although Hg-197 chlormerodrin is entirely bound to protein in plasma, its blood clearance is considerably faster than that of Tc-99m DMSA, in keeping with its higher EE.

The cumulative urinary excretion for the various radioactive agents during the first hour after injection is listed in Table 2. The excretion is greatest for [^{131}I]*o*-iodohippurate and higher for Tc-99m DTPA than for the other technetium agents. The excretion of the complex of AC is lower than that of Tc-99m GHA because its volume of distribution in extrarenal tissues is relatively higher. The urinary excretions of Tc-99m DMSA and Hg-197 chlormerodrin are relatively low in contrast to their high retention in the renal cortex (*6*).

DISCUSSION

In the present study the EE was measured after single intravenous injection of the agents without added carrier, rather than during continuous infusion with added carrier, because most clinical studies are now performed by the single-injection technique. The EEs of the Tc-99m agents were measured in whole-blood samples rather than plasma. For such Tc-99m complexes as GHA, DTPA, DMSA, MDP, and HEDP, which do not diffuse into the red cells, the blood and plasma extraction ratios

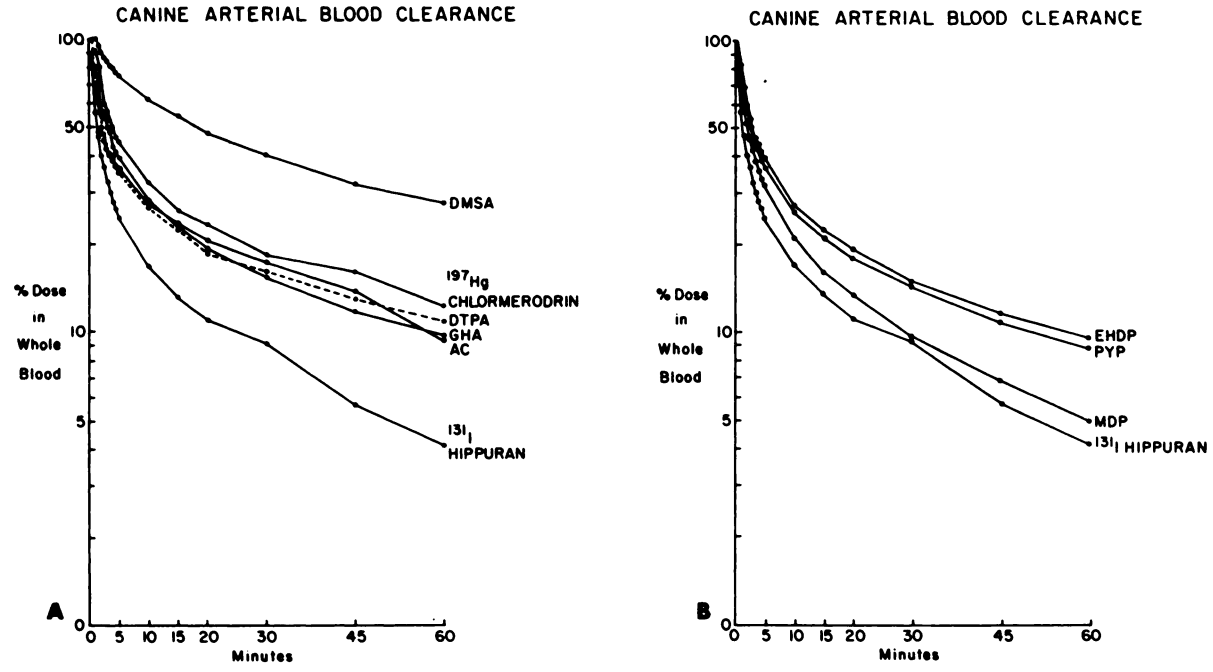

FIG. 3. (A, B) Clearance of renal and skeletal agents from canine arterial blood during first hour after single intravenous injection.

are identical because the hematocrit for arterial and renal venous blood are the same. For Tc-99m PPi, the diffusion into the red-cell fraction is about 10% (*8*).

For [^{131}I]*o*-iodohippurate, a significant fraction (16%) of the whole-blood activity entered the red cells but was similar in arterial and venous blood. The average EE of plasma activity was higher than that of whole blood. Back-diffusion of red-cell activity, however, may cause errors in the measurement of plasma activity, even when the blood is spun immediately. According to Phillips et al. (*4*), the renal transit time is too short for significant back-diffusion in vivo from red cells to plasma. For continuous infusions of PAH, the EE from plasma in dogs was about 5% too low (*4*), because back-diffusion occurred in vitro as the blood specimens were being centrifuged to separate the plasma fraction. As much as 50% of the blood concentration of PAH diffused in vivo into the red-cell fraction in dogs, but no diffusion occurred in man (*2*). For continuous infusions of carrier *o*-iodohippurate, the red-cell fraction contained about 40% in the dog and 50% in man (*2*), but only 30% for [^{131}I]*o*-iodohippurate without carrier (*10*).

According to Smith (*2*), EEs in both dog and man may be lower on the left side than the right because the left-renal venous blood is partially diluted with effluent from the spermatic or ovarian veins. In this study, however, there was no difference in EE of [^{131}I]*o*-iodohippurate between samples from the left or right renal veins.

The EEs obtained in this study apply only to normal kidneys in anesthetized dogs. The ratios are known to be markedly altered in disease states (*2*). The average ratios from blood—61% for a single injection of commercial [^{131}I]*o*-iodohippurate and 65% for "purified Hippuran"—were lower than anticipated. The average ratio for continuous infusions of para-aminohippurate (PAH) was 91% in man and somewhat lower in the dog (*2*). Phillips et al. (*4*) obtained an average value of 87% for PAH in explanted kidneys in anesthetized dogs, but the validity of results in explanted kidneys has been questioned (*2*). With the kidneys in situ, Valesquez et al. (*11*) obtained PAH extraction ratios of only 61-66%. These workers found that EE was markedly lowered by saline infusions or hyperosmolar infusions that produced vasodilatation and shortening of the renal transit time. In simultaneous continuous infusion in dogs, Mailloux and Gagnon (*12*) obtained a ratio of 77% for PAH and 67% for [^{131}I]*o*-iodohippurate containing less than 2% free I-131. In normal man, also, the EE from plasma for *o*-iodohippurate is lower than for PAH. For example, Maher et al. (*13*), using continuous infusion, obtained values of 88% for PAH and 73% for [^{131}I]*o*-iodohippurate with added carrier, and 80% and 66% without added carrier. Ultrafiltrates of plasma yielded values identical to those of plasma; hence, plasma-protein binding did not appear to lower the EE significantly.

Although only a few direct measurements of EE for [^{131}I]*o*-iodohippurate have been reported, numerous comparisons of its clearance relative to PAH have been made. In a review (*12*), the clearance of [^{131}I]*o*-iodohippurate varied in different reports from 71 to 97% of the PAH clearance in dogs, averaging about 87%. From this review, it may be inferred that the EE of [^{131}I]*o*-iodohippurate in the dog and in man is consistently less than that of PAH.

An important factor in the decline in EE of commercial [^{131}I]*o*-iodohippurate is free radioiodide in the preparation. Although initially this represents only a small fraction of the total activity, during the first hour most of the *o*-iodohippurate-bound activity is cleared by the kidneys, leaving an increasingly large fraction of remaining radioiodide activity to be slowly extracted by the kidneys (*14*) and thyroid. The decline was reduced but not prevented by eliminating radioiodide from the radiopharmaceutical. Other minor factors that may contribute to the fall in EE include the absence of carrier *o*-iodohippurate, protein binding, and a species difference between canine and human kidneys. The relatively low EE from plasma of the purified *o*-iodohippurate (82%) tends to discourage its use by single-injection technique for estimation of effective renal plasma flow. Moreover, renal physiologists have previously criticized its use for the reliable assessment of effective renal plasma flow because of the lack of equilibration between plasma and extravascular fluid activity (*15*).

The average EE for the DTPA complex from 1-60 min after a single injection was 27%. This is close to the value of 24% given by Shipley et al. (*16*) for continuous infusions of inulin in dogs. This, together with the evidence that DTPA is minimally accumulated in the renal cortex, again suggests that this complex is excreted purely by glomerular filtration. The "filtration fraction" is defined as the glomerular filtration rate (GFR) divided by the renal plasma flow. It has been measured by some workers (*16*) as the GFR ÷ (renal blood flow obtained with a flowmeter × plasmacrit). The EE of a glomerular agent and the filtration fraction are then equivalent. On the other hand, the filtration fraction has also been measured as the EE of inulin ÷ the EE of PAH (*2*). With the present data, if DTPA were considered a glomerular agent like inulin, and [^{131}I]*o*-iodohippurate a substitute for PAH, the average filtration fraction would be falsely high because the EE of [^{131}I]*o*-iodohippurate was so low that it did not reflect effective renal plasma flow.

None of the Tc-99m complexes included in the present report have an EE approaching that of [^{131}I]*o*-iodohippurate. Nonetheless, their ratios must be considerably greater than that of pertechnetate. The latter has not been directly measured. In one report (*17*), however, the renal clearance of pertechnetate in man is only 13.5% of the inulin clearance. Since the former is not accumulated in the kidney, we can assume that it undergoes consid-

erable tubular resorption and that its EE is only about 2.7%.

For several renal agents—including the Tc-99m complexes of DTPA, GHA, AC and Hg-197 chlormerodrin—the EE during the first 30-sec interval was much higher than at subsequent times. The first-transit EE has been measured in dogs both by direct methods and by dual-tracer techniques in which one of the tracers remains intravascular (*18*). Crone (*19*) obtained a first-transit extraction ratio as high as 73% for inulin and estimated that 20% was due to glomerular filtration and ~50% due to immediate diffusion through the peritubular capillaries, presumably into the interstitial spaces. This high extraction fell within a few seconds. Chinard (*18*) likewise obtained an average initial EE of 69% for inulin and 96% for PAH. Weich et al. (*20*) obtained a value of 48% for Hg-203 chlormerodrin and 81% for Tl-201. The high ratios during the first 30 sec in the current study further support the concept that the initial rapid loss of tracer in the kidney may be due to peritubular capillary diffusion, since it occurs for both "glomerular" and "tubular" agents.

FOOTNOTES

* E. R. Squibb & Sons, Inc , Princeton, NJ.

† Spectra-Physics model 3500 B HPLC with R Sil-C-18-HL column (25 cm × 4.6 mm), Altech Assoc, Arlington Heights, IL. Solvent 4 parts methanol to 6 parts 1% acetic acid, flow rate 1 ml/min.

‡ Diagnostic Isotopes, Inc., Bloomfield, NJ.

‖ Medi-Physics, Inc., Emeryville, CA.

§ Mallinckrodt, Inc., St. Louis, MO.

¶ Union Carbide Corp., Medical Products Division, Rye, NY.

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