Detection of Diffuse Glomerular Lesions in Rats: I. Comparisons of Conventional Radioactive Agents

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Conventional renal diagnostic agents, [^{131}I]hippuran, [^{99m}Tc]glucoheptonate (GHA), and [^{99m}Tc] dimercaptosuccinate (DMS) were compared with [^{99m}Tc] or [^{111}In] diethylenetriaminepentaacetic (DTPA) for the detection of glomerular damage in rats compared with controls. The glomerular lesions were induced by the i.v. injection of puromycin aminonucleoside (PA) 9 days before the radionuclide studies, a model of spontaneous "minimal change" glomerulonephritis in humans. Computer-generated early renal uptake of [^{99m}Tc]DTPA or GHA correlated with the glomerular filtration rate (GFR) quantitated by biexponential plasma clearance of DTPA administered by single i.v. injection. The early renal uptake of hippuran and DMS correlated poorly with GFR as assessed by DTPA clearance. However, the 2-hr renal retention of DMS correlated well with the DTPA clearance. None of the parameters measured with [^{131}I]hippuran correlated well with DTPA clearance, probably because of decreased protein plasma binding of hippuran secondary to hypoproteinemia in this experimental model. It was concluded that none of these agents was superior to labeled DTPA for the detection of glomerular damage in this experimental model.

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End-stage renal disease in the USA results in one death per 10,000 population each year; one per 4,000 population is dependent on renal dialysis for survival, and approximately one in 50,000 has a renal transplant. Glomerulonephritis remains the commonest cause of renal failure (42% of the total), compared with only 11% for hypertensive renal disease and 7% for diabetic nephropathy. Because of the poor correlation between clinical and laboratory findings with the glomerular pathology, initial biopsy is often required for accurate classification (*1*). An increasing number of treatment regimes for different lesions now includes not only steroids and diuretics but cytotoxic drugs, anticoagulants, anti-platelet agents, and angiotensin-converting enzyme inhibitors. Hence, defining the type and extent of glomerular lesions is becoming more important.

The time-honored method for following the course of glomerular disease is the creatinine clearance. However, only about half the patients with biopsy-proven glomerular disease exhibit a significant decrease in creatinine, inulin, or PAH clearance (*2*), and these measurements correlate well only with interstitial and vascular involvement. The glomerular filtration rate (GFR) may be maintained by hyperfiltration of the surviving functional glomeruli through efferent arteriolar vasoconstriction and increased intraglomerular pressure, leading eventually to progressive glomerular damage (*3*). Gates (*4,5*) and others (*6*) have developed a gamma camera-computed method for quantitating total and individual renal function from the initial renal uptake 2 and 3 min after injection of radioactive renal agents. Using [^{99m}Tc]DTPA, for example, the GFR could be estimated by linear regression with the creatinine clearance. In this report, we have compared four conventional renal agents for the detection of diffuse glomerular disease using a rat model. They have not been systematically tested previously for this application, to our knowledge. The purpose of this study was to ensure that none of the widely used renal agents was better than diethylenetriaminepentaacetic acid (DTPA) for glomerular disease before embarking on the development of newer agents.

Puromycin aminonucleoside (PA) has been used ex-

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tensively to produce transient or permanent glomerular damage in rats because the induced changes closely mimic those of spontaneous minimal change glomerulonephritis (nephrotic syndrome) common in childhood. It is often administered intravenously in doses of 10 mg/100 g body weight (*7*), producing proteinuria in Sprague-Dawley rats in ~5 days. Repeated doses combined with unilateral nephrectomy produce focal glomerulosclerosis (*8*). Typically, with single doses, the glomeruli appear normal by light microscopy. By electron microscopy (*9*), there is fusion of the foot processes of the epithelial cells and detachment from the glomerular basement membrane, with disappearance of the slit pores and diaphragms. Later changes in the proximal tubules are secondary to continued albuminuria, including loss of brush border, dilated lumina, thin walls, and dense luminal casts. Functionally, PA produces only a minimal reduction in glomerular plasma flow rate, no change in effective pore radius or in the number of pores. Proteinuria is due to the dimunition of the electrostatic barrier function of the glomerular membrane (*10*). PA removes the anionic sialic acid coat covering the surface of glomerular epithelial cells, slit pores, and diaphragms (*1*), leaving the heparan sulphate-proteoglycan anionic sites intact (*11*). In the spontaneous human disease, similar membrane damage is probably produced by lymphokines secreted by extrarenal activated T suppressor cells (*1*).

MATERIALS AND METHODS

Commercial iodine-131 (^{131}I) hippuran could not be used because its low radioactive concentration resulted in excessive injection volumes. Sodium orthoiodohippurate (OIH, hippuran) was purified by high performance liquid chromatography (HPLC) and labeled with [^{131}I]sodium iodide by exchange iodination. Ten milligrams of OIH in a serum vial were dissolved in 500 μl of 0.2*M* sodium acetate buffer (pH 4.0) and mixed with 2 mg $CuSO_4 \cdot 5H_2O$ in 100 μl of water. Three to 5 mCi of [^{131}I]sodium iodide* (40–200 mCi/ml) in a 200 μl volume were added and the vial was autoclaved at 15 psig for 15 min. It was cooled to room temperature and 2 ml of 0.2*M* sodium phosphate buffer (pH 7.5) were added to precipitate the copper as copper phosphate. The radioactive mixture was then passed through a 0.2-μ Gelman Acrodisc membrane filter. The filtrate containing labeled hippuran was collected and assayed. Its purity was >99%, as determined by HPLC using a Spherisorb-5 ODS-2 column (0.46 cm × 27 cm) and methanol and 1% acetic acid in water (65:35) as elutant. At a flow rate of 1 ml/min, [^{131}I]OIH had a retention time of 3.5 min. Iodine-131 OIH was diluted with pH 7.5, 0.2*M* sodium phosphate buffer and stored in a refrigerator.

Commercial [^{111}In]DTPA* and kits of dimercaptosuccinic acid (DMS)† were used. The quality of technetium-99m dimercaptosuccinate ([^{99m}Tc]DMS) was determined by ITLC using Gelman ITLC-SA medium and butanol saturated with 0.3*M* HCl as the solvent. In this system, [^{99m}Tc]DMS had an R_f value of 0.5 while pertechnetate migrated to the solvent front. Technetium-99m glucoheptonate (GHA) and [^{99m}Tc]DTPA were prepared from freeze-dried kits made in our laboratory. The GHA kit contained 200 mg GHA and 100 μg $SnCl_2 \cdot 2H_2O$ at pH 7.2. The DTPA kit contained 10 mg of DTPA and 400 μg $SnCl_2 \cdot 2H_2O$ at pH 6.8. Required activities (1–10 mCi) of [^{99m}Tc]pertechnetate were added to the kits and mixed well. The quality of labeled products was ascertained by ITLC. The free pertechnetate was determined with Whatman 31-ET paper and acetone as the solvent. In this system, the labeled product stayed at the origin and free pertechnetate moved with the solvent front. Any colloids or reduced unbound ^{99m}Tc in the labeled product were determined with Gelman ITLC-SG strips and isotonic saline as the solvent. The impurities stayed at the origin and the labeled product (with free pertechnetate, if any) moved with the solvent. The purity of all three ^{99m}Tc complexes was >97%.

Male Sprague-Dawley rats, acquired at a weight of ~200 g were studied in groups of six, three controls and three with glomerular damage induced by a 2% solution of puromycin aminonucleoside‡ (PA) injected intravenously 9 days previously. Initially, experiments were performed on 60 animals with [^{99m}Tc]DTPA with varying doses of PA (0, 2.5, 5, and 10 mg/100 g body weight) to determine whether or not the reduction in estimated GFR was reproducibile and/or dose dependent. In subsequent experiments, each agent was compared with small doses of [^{99m}Tc] or [^{111}In]DTPA in 12 control and 12 PA treated animals (5 mg/100 g). To obtain data on this number, up to 30 animals had to be injected to obtain 24 in each group because of instances of death or excessive renal damage.

The rats were anesthetized with pentabarbital 0.1 ml/100 g intraperitoneally. The tail was washed and the animals weighed. In 25% of the animals, the urethra was tied off to obtain the cumulative 2-hr urine sample. The early renal uptake of the radioactive agent under study was quantiated by modifications of the Gates technique using a small field-of-view camera§ facing upwards, with parallel hole collimators and a DEC 11/34 computer. The injection syringe (containing 0.3 ml) was counted for 1 min in a stainless steel boat lined with 1/16″ of lead (transmission factots 0.0178 for ^{99m}Tc, and 0.333 for ^{111}In) to avoid count losses at high counting rates. This lead attenuation was not needed for [^{131}I] hippuran because a less sensitive, high-energy collimator was used. The tail was warmed and the radioactive agent (1.2 mCi of [^{99m}Tc]GHA or DMS, 90 μCi of [^{111}In]DTPA or 180 μCi of [^{131}I]Hippuran) was injected into a vein, followed immediately by the "standard"

agent, 20 μCi of ^{111}In or [^{99m}Tc] DTPA in 0.2 ml. Counts were acquired in a 64 × 64 matrix at one frame every 15 sec for 3 min. At 3 min, the tail was counted for residual activity and the postinjection syringe counted for 1 min. With the tail constantly warmed, 0.2-ml heparinized blood samples were taken from a tail vein at 5, 10, 20, 30, 40, 60, 80, and 100 min after injection. At 2 hr, a 2-ml blood sample was taken from the inferior vena cava and the animal killed. The blood samples were transferred to microcapillary tubes and centrifuged for counting weighed plasma samples. The plasma clearances of the agent under study and reference DTPA were calculated by computer, using either the double exponential method (*12,13*) or the stimulated continuous infusion method (*14*). For hippuran, the clearance was calculated from plasma samples taken up to 1 hr.

After death, the depth of the centroid of the two kidneys was measured in vivo by direct needle puncture. The renal uptakes were corrected for depth using linear attenuation coefficients in tissue of 0.153, 0.140, and 0.116 cm^{-1} for ^{99m}Tc, ^{111}In, and ^{131}I, respectively. These uptakes were analyzed by computer for the interval from 0.5 to 1.5 min after injection. For [^{99m}Tc] DMS, uptakes were calculated also for the interval from 2.0 to 3.0 min.

The kidneys were removed and weighed. The liver was removed, weighed, and three representative samples were weighed. The bladder and its urinary contents were removed in toto, placed in a preweighed counting vial and reweighed. All samples were radioassayed in a LKB well scintillation spectrometer together with appropriate dilute standards, and Compton corrections made. The data for the two radionuclides were expressed as a percent of the administered activity per organ. The blood volume was assumed to be 6% of animal weight (*15*) and the plasma volume calculated from this value and the plasmacrit.

Because of the anomalous increase in clearance of hippuran encountered in PA compared with control rats, the studies described above were repeated with 2.5 and 3.75 mg/100 g doses of PA. In another group of ten rats (five given 5 mg/100 g PA and five controls), the above studies were repeated, but modified. To obtain an independent indication of change in renal blood flow, just before the 2-hr killing, 5 μCi (0.2 ml) of rubidium-86 (^{86}Rb) chloride* were injected through the right femoral vein, and exactly 30 sec later, the rat was killed by 1 ml of 36% KCl intravenously. The fraction of the cardiac output to the kidneys and liver, renal blood and plasma flows were calculated, according to modifications of Sapirstein's method (*16,17*). In another group of 14 rats with and without PA treatment, clearances performed with 1–1.5 mg added carrier hippuran were compared to those with no added carrier [^{131}I]hippuran. This experiment was done to explore the possibility that the measured clearances of hippuran in the normal rat could be improved by decreasing its binding to plasma proteins with added carrier, without exceeding the maximum tubular transport in rats.

RESULTS

Among the 60 rats studied with [^{99m}Tc]DTPA, the mean clearance in the control group was 0.88 ± 0.25 (s.d.) ml/min/100 g. Progressively increasing doses of puromycin aminonucleoside (PA) administered 9 days previously produced a progressive decrease in both plasma clearance and renal uptake quantitated by computer 0.5 to 1.5 min after injection (Fig. 1A). This initial uptake, measured before radioactivity appeared in the pelvocalyceal collecting system correlated well with the plasma clearance (Fig. 1B, Table 1). The differences between controls and rats given the smallest dose of 2.5 mg PA/100 g, however, were slight, and there was considerable overlap in individual values between these two groups. The 2-hr renal retention, measured by direct organ radioassay increased progressively with more severe glomerular injury (Fig. 1A). The abnormal kidneys were brownish in color and increased in weight, probably due to edema. The urines of the PA-treated animals consistently showed 2 to 4+ albuminuria.¶ The PA treatment produced a decrease in body weight; however, the animals receiving the largest dose of 10 mg/100 g had ascites.

The "normal" clearance of hippuran, obtained by individual calculations from plasma levels in each of 12 control rats was 2.06 ± 0.402 (s.d.) ml/min/100 g. In the 36 treated animals given [^{131}I]hippuran (Fig. 2A), the expected incremental decreases in [^{111}In]DTPA clearance with increasing doses of PA were accompanied by paradoxical increases in hippuran clearance and computer estimated early renal uptake. Thus, at the dose level of 5 mg PA/100 g, the mean DTPA clearance declined 46% compared with the control value, whereas the hippuran clearance increased by 48%. The 2-hr retention of both agents was increased in the PA-treated animals. There was a moderately good correlation between hippuran clearance and its computer-generated renal uptake (Fig. 2B), but poor correlation between hippuran and DTPA clearances and between other measured parameters (Table 1). In the computer-generated biexponential analysis of the mean hippuran plasma values for controls (Fig. 2C) and PA rats (Fig. 2D), the half-times of the two components were increased in the PA rats, but their zero time intercepts (A and B) were lower, resulting in increased clearance and a very high hypothetical "volume of distribution" (100 ÷ [A + B]).

In the additional series of rats given ^{86}Rb 30 sec prior to killing, the large increases in hippuran clearance and

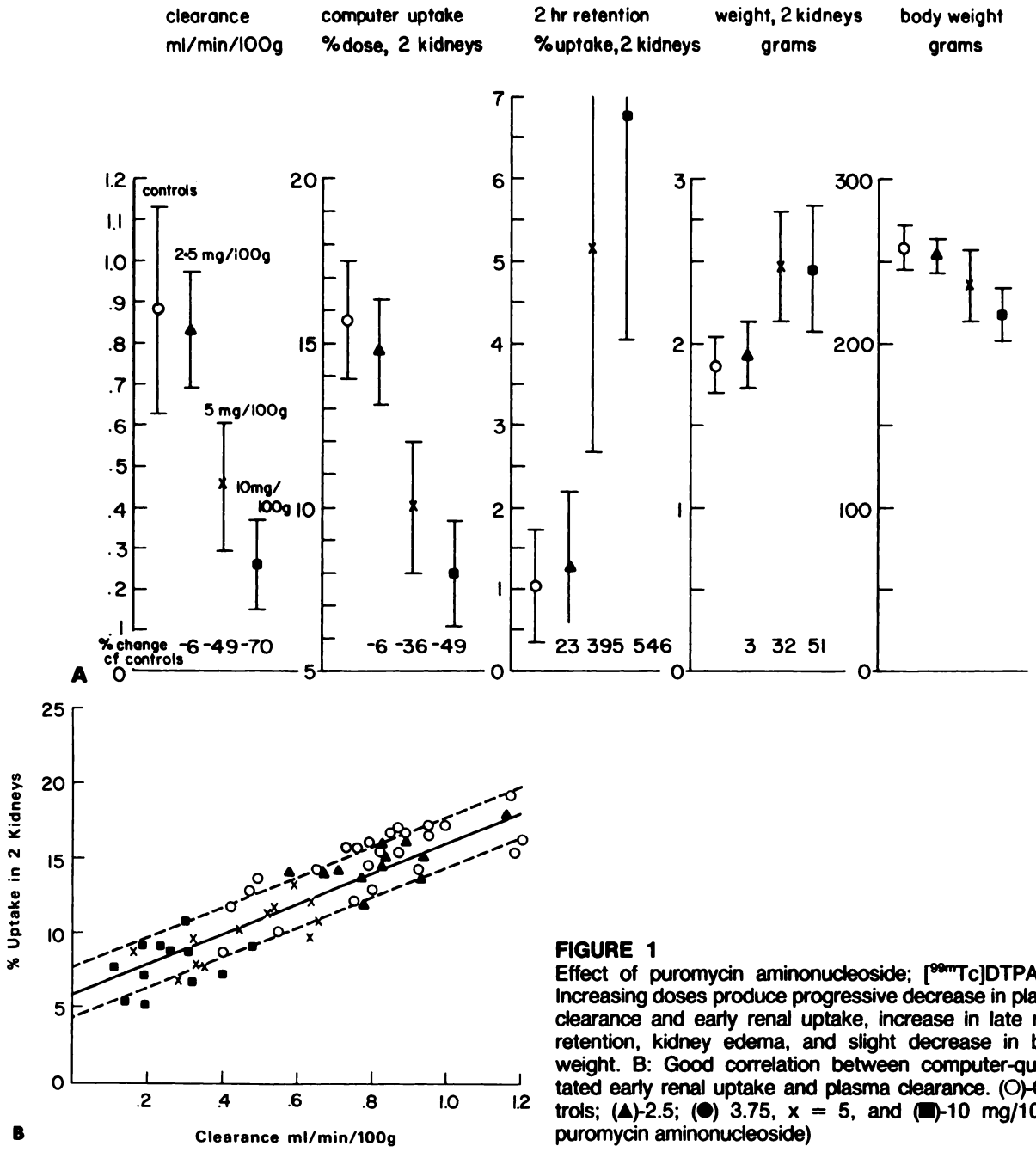

FIGURE 1
Effect of puromycin aminonucleoside; [^{99m}Tc]DTPA. A: Increasing doses produce progressive decrease in plasma clearance and early renal uptake, increase in late renal retention, kidney edema, and slight decrease in body weight. B: Good correlation between computer-quantitated early renal uptake and plasma clearance. (○)-Controls; (▲)-2.5; (●) 3.75, x = 5, and (■)-10 mg/100 g puromycin aminonucleoside)

renal retention in the PA rats could not be explained by the minimal increase in the Rb-estimated renal blood flow (Table 2). The 2-hr liver, GI, and urine levels of hippuran were somewhat higher in the PA rats than in the controls. The total serum protein** averaged 3.7 g/dl in the PA rats compared with 4.8 g/dl in controls, and the corresponding values for serum albumin were 2.0 and 2.8 g/dl, respectively.

In the group of animals given 1–1.5 mg of added carrier hippuran, the mean clearance of [^{131}I]hippuran in controls (1.99 ml/min/100 g) was not noticeably different from that obtained without added carrier. With the added carrier, the paradoxical increase in the mean clearance of [^{131}I]hippuran again was observed (3.03 ml/min/100 g). The fraction of the whole blood hippuran activity in the red cell fraction ranged from 20 to 32% during the first hour with and without added carrier, and was not noticeably different in the PA and control animals.

The mean clearance of [^{99m}Tc]GHA in the control rats (0.65 ± 0.15 s.d.) ml/min/100 g) was lower than that of [^{111}In]DTPA in the same animals (Fig. 3A). Nonetheless, the degree of reduction in their clearances and in the computer-generated renal uptake of GHA was similar (25 to 28%) in the PA-treated rats compared with the controls. The 2-hr renal uptake of GHA de-

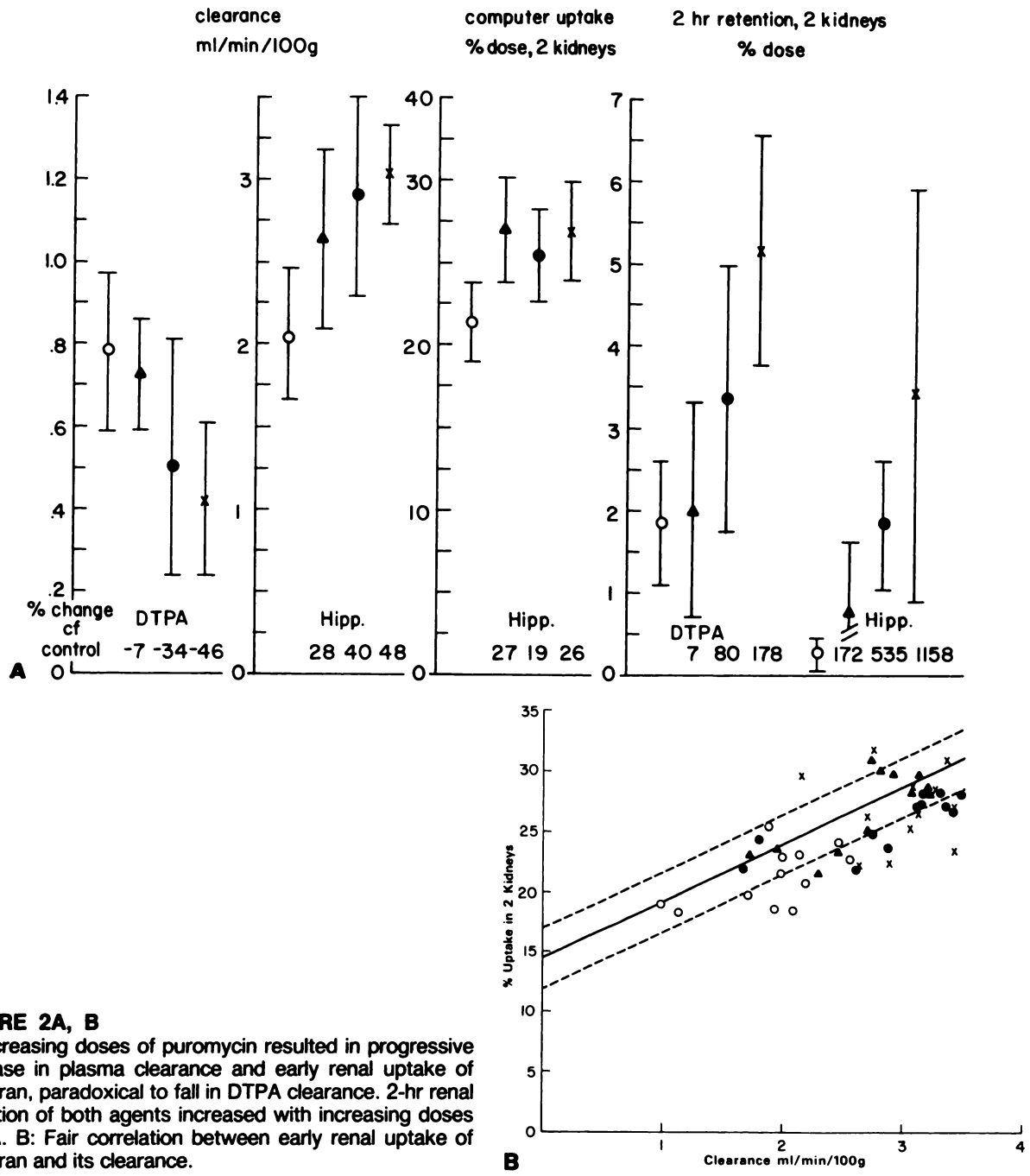

FIGURE 2A, B
A: Increasing doses of puromycin resulted in progressive increase in plasma clearance and early renal uptake of hippuran, paradoxical to fall in DTPA clearance. 2-hr renal retention of both agents increased with increasing doses of PA. B: Fair correlation between early renal uptake of hippuran and its clearance.

creased minimally by the PA treatment. The computer-generated early renal uptake correlated to some extent with the GHA clearance (Fig. 3B), and better with the DTPA clearance (Fig. 3C, Table 1).

The mean clearance of [^{99m}Tc]DMS in controls was much lower (0.20 ± 0.032 s.d. ml/min/100 g) than that of the other agents (Fig. 4A), probably because its renal extraction efficiency is lower. The clearance decreased only 20% in the PA-treated group. The computer-generated early renal uptake correlated poorly with the clearance (Table 1), and the clearance in turn correlated poorly with the [^{111}In]DTPA clearance (Fig. 4B). The computer uptake measured at 2 to 3 min decreased only 20% in the PA-treated group, but this correlated somewhat better than the DTPA clearance (r = 0.69, Fig. 4C) than the earlier uptake at 0.5 to 1.5 minutes. A close correlation was found between the 2-hr renal retention of DMS and the DTPA clearance (r = 0.92, Fig. 4D). The decrease in DTPA clearance and 2-hr DMS renal uptake from the PA treatment was similar (36% and 33%, respectively).

DISCUSSION

Our mean control value for [^{99m}Tc]DTPA (0.88 ± 0.25 ml/min/100 g) is higher than some previous esti-

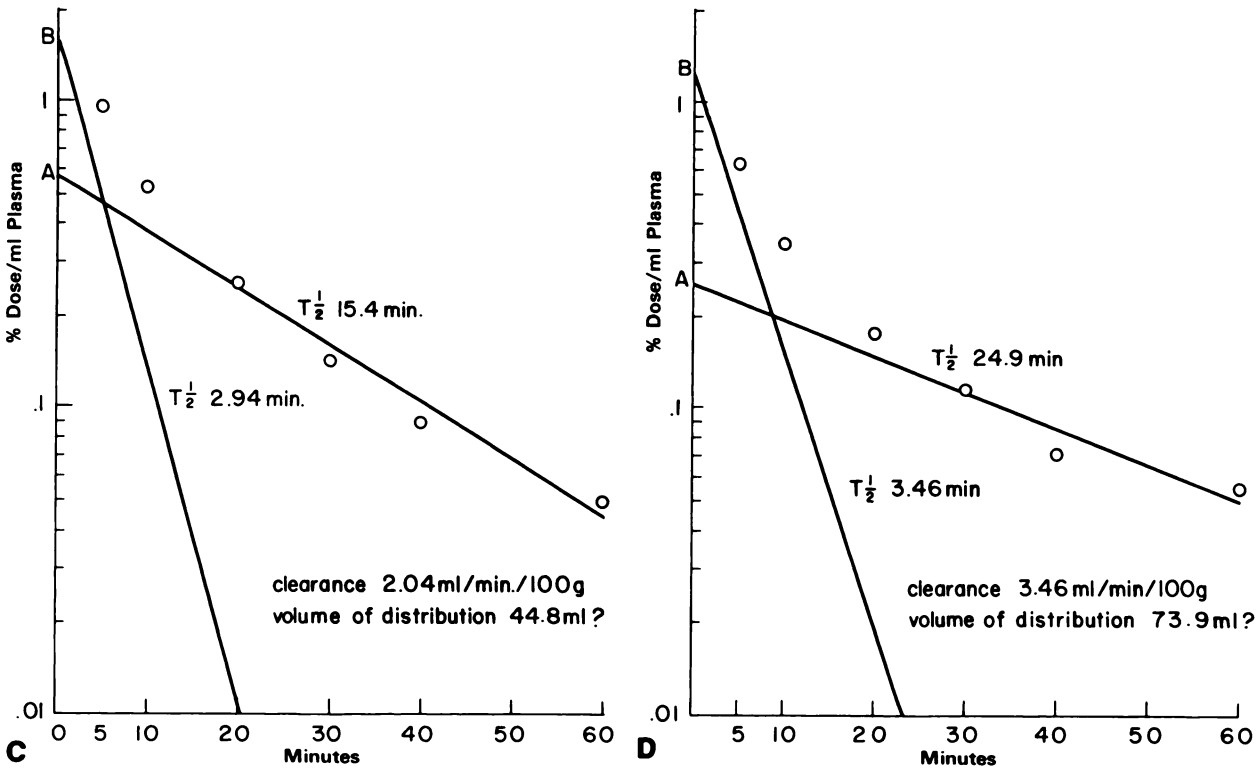

FIGURE 2C, D

C: Bi-exponential analysis of hippuran clearance in control rats. D: In PA treated rats, calculated hippuran clearance is increased despite slower half-times because intercepts are lower, probably due to early extravascular diffusion accompanying hypoalbuminemia

TABLE 1
Linear Correlations

Relations		Linear equation		Coefficient correlation	Regression
x	y	(y)		(r)	equation* (x)
[^{99m}Tc]DTPA (60)†					
Clearance	Computer uptake	11.1x + 5.53	1.56	0.90	0.0735y − 0.289
Clearance	2-hr Uptake	7.33x + 7.66	2.10	0.72	−0.0699y + 0.843
[^{131}I]hippuran (48)					
Clearance	Computer uptake	4.11x + 14.5	2.64	0.71	0.123y + 0.493
Clearance	2-hr Uptake	0.493x + 0.25	1.83	0.17	0.123y − 0.493
DTPA clearance	Clearance	−0.627x + 2.99	0.629	0.24	
DTPA clearance	Computer uptake	−1.89x + 26.3	3.72	0.13	
DTPA clearance	2-hr Uptake	−4.21x + 4.11	1.52	0.57	
[^{99m}Tc]GHA (24)					
Clearance	Computer uptake	10.6x + 6.50	1.86	0.71	0.0474y − 0.026
Clearance	2-hr Uptake	−1.04x + 13.5	1.55	0.12	
DTPA clearance	Clearance	0.589x + 0.12	0.0960	0.84	
DTPA clearance	Computer uptake	8.08x + 6.36	1.68	0.77	0.0735y − 0.159
DTPA clearance	2-hr Uptake	−0.35x + 13.2	1.56	0.055	
[^{99m}Tc]DMS (24)					
Clearance	Computer uptake (0.5–1.5′)	17.7x + 7.83	1.96	0.34	
Clearance	Computer uptake (2–3′)	38.9x + 10.0	2.66	0.50	
Clearance	2-hr Uptake	182x + 14.2	9.73	0.60	
DTPA clearance	Clearance	0.098x + 0.102	0.0318	0.60	
DTPA clearance	Computer uptake (0.5–1.5′)	2.97x + 8.64	1.95	0.35	
DTPA clearance	Computer uptake (2–3′)	8.71x + 10.0	2.22	0.69	
DTPA clearance	2-hr Uptake	45.8x + 9.96	4.63	0.29	0.0179 − 0.0390

* For calculating clearance from renal uptake.
† Number of rats in parentheses.

TABLE 2
^{86}Rb Experiment
(Mean Values, Five Controls, Five Given PA 5 mg/100 g 9 Days Previously)

	^{86}Rb			[^{131}I]hippuran			[^{99m}Tc]DTPA		
	C	PA	% Change	C	PA	% Change	C	PA	% Change
				% Dose/Organ at 2 hr killing					
Two kidneys	17.1	18.2	6	0.259	1.98	660	1.53	3.52	130
Liver	6.08	6.31	4	0.118	0.212	80	0.328	0.605	84
GI + contents	—	—	—	2.45	2.51	2	1.10	1.48	34
Urine	5.4	11	103	60	69	15	79	64	−19
Plasma		—	—	0.179	0.217	21	0.643	2.34	263
Computer uptake (% two kidneys)				22.8	28.7	26			
				ml/min/100 g					
*RBF	4.74	5.04	6						
†RPF	2.71	3.13	15						
Clearance				1.72	2.51	46	0.620	0.407	−34

* Renal blood flow assuming cardiac output 27.7 ml/min/100 g.
† Renal plasma flow, from renal blood flow and plasmacrit.

mates of glomerular filtration rate (GFR) in rats. In anesthetized male Sprague-Dawley rats 250–300 g, inulin clearance values reported include 0.72 (*18*), 0.83 (*19,20*), 0.87 (*21*), 0.92 (*22*), and 0.94 (*23*) ml/min/100 g. Lower values (0.5 ml/min/100 g) are reported for larger rats (*24*). Our mean control value for [^{131}I] hippuran clearance (2.06 ± 0.40 ml/min/m100 g) is close to previous estimates of 2.15 obtained with non-radioactive hippuran (*24*) and 2.11 with single injection (*12*) and continuous infusion (*25*) of [^{131}I]hippuran.

Single nephron GFR is determined by four factors (*3*)–the glomerular plasma flow rate, the systemic oncotic pressure, the transcapillary hydraulic pressure differences, and the ultrafiltration coefficient (product of the glomerular capillary hydraulic permeability and filtering surface area). Whole kidney GFR is obviously dependent also on the number of surviving functioning nephrons. In milder glomerular damage induced by PA, both whole kidney and single nephron GFR are homogeneously decreased because of a fall in the ultrafiltration coefficient and glomerular plasma flow rate. With more severe injury, a heterogenity occurs—i.e., some glomeruli are structurally damaged and some surviving glomeruli undergo hyperfiltration.

In our rat model, a good linear relation was found between GFR measured by single injection plasma clearance of ^{99m}Tc or [^{111}In]DTPA over a wide range of values, and the early computer-generated renal uptake of either DTPA (r = 0.90) or [^{99m}Tc]GHA (r = 0.71). These results confirm the studies of Gates (*5*), who predicted creatinine clearance from the early renal uptake of [^{99m}Tc]DTPA in 47 patients, but his coefficient of correlation was higher (r = 0.97). In earlier work (*26*), the correlation between GFR measured by single injection clearance of chromium-51 ethylenediaminetetraacetic acid [^{99m}Tc]DTPA and [^{131}I]hippuran was not as good (r = 0.67 and 0.69, respectively). We found that the early renal uptake of [^{131}I]hippuran or [^{99m}Tc] DMS was poor for predicting GFR. Hippuran and DMS showed a higher computer uptake in the right kidney than in the left (up to 19% and 12%, respectively, in PA rats), because of overlap of liver and right kidney in the rat. GHA and DTPA had less than a 2% difference in uptake between the two kidneys.

The indirect technique of estimating clearance from quantitative measurements of the early renal uptake has been criticized (*27,28*), because the uptake varies with different volumes of distribution in patients of different size. Hence, such estimates could be invalid with large extracellular volumes, especially in edematous patients. However, a markedly expanded volume of distribution with reversible back diffusion into the bloodstream also slows the clearance measured from multiple plasma sampling after single i.v. injection (*29*). Hence, in this situation, accurate results require continuous infusion methods.

The 2-hr renal retention of DMS correlated closely with the GFR, whereas the same measurement with GHA did not. Clinically, a high correlation between delayed renal uptake of DMS and creatinine clearance was found (r = 0.90), but in obstructive uropathy, the uptake should be determined at 24 hr or later, after the pelvocalyceal activity has drained (*30*). The 2-hr renal retention of DTPA and hippuran discriminated well between control and PA treated rats, but this would be more difficult to quantitate in vivo because the uptakes are so low at this time (0.5 to 2.5%/kidney). The cumulative 2-hr urinary excretion appeared to be a poor discrimination for all four agents; however, earlier urine measurements were not performed.

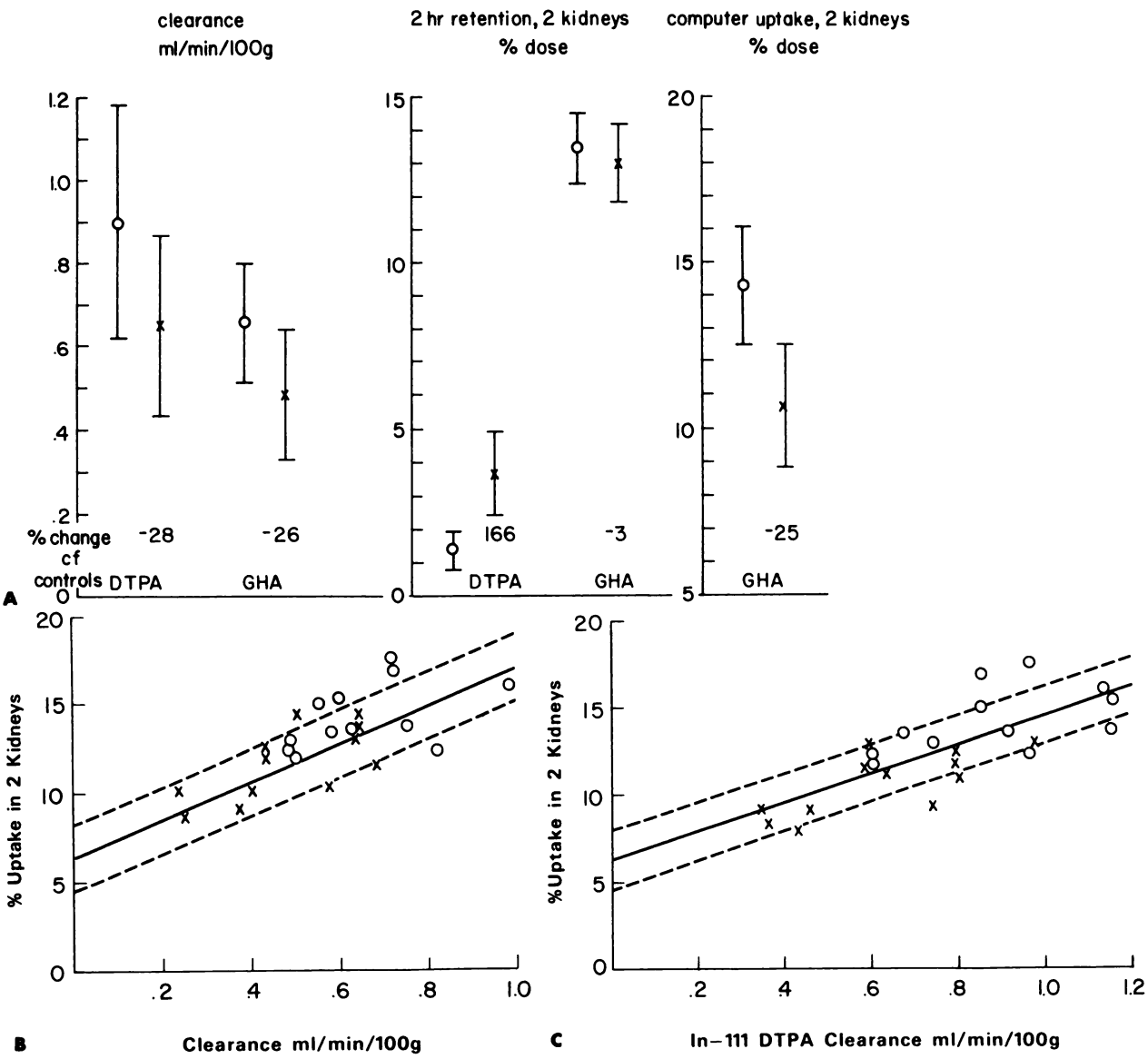

FIGURE 3

[^{99m}Tc]GHA compared with [^{111}In]DTPA. A: Percent reduction in clearance in PA treated rats was similar for two agents. PA treatment had little effect on 2-hr renal retention of GHA. Reduction in computer uptake of GHA in PA rats was similar to reduction in clearance. B: Fair correlation between early renal uptake of GHA and its clearance. C: Somewhat better correlation between early renal uptake of GHA and [^{111}In]DTPA clearance

Sapirstein (*16*) recovered 16% of the injected dose of ^{86}Rb in rat kidneys killed at 30 sec after injection, and considered this an indication of the fractional distribution of the cardiac output, estimated at 20.5 ml/min/100 g. Subsequent workers (*17*) found that ^{86}Rb underestimated renal blood flow by 18% because of incomplete extraction, in comparison with the microsphere method, and that the cardiac output in rats averaged 27.7 ml/min/100 g. Using this value, our estimate of mean renal plasma flow with ^{86}Rb in controls, 2.71, agrees with published values of 2.65 (*18*) and 2.74 (*22*). Therefore, it appears that hippuran clearance significantly underestimates renal plasma flow in rats. Recently, Eshima et al. (*25*) obtained a renal extraction efficiency of only 69% for hippuran in rats.

The quantitation of plasma clearance of labeled DTPA and hippuran by two-exponential curve fitting has been widely used in man for many years. A good correlation has been established between single injection [^{131}I]hippuran quantitated by this method and continuous infusion of para-aminohippurate (PAH) (*13*). In normal man, the zero intercepts for hippuran account for only about half of the dose injected, and for DTPA, only about 40% (Table 3). For hippuran, the half-times are ~3 and 25 min (*31*) and the intercept of the fast component is about three times greater than that of the slow component. For DTPA, the normal half-times are about 15 and 120 min, and the fast component intercept about 1.5 times greater than that of the slow component. The hypothetical "volumes of

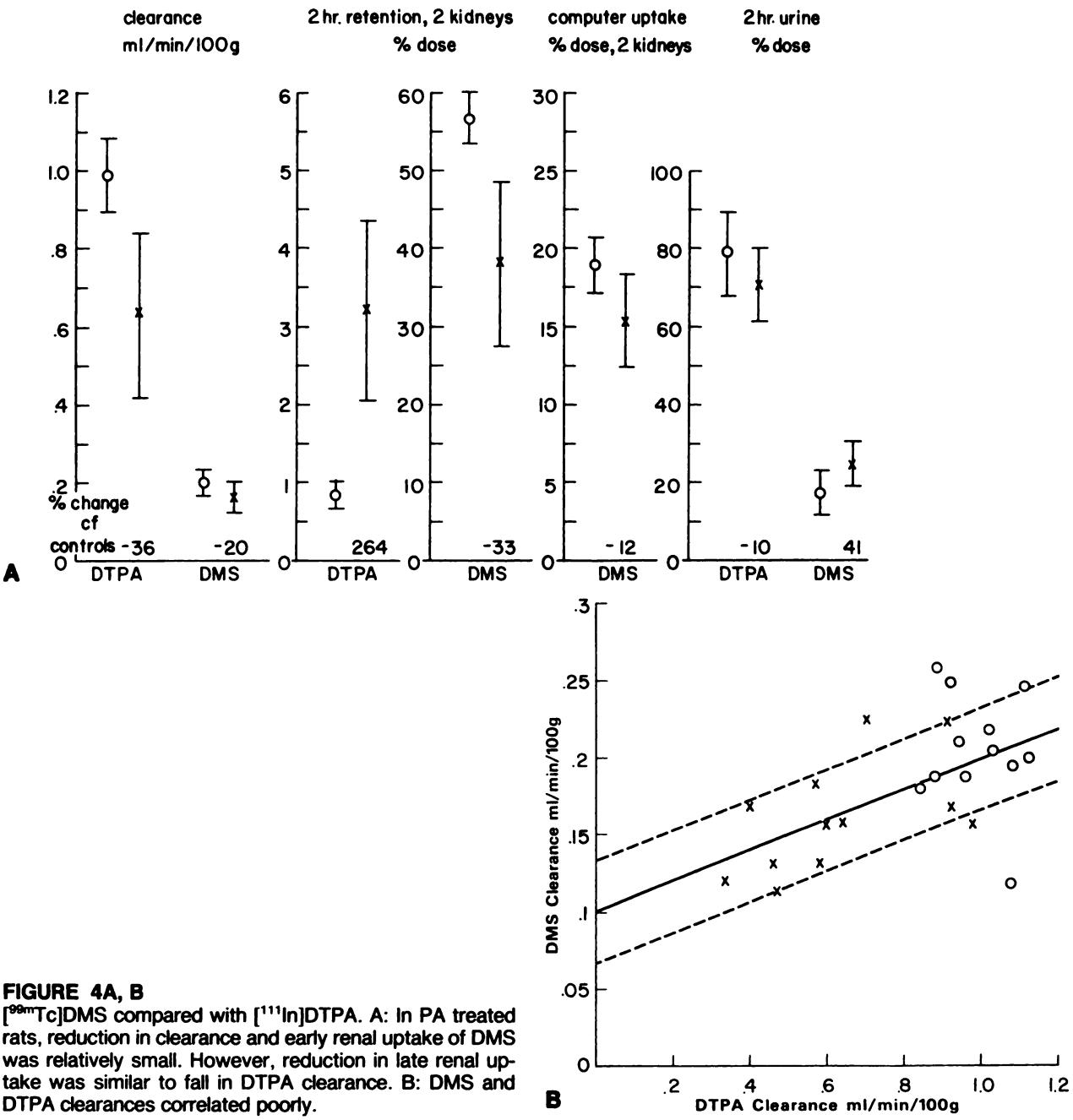

FIGURE 4A, B
[^{99m}Tc]DMS compared with [^{111}In]DTPA. A: In PA treated rats, reduction in clearance and early renal uptake of DMS was relatively small. However, reduction in late renal uptake was similar to fall in DTPA clearance. B: DMS and DTPA clearances correlated poorly.

distribution" are about 7–8 l for both agents, or about half the estimated extracellular fluid volume. To account for the rapid disappearance of 50% of the injected dose of these agents, van Steckelenburg et al. (*31*) have proposed a third compartment into which rapid diffusion occurs, with a half-time less than a minute. Tracers in this compartment may not be immediately subject to diffusion back into the circulation.

In the normal rat, the conventional two-exponential components account for only 19% of the injected dose for hippuran, and in the PA rats for only 11%. The two components for DTPA in both normal and PA rats account for 13% of the administered dose. The hypothetical volumes of distribution are very large and probably have little physiologic meaning (*29*). The extracellular fluid volume in the rat is about 28 ml/100 g (*20*). For hippuran, the half-times are 3 and 15 min and the fast component intercept three times greater than the slow component intercept (Table 3). For DTPA, the half-times are 15 and 68 min, and the intercept of the fast component about five times greater than that of the slow component.

The marked paradoxical increase in the plasma clearance and early renal uptake of [^{131}I]hippuran with increasing doses of PA could not be explained by an increase in renal blood flow, or by any change in diffusibility into the circulating red cells. The increase may be due in part to an increased renal extraction

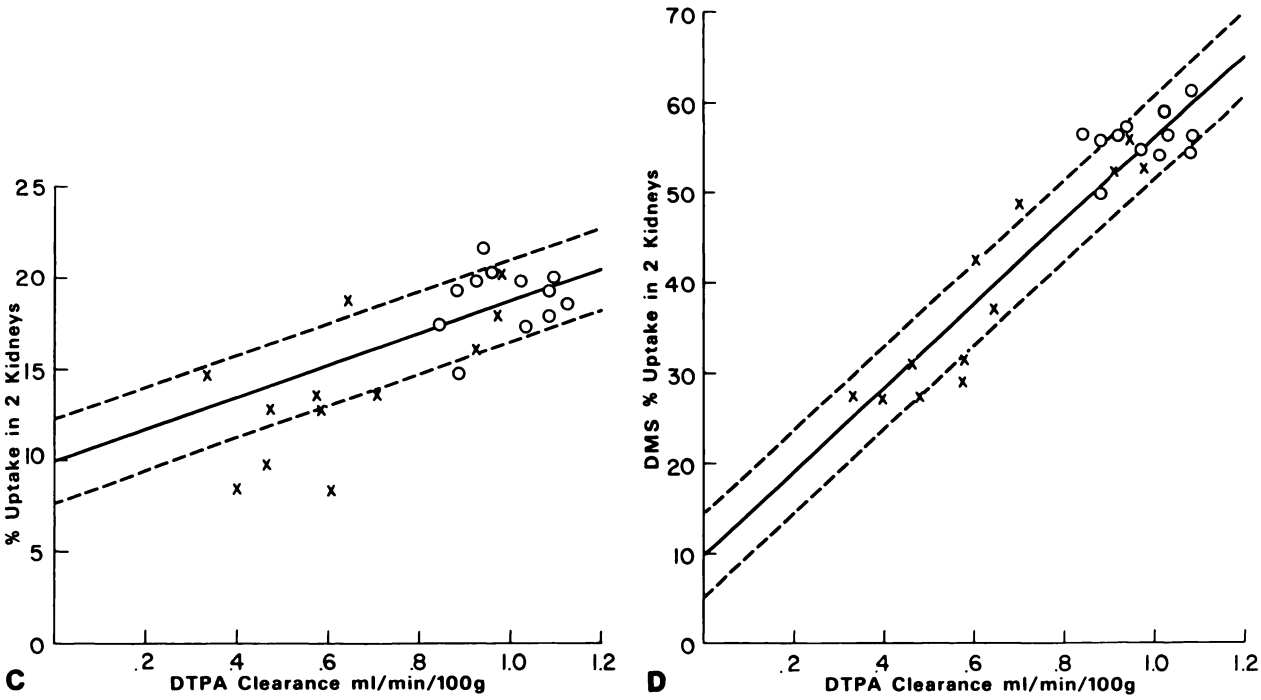

FIGURE 4C, D
C: Fair correlation between DMS early renal uptake and DTPA clearance. D: Excellent correlation between DMS late renal uptake and DTPA clearance

efficiency for Hippuran from reduced protein binding associated with the hypoproteinemia. This would explain the increased urinary excretion also. The most important factor may be increased immediate diffusion into the extracellular extravascular fluid space, again due to the decreased protein binding from hypoproteinemia. The tracer in at least part of this "space" may not be accessible for back diffusion into the circulation during the total time of the study.

According to Bryan et al. (*32*), the ultrafilterable fraction of [^{131}I]hippuran measured in vitro is highly dependent on plasma protein concentration and virtually doubles from a concentration of 7 g/dl to 0 g/dl. Hence, significant errors occur in assuming that diffusibility of this agent is constant in states of hypoproteinemia, as in saline expansion, liver disease and the nephrotic syndrome, and in hyperproteinemia, as in dehydration and myelomatosis. In any case, single injection techniques using [^{131}I]hippuran appear unsatisfactory for investigating this model of "minimal change" glomerulonephritis.

TABLE 3
Comparison of Clearance Parameters Between Normal Man and Rat

	Man* 80 kg 1.98 M²		Rat† 250 g 0.0357 M²	
Body surface area	Hippuran	DTPA	Hippuran	DTPA
Clearance ml/min	533	94	5.07	1.872
ml/min/100 g	0.666	0.118	2.03	0.75
ml/min/M²	269	47	142	52
Sum A + B intercepts (% in plasma volume)	50	40	19	13
T½ fast component (min)	3	15	3	15
T½ slow component (min)	25	120	15	68
Fast/slow intercepts	3:1	1.5:1	3:1	5:1
Theoretical volume of distribution	7–8 l	7–8 l	45 ml?	67 ml?
Extracellular fluid volume	14–16 l		70 ml	
ml/100 g	20–23		28	

* Data from various authors, including (*13,30*).
† Data from various authors; analysis of plasma curves from this work.

FOOTNOTES

* Amersham Corp., Arlington Heights, IL.
† Medi-Physics, Inc., Richmond, CA.
‡ Sigma Chemical Co., St. Louis, MO.
§ Technicare, Solon, OH.
¶ Albustix, Ames Division, Miles Laboratories, Elkhart, IN.
** Vet Path Clinical Laboratory Facility, Teterboro, NJ.

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