

# Measurement of glomerular filtration rate via urinary clearance of inulin and plasma clearance of technetium Tc 99m pentetate and exogenous creatinine in dogs

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**Objective**—To compare glomerular filtration rate (GFR) measured via urinary clearance of inulin (UCI) with plasma clearance of technetium Tc 99m pentetate ( $^{99m}\text{Tc}$ -pentetate) and creatinine in dogs.

**Animals**—6 healthy Beagles and 18 Beagles with reduced renal function.

**Procedure**—13 blood samples were obtained between 5 and 600 minutes after IV bolus injections of  $^{99m}\text{Tc}$ -pentetate and creatinine. Plasma clearance of  $^{99m}\text{Tc}$ -pentetate was computed on the basis of 1, 2, or 13 samples, and plasma clearance of creatinine was computed on the basis of 2, 5, or 13 samples. During plasma clearance procedures, constant IV infusion of carboxyl carbon 14 inulin was begun and UCI was determined in urine collected from 90 to 120, 120 to 180, and 180 to 240 minutes. Clearance procedures were repeated in 12 dogs to evaluate reproducibility of results.

**Results**—Significant association between UCI and plasma clearance was determined via all methods. However, plasma clearances were moderately to markedly different from UCI, depending on test substance, GFR, and sample numbers used for plasma clearance computations. Comparisons were particularly discordant when some methods of limiting samples were used to define plasma clearance.

**Conclusions and Clinical Relevance**—Values derived from plasma clearance methods for  $^{99m}\text{Tc}$ -pentetate and creatinine were not interchangeable with UCI results, which raises questions about their reliability as clinical research tools for measurement of GFR. Plasma clearance methods that are relative indices of renal function should not be interpreted as accurate measures of GFR without validation. (*Am J Vet Res* 2005;66:1046–1055)

Glomerular filtration rate (GFR) is considered the best index of renal function in both health and disease.<sup>1</sup> Urinary clearance of inulin (UCI) is the most widely used standard for precise measurement of GFR,<sup>2,3</sup> but this procedure is rarely if ever used by veterinarians because it is time-consuming and requires analytical methods that are not routinely available. Analysis of blood, plasma, or serum for urea or creatinine concentration is performed clinically to evaluate renal function, but these tests are insensitive and do

not detect dysfunction until GFR is reduced to < 25% of values in healthy animals.<sup>4</sup>

The impracticality of measuring UCI and the lack of sensitivity of BUN and creatinine measurements has led to investigation of alternatives for monitoring renal function in clinical practice and research. Plasma clearance is measured via pharmacokinetic analysis of the plasma decay curve derived from bolus IV injection of test material. Results from computation of plasma clearance should be an accurate measure of GFR if the test substance meets defined criteria and is used properly. Characterization of the plasma decay curve is achieved by determining the concentration of test substance in multiple serial blood samples. Because multiple sampling is impractical in clinical patients, protocols have been reported in which sampling is limited. One method used bolus injection of technetium Tc 99m pentetate ( $^{99m}\text{Tc}$ -pentetate) and procurement of 1 or 2 blood samples thereafter.<sup>5</sup> Another method used a bolus injection of creatinine and limited sampling thereafter.<sup>6</sup> The ease and reported accuracy of those tests make them appealing.

New tests are compared with established standards to determine whether results obtained with the new tests are reliable. Linear regression analysis is commonly used to compare test results, but limitations of this method of comparison have been emphasized.<sup>7,8</sup> Comparison of methods graphically and computation of agreement limits and their confidence intervals has been advocated as being more discriminating than regression analysis.<sup>7,8</sup> The objective of the study reported here was to compare urinary clearance of inulin with plasma clearance procedures for  $^{99m}\text{Tc}$ -pentetate and creatinine in dogs by use of linear regression and limits-of-agreement techniques.

## Materials and Methods

**Preparation of test materials**—A  $^{99m}\text{Tc}$ -pentetate solution was prepared by a commercial supplier<sup>a</sup> within 1 hour of use. The solution was diluted with sterile lactated Ringer's solution, mixed, and aspirated into syringes to provide each dog with approximately 300  $\mu\text{Ci}$  of  $^{99m}\text{Tc}$ -pentetate in a volume of 5.6 mL of mixture. For confirmation of estimated dose, a dosimeter was used to measure syringe contents of  $^{99m}\text{Tc}$ -pentetate before and after injection. For precise measurement of dose administered, a 0.5-mL volume of mixture was used as a standard in counting procedures.

Creatinine solution for injection was prepared by dissolving anhydrous creatinine<sup>b</sup> in distilled water (80 mg/mL). Preliminary trials revealed that autoclaving caused a decrease in creatinine concentration, so the solution was sterilized by filtration into sterile bottles through a 0.2- $\mu\text{m}$  filter. The

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solution was tested for creatinine concentration and sterility at intervals over time.

Carboxyl carbon 14 (<sup>14</sup>C) inulin powder supplied by the manufacturers<sup>cd</sup> was dissolved in sufficient distilled water to give a concentration of 1  $\mu$ Ci/mL. The solution was sterilized by passage through a 0.2- $\mu$ m filter and stored at -80°C until thawed for use.

**Dogs and dog handling**—Twenty-four purpose-bred young adult male and female Beagles that weighed 8.1 to 14.0 kg were studied. Six clinically normal dogs had intact kidneys, and 18 clinically normal dogs had renal mass reduced surgically, as previously described<sup>9</sup> for studies unrelated to this report. The interval (mean  $\pm$  SD) between surgical reduction of renal mass and study of kidney function was 282  $\pm$  129 days. Dogs were housed individually in inside runs with controlled temperature and light-dark cycles. Commercially available dry dog food was fed, and water was available ad libitum.

Dogs were acclimated to rest in Pavlov slings. Clearance procedures were performed on groups of 3 to 6 dogs/d, without use of sedatives or anesthetics. Food was withheld for 15 hours before study, but water was available ad libitum. Clearance procedures were repeated in 12 dogs (6 clinically normal and 6 with reduced renal mass) at least 1 week after initial measurements. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

**Plasma clearance procedures**—On the day of study, each dog was weighed and placed in a Pavlov sling. Saphenous and jugular vein catheters were inserted, and a quantity of water equal to 3% of body weight was administered by gavage to ensure adequate hydration. One hour after gavage a preinjection blood sample was taken from the jugular catheter. Bolus injection of <sup>99m</sup>Tc-pentetate was made via the saphenous vein catheter, followed immediately by bolus injection of 1.0 mL of creatinine solution/kg of body weight. At the conclusion of each bolus injection, a few milliliters of blood was drawn quickly into the injection syringe and reinjected. Finally, 20 mL of lactated Ringer's solution was injected to rinse the saphenous vein catheter. The end of the <sup>99m</sup>Tc-pentetate injection was considered time 0. Blood samples (3 mL) were obtained in heparinized syringes from the jugular catheter at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 420, and 600 minutes; the exact time (minutes and seconds) of sampling of each dog was recorded.

**Urinary clearance procedures**—At 45 minutes, each dog received a bolus injection of 2.5  $\mu$ Ci of carboxyl <sup>14</sup>C-inulin (1  $\mu$ Ci/mL) via the saphenous vein catheter, and constant infusion (0.5 mL/min) of carboxyl <sup>14</sup>C-inulin solution was initiated with an infusion pump. Rate of carboxyl <sup>14</sup>C-inulin infusion (0.04 to 0.07  $\mu$ Ci/min) was individualized for each dog on the basis of body weight and GFR, which had been measured previously in unrelated studies. An indwelling urinary catheter was put in place in preparation for collection of urine during the 90 to 120-, 120 to 180-, and 180- to 240-minute intervals. To ensure accurate collection of urine, the bladder was emptied and rinsed with several washes of sterile saline (0.9% NaCl) solution prior to the first collection. The time (minutes and seconds) that rinsing was concluded to begin the first urine collection period was recorded. Approximately 3 minutes before the end of each urine collection period, the bladder was emptied and rinsed several times with sterile saline solution. The last rinse and procurement of blood were coordinated, and the time (minutes and seconds) was recorded. Urine and rinse solutions were both placed in a graduated cylinder, and the volume was recorded. The graduated cylinder was sealed, the contents were thoroughly mixed, and an aliquot was saved for assay. After the 240-minute collection of blood and urine the

infusion of carboxyl <sup>14</sup>C-inulin was stopped and urinary and saphenous vein catheters were removed. Dogs were provided with drinking water ad libitum for the remainder of the experiment.

**Plasma and urine analyses**—The <sup>99m</sup>Tc-pentetate activities (counts per minute) in 0.5-mL samples of plasma, urine, and standard were measured in a well counter shortly after 600-minute samples were obtained. The counts per minute of each sample were corrected for radioisotope decay that occurred during serial counting of tubes by applying a mathematical formula that accounted for decay rate (half-life of 6.05 hours) and time interval between sample counts (1.2 minutes). The same standard samples were counted at the beginning and end of each series to verify the accuracy of the correction formula. Corrected counts per minute were used for computations.

Creatinine concentrations of plasma, urine, and injection solution were measured with an automated analyzer<sup>e</sup> via the kinetic alkaline picrate method. For carboxyl <sup>14</sup>C-inulin measurement, plasma and urine samples (0.5 mL) were mixed with scintillation fluid (10 mL) and stored until 16 half-lives of <sup>99m</sup>Tc-pentetate had elapsed. Activity of carboxyl <sup>14</sup>C-inulin was then measured in a liquid scintillation counter, and measurements were repeated at least 24 hours later to confirm the absence of interference from <sup>99m</sup>Tc-pentetate.

**Data and statistical analyses**—Constancy of plasma inulin concentration in each dog was evaluated by expressing the counts per minute at each plasma collection time (90, 120, 180, and 240 minutes) as a percentage of the mean counts per minute of the 4 samples. Urinary clearance of carboxyl <sup>14</sup>C-inulin was computed separately for the 90- to 120-, 120 to 180-, and 180 to 240-minute intervals by use of the standard clearance formula  $[C = (Uv \times Uc)/Pc]$ , where C is clearance, U is urine, P is plasma, c is concentration, and v is volume. The Pc was computed as the mean counts per minute in plasma obtained from blood taken at the beginning and end of each urine collection period. For comparison with plasma clearance methods, the urinary clearance of carboxyl <sup>14</sup>C-inulin for each dog was computed as the mean of the 3 collection periods and identified as UCI. For urine and plasma clearance computations, clearance was expressed as milliliters per minute per kilogram of body weight.

A commercially available software program<sup>f</sup> was used to compute plasma clearance of <sup>99m</sup>Tc-pentetate and creatinine via noncompartmental data analyses. For these computations, area under the curve (AUC) was determined with data from plasma obtained at 13 time points (5 to 600 minutes) and clearance was termed Tc-13 for <sup>99m</sup>Tc-pentetate and Cr-13 for creatinine. For creatinine clearance computations, baseline plasma creatinine concentration was subtracted from postinjection values.

Limited sampling strategies reported for <sup>99m</sup>Tc-pentetate<sup>5</sup> also were evaluated. Plasma clearance of <sup>99m</sup>Tc-pentetate was calculated on the basis of 2 plasma samples (20 and 180 minutes). This computation was made on the basis of a 1-compartment model with monoexponential decay. Zero-time plasma concentration of <sup>99m</sup>Tc-pentetate was extrapolated from ln 20-minute and ln 180-minute samples, and AUC was computed as zero-time concentration/ln slope. Clearance was calculated as dose of test substance/AUC and termed Tc-2. Plasma clearance of <sup>99m</sup>Tc-pentetate also was calculated on the basis of a 1-point sampling time (120 minutes) and a linear quadratic model.<sup>5</sup> Clearance by this method was calculated, as reported,<sup>5</sup> from the following regression equation:

$$Cl = (-4.69 \times 10^{-2})Vt + 3.89Vt - 8.21,$$

where Cl is clearance and Vt is volume of distribution of the tracer. The Vt was calculated as <sup>99m</sup>Tc-pentetate dose/<sup>99m</sup>Tc-

pentetate count at 120 minutes. Clearance as calculated by use of this method was termed Tc-1.

Plasma clearance of creatinine was calculated from 5 plasma samples, as described,<sup>6</sup> and computations obtained by this method were termed Cr-5. This method limited AUC to the 0 to 600-minute interval, ignoring AUC generated after that time.

Creatinine clearance also was computed on the basis of calculation of AUC from various combinations of 2 plasma samples obtained during the elimination phase (60 to 420 minutes). Creatinine clearance values derived from 1 set of 2 samples (90 and 420 minutes) were termed Cr-2.

**Statistical analyses**—Commercially available software was used for statistical analyses.<sup>8</sup> Mean  $\pm$  SD values are reported; ANOVA with repeated measures was used to compare UCI for the 3 collection periods, and 1-way ANOVA was used to compare results from nonazotemic dogs (renal-intact and nonazotemic dogs with reduced renal mass) with azotemic dogs with reduced renal mass. Regression analyses were used to determine the degree of association among values obtained by different clearance computations. A value of  $P \leq 0.05$  was considered significant.

Agreement between UCI and each plasma clearance method was evaluated graphically via limits-of-agreement methods.<sup>7,8</sup> Briefly, to evaluate absolute differences between each plasma clearance method and UCI, the plasma clearance minus UCI values were plotted against mean clearance ( $[\text{plasma clearance} + \text{UCI}]/2$ ). In addition, to determine whether the relationship between UCI and plasma clearance methods could be improved, data were transformed to the percentage difference between each plasma clearance method and UCI, and  $([\text{plasma clearance} - \text{UCI}]/\text{UCI})$  was plotted against the value of  $([\text{plasma clearance} + \text{UCI}]/2)$ .

For the percentage difference data, the Lilliefors modification of the Kolmogorov-Smirnoff test was used to determine whether normal distribution of differences existed. Normal distribution of differences existed for the nonazotemic subset but not for all 24 dogs or the azotemic subset. Consequently, the mean percentage difference, confidence interval, and 95% confidence limits of the interval were calculated only for the nonazotemic subset.

## Results

**Azotemia**—Baseline (preinjection) plasma creatinine concentration was within the laboratory reference range of 0.5 to 1.7 mg/dL in 6 clinically normal dogs and in 8 dogs with reduced renal mass. In this nonazotemic group, plasma creatinine concentration was  $1.25 \pm 0.34$  mg/dL. The other 10 dogs with reduced renal mass had azotemia (plasma creatinine concentration,  $2.82 \pm 1.07$  mg/dL; range, 1.9 to 5.4 mg/dL).

**Urinary clearance of carboxyl <sup>14</sup>C-inulin**—A fairly constant plasma inulin concentration was achieved in each dog during the period of urine collections (Figure 1). Compared with the mean of the 4 measured values, values were  $98 \pm 13\%$  at 90 minutes,  $101 \pm 4\%$  at 120 minutes,  $105 \pm 15\%$  at 180 minutes, and  $101 \pm 10\%$  at 240 minutes.

The range of values for UCI was 0.26 to 4.45 mL/min/kg. For 6 clinically normal dogs, the range was 3.17 to 4.47 mL/min/kg, and for 18 dogs with reduced renal mass, the range was 0.26 to 2.09 mL/min/kg. For 14 nonazotemic dogs, the range of UCI was 1.18 to 4.47 mL/min/kg, and for 10 azotemic dogs, the range was 0.26 to 1.58 mL/min/kg. The UCI

measurements were not significantly different among collection periods for clinically normal dogs (90 to 120 minutes,  $3.89 \pm 0.44$  mL/min/kg; 120 to 180 minutes,  $3.85 \pm 0.51$  mL/min/kg; and 180 to 240 minutes,  $3.96 \pm 0.54$  mL/min/kg) or for dogs with reduced renal mass (90 to 120 minutes,  $1.31 \pm 0.55$  mL/min/kg; 120 to 180 minutes,  $1.30 \pm 0.55$  mL/min/kg; and 180 to 240 minutes,  $1.31 \pm 0.54$  mL/min/kg).

**Association between clearance measurements**—Linear regression analyses revealed that the association between UCI and plasma methods was significant ( $P \leq 0.001$ ). The  $R^2$  values were Tc-13, 0.986; Tc-2, 0.991; Tc-1, 0.925; Cr-13, 0.986; and Cr-5, 0.984. The relationship between UCI and Cr-2 was significant for all time combinations tested, but clearance difference and SD were large for many time combinations (Table 1).

The association between results from Tc-13 and Tc-2 was significant ( $R^2 = 0.996$ ). The relationship between Tc-13 and Tc-1 was better described by a quadratic formula ( $R^2 = 0.982$ ) than by a linear analysis ( $R^2 = 0.910$ ). A significant linear association existed between Cr-13 and Cr-5 ( $R^2 = 0.991$ ) as well as between Cr-13 and Cr-2 (90 and 420 minutes,  $R^2 = 0.996$ ).

**Agreement between UCI and plasma clearance methods**—Absolute and percentage difference comparisons between UCI and plasma clearance revealed discordance in clearance values that varied with test material, sample numbers used for computations, and dog groups used for comparisons (Figures 2–7).

The values for Tc-13 minus UCI (mL/min/kg of body weight) were usually negative, but the magnitude of difference increased markedly as clearance rate ( $[\text{Tc-13} + \text{UCI}]/2$ ) increased. When clearance difference was expressed as percentage of UCI, the agreement between tests was more consistent but still changed over the span of clearance values (Figure 2).

Clearances of Tc-2 (mL/min/kg of body weight) exceeded UCI at low clearance values but were markedly less than UCI at high clearance values. When clearance difference was expressed as percentage of UCI, Tc-

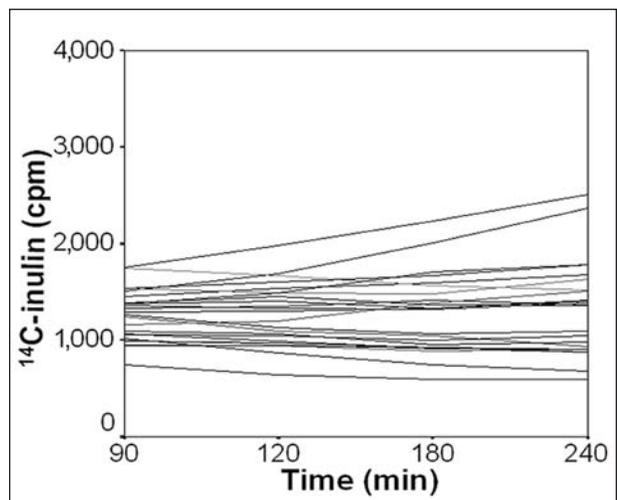


Figure 1—Carboxyl carbon 14 (<sup>14</sup>C) inulin concentrations in plasma samples obtained from dogs at 90, 120, 180, and 240 minutes after bolus injection and constant infusion of carboxyl <sup>14</sup>C-inulin. cpm = Counts per minute.

2 markedly exceeded UCI at lower clearance but was less than UCI at higher clearance values (Figure 3). Although regression analysis revealed a significant relationship between UCI and Tc-1, differences between values from the 2 tests were large and increased in magnitude as clearance increased (Figure 4).

Regardless of level of clearance, most values for Cr-13 were less than UCI whether absolute or percent difference was compared (Figure 5). However, percentage differences were fairly uniform over the range of clearance values tested.

When plasma creatinine clearance calculations were based on 5 plasma samples, Cr-5 clearance (mL/min/kg of body weight) overestimated clearance at low clearance values and underestimated it at high clearance values. When Cr-5 was expressed as percentage difference between it and UCI, CR-5 markedly exceeded (> 200%) UCI at low clearance levels (Figure 6).

A significant correlation between Cr-2 and UCI was detected for many sample pairs (Table 1). For computations based on the 90- and 420-minute samples, Cr-2 clearance was nearly the same as UCI at lower clearance values but had more variation at higher clearance values. Expressing the difference as percentage, Cr-2 values were fairly constant over the range of clearance values tested (Figure 7).

Separation of data according to azotemia status (Table 2) confirmed interpretation of graphic data, which indicated that the difference between UCI and plasma methods varied with magnitude of clearance. Comparison of absolute differences from nonazotemic and azotemic dogs by use of ANOVA indicated groups differed for Tc-13, Tc-2, Tc-1, and Cr-5 methods. For Tc-13 and Tc-2, the values in the azotemic group were numerically closer to UCI values, whereas for Cr-5, values from the nonazotemic group were closer to UCI values (Table 2). Comparison of percentage differences indicated that values from the nonazotemic group were significantly different from the azotemic group for Tc-2,

Table 1—Results of linear regression analysis of urinary clearance of carboxyl carbon 14 inulin (UCI) versus plasma clearance of creatinine in 24 dogs when 2 plasma creatinine samples were used to compute plasma creatinine clearance.

Sample times (min)	R <sup>2</sup> value	Clearance difference (%)*
60, 120	0.938	76 ± 80
60, 180	0.982	45 ± 34
60, 240	0.987	30 ± 19
60, 300	0.982	22 ± 30
60, 420	0.967	8 ± 13
90, 180	0.983	30 ± 25
90, 240	0.990	21 ± 14
90, 300	0.990	17 ± 19
90, 420	0.977	6 ± 10
120, 180	0.951	15 ± 42
120, 240	0.988	14 ± 28
120, 300	0.989	14 ± 19
120, 420	0.980	5 ± 13
180, 240	0.942	8 ± 49
180, 300	0.984	13 ± 36
180, 420	0.990	7 ± 14
240, 300	0.956	9 ± 63
240, 420	0.978	7 ± 17
300, 420	0.986	3 ± 59

\*Clearance difference (mean ± SD) = (Computed creatinine clearance - UCI)/UCI × 100%.

Tc-1, and Cr-5. Values from the nonazotemic group were in better accord with UCI for Tc-2 and Cr-5, whereas Tc-1 better corresponded to UCI in the azotemic group.

For the nonazotemic subset of dogs, 95% confidence intervals on lower and upper limits of agreement revealed that large differences between UCI and plasma clearance methods would be expected if results from the present study were applied to another population (Table 3). However, for Tc-2 and Cr-5, limits-of-agreement values were deceptive because plasma clearance progressively increased as UCI declined (Figures 8 and 9).

Noncompartmental pharmacokinetic analysis of <sup>99m</sup>Tc-pentetate clearance revealed that the percentage of AUC derived from extrapolation beyond 600 minutes was 3.16 ± 1.82% for nonazotemic dogs and 18.65 ± 18.40% for azotemic dogs. The steady state volume of distribution (V<sub>ss</sub>) of <sup>99m</sup>Tc-pentetate was 24.74 ± 2.93% in nonazotemic dogs and 25.09 ± 2.60% in azotemic dogs. For creatinine, the percentage of the AUC attributed to extrapolation beyond 600 minutes was 14.40 ± 13.11% in the nonazotemic group and 40.10 ± 20.06% in the azotemic group. The V<sub>ss</sub> of creatinine was 63.88 ± 6.89 in nonazotemic dogs and 64.27 ± 8.38 in azotemic dogs.

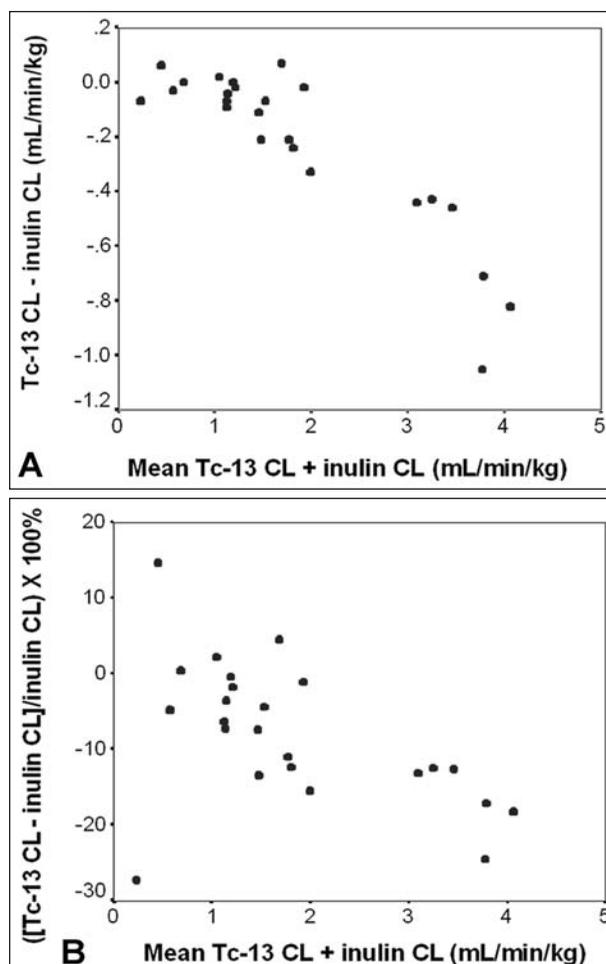


Figure 2—Absolute difference (A) and percentage difference (B) between urinary clearance (CL) of inulin and plasma CL of technetium Tc 99m pentetate (<sup>99m</sup>Tc-pentetate) calculated with a 13-sample (Tc-13) definition of area under the curve (AUC) in 24 dogs.

Repeatability of clearance values—For the 12 dogs tested, the ratio of the first to second clearance determination typically was within 3% for all methods except Cr-5, which was 11% different when repeated

(Table 4). However, the SD differed between tests and was lowest for the Tc-13 method and highest for the Tc-1 method. Lack of normal distribution of differences, particularly for Cr-5, precluded calculation of

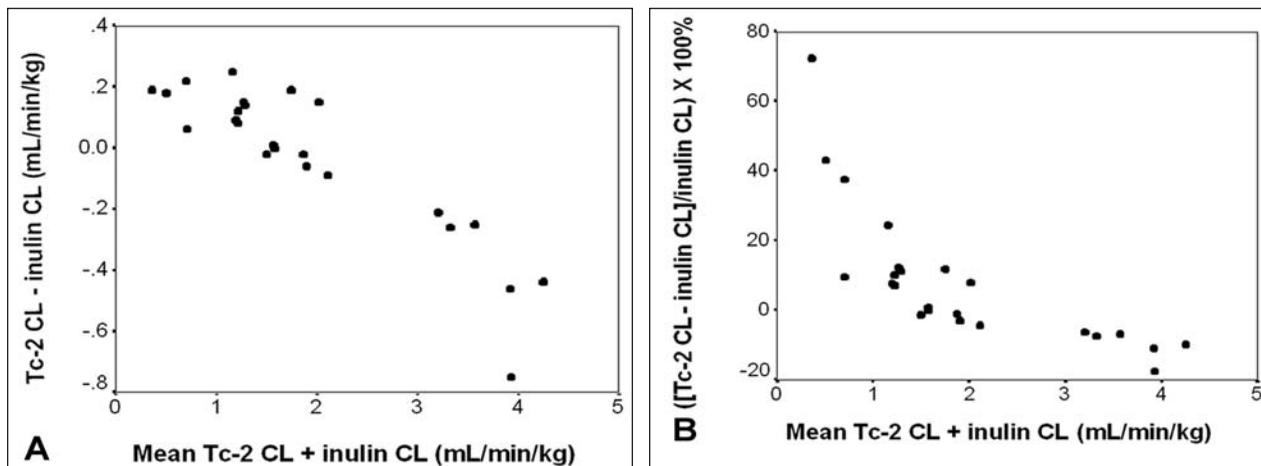


Figure 3—Absolute difference (A) and percentage difference (B) between urinary CL of inulin and plasma CL of <sup>99m</sup>Tc-pentetate with 2-sample (Tc-2) definition of AUC in 24 dogs. Notice scale differences between Figures 2 and 3.

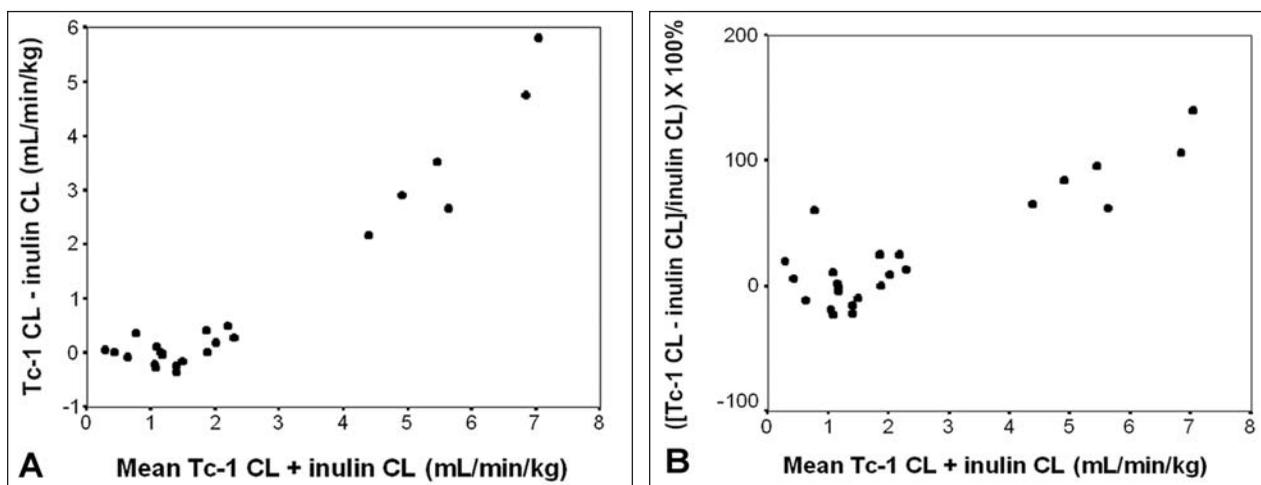


Figure 4—Absolute difference (A) and percentage difference (B) between urinary CL of inulin and plasma CL of <sup>99m</sup>Tc-pentetate with 1-sample (Tc-1) definition of AUC in 24 dogs. Notice scale differences between Figures 2 and 4.

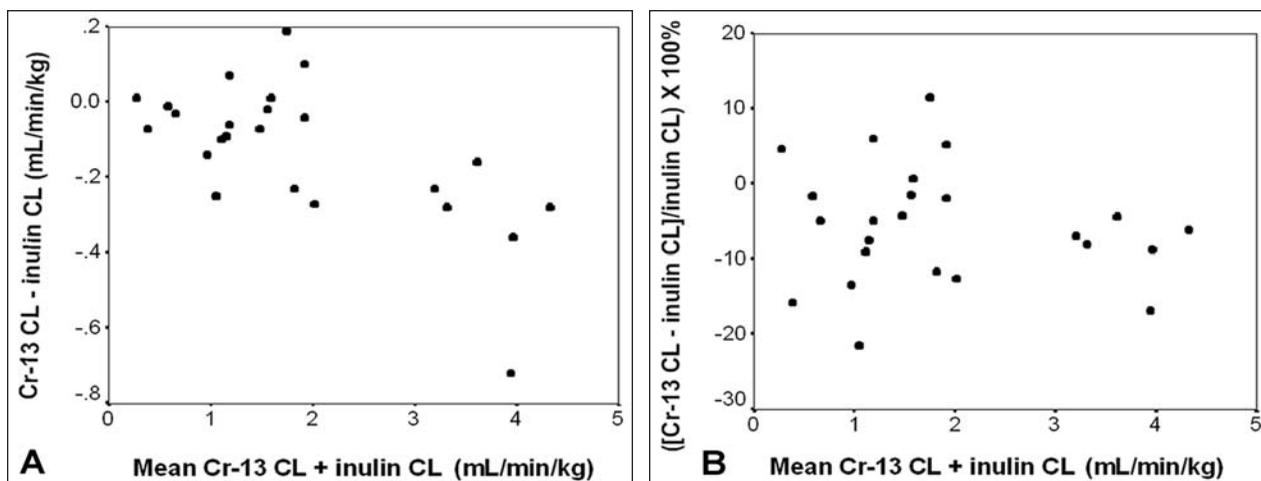


Figure 5—Absolute difference (A) and percentage difference (B) between urinary CL of inulin and creatinine CL with 13-sample (Cr-13) definition of AUC in 24 dogs.

repeatability coefficients as recommended by others.<sup>7,8</sup> For UCI, percentage differences between first and second clearance determinations were fairly uniformly distributed over the range of clearance values tested (Figure 10).

**Stability of creatinine solution**—A decrease in creatinine concentration occurred in vials after 60 days, and in some vials, crystals were visible. The decrement in creatinine concentration was not attributable to precipitation because warming the solutions

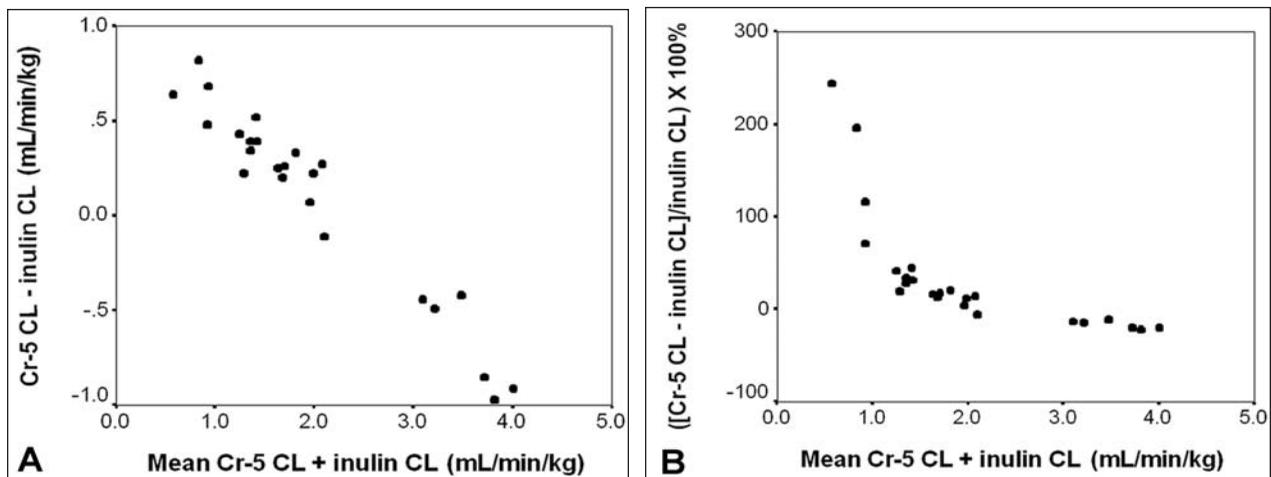


Figure 6—Absolute difference (A) and percentage difference (B) between urinary CL of inulin and creatinine CL with 5-sample (Cr-5) definition of AUC in 24 dogs. Notice the scale differences between Figures 5 and 6.

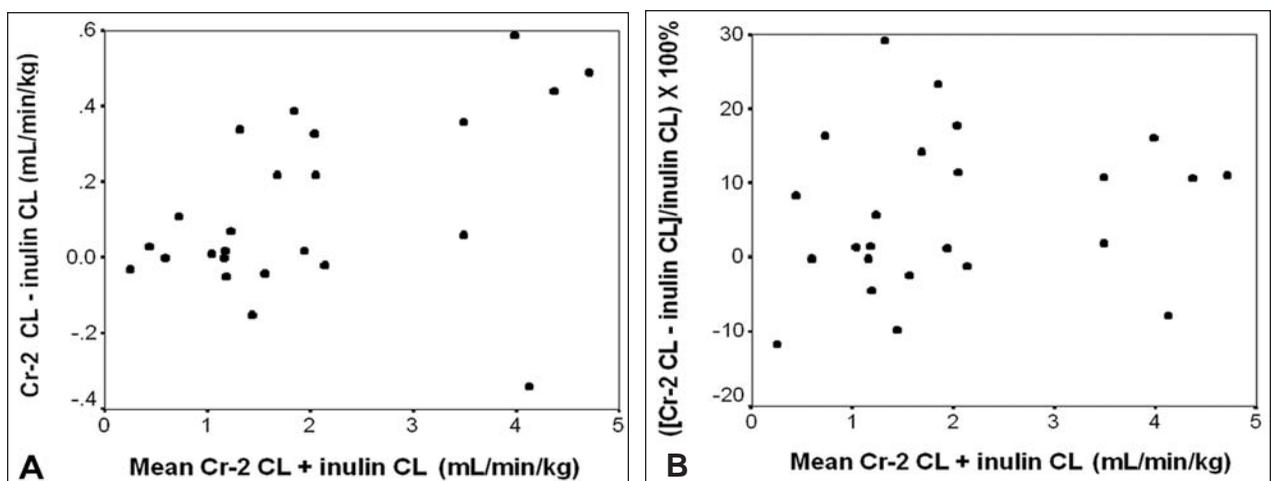


Figure 7—Absolute difference (A) and percentage difference (B) between urinary CL of inulin and creatinine CL with 2-sample (Cr-2) definition of AUC in 24 dogs.

Table 2—Differences between UCI and plasma clearance methods measured in nonazotemic and azotemic dogs.

Measurement	Clearance	All dogs (n = 24)	Nonazotemic (14)	Azotemic (10)
Absolute difference (mL/min/kg)	<sup>99m</sup> Tc-pentetate-13 sample	-0.22 ± 0.30	-0.35 ± 0.33	-0.04 ± 0.07*
	<sup>99m</sup> Tc-pentetate-2 sample	-0.03 ± 0.25	-0.15 ± 0.26	0.14 ± 0.08*
	<sup>99m</sup> Tc-pentetate-1 sample	0.93 ± 1.73	1.61 ± 2.02	-0.02 ± 0.18*
	Creatinine-13 sample	-0.13 ± 0.19	-0.19 ± 0.22	-0.04 ± 0.06
	Creatinine-5 sample	0.10 ± 0.51	-0.18 ± 0.48	0.49 ± 0.18*
	Creatinine-2 sample	0.13 ± 0.22	0.19 ± 0.27	0.04 ± 0.12
Percentage difference†	<sup>99m</sup> Tc-pentetate-13 sample	-8.0 ± 9.5	-10.9 ± 7.4	-4.0 ± 10.8
	<sup>99m</sup> Tc-pentetate-2 sample	7.8 ± 19.9	-2.9 ± 8.0	22.8 ± 22.1*
	<sup>99m</sup> Tc-pentetate-1 sample	25.8 ± 45.1	42.0 ± 50.1	3.2 ± 24.1*
	Creatinine-13 sample	-5.6 ± 7.8	-6.3 ± 8.4	-4.6 ± 7.2
	Creatinine-5 sample	33.9 ± 65.8	-0.39 ± 16.5	82.0 ± 79.1*
	Creatinine-2 sample	6.0 ± 10.4	7.2 ± 9.8	4.2 ± 11.6

Values are mean ± SD.  
 \*Significant ( $P < 0.05$ ) difference between values for nonazotemic and azotemic groups. †Percentage difference = (plasma clearance - UCI)/UCI × 100%.  
<sup>99m</sup>Tc-pentetate = Technetium Tc 99m pentetate.

Table 3—Lower and upper limits of agreement (95% confidence interval [CI]) for percentage differences between plasma clearance methods and UCI in 14 nonazotemic dogs.

Clearance method	Limits of agreement (95% CI)	
	Lower limit	Upper limit
<sup>99m</sup> Tc-pentetate-13 sample	-10.9 (-32.9 to -18.1)	7.4 (-3.7 to 10.9)
<sup>99m</sup> Tc-pentetate-2 sample	-18.6 (-23.4 to -10.7)	12.6 (4.7 to 20.4)
<sup>99m</sup> Tc-pentetate-1 sample	-56.5 (-106.0 to -6.9)	140.2 (90.7 to 189.8)
Creatinine-13 sample	-22.8 (-31.1 to -14.5)	10.2 (1.9 to 18.5)
Creatinine-5 sample	-32.7 (-48.9 to -16.4)	31.7 (15.5 to 47.9)
Creatinine-2 sample	-12.0 (-21.6 to -2.4)	26.1 (16.6 to 35.7)

See Table 2 for key.

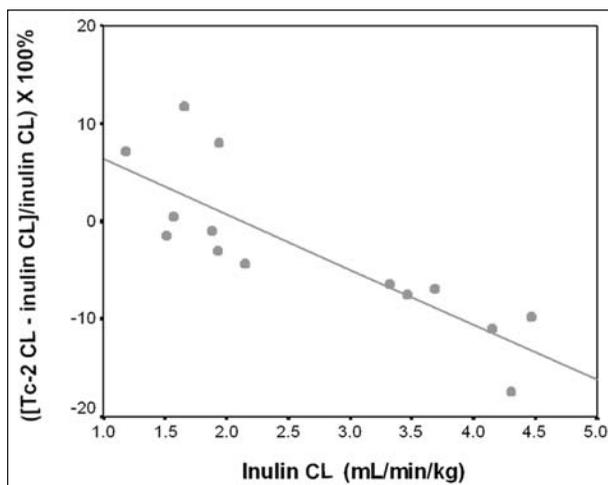


Figure 8—Association between 2-sample plasma CL of <sup>99m</sup>Tc-pentetate acid and urinary CL of inulin in 14 nonazotemic dogs ( $R^2 = 0.68$ ;  $P < 0.05$ ).

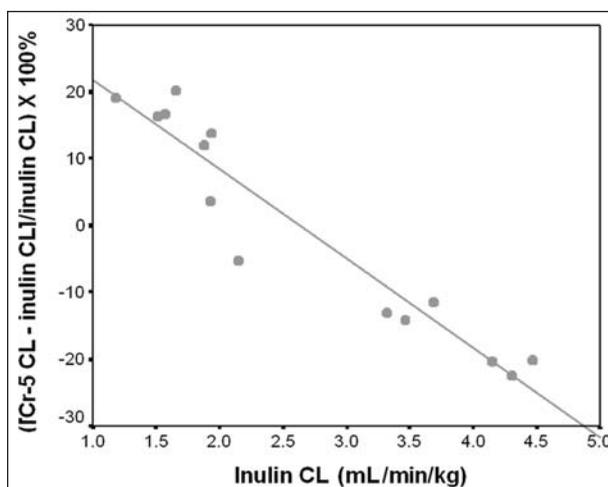


Figure 9—Association between 5-sample plasma CL of creatinine and urinary CL of inulin in 14 nonazotemic dogs ( $R^2 = 0.93$ ;  $P < 0.05$ ).

at 60°C until crystals were dissolved left the decrement unchanged. Bacteriologic culture of creatinine solution revealed no growth. Between 90 and 150 days after preparation, creatinine concentration had declined from 80 mg/mL to a range of 66 to 70 mg/mL.

Table 4—Repeatability of clearance methods in 12 dogs.\*

Clearance method	Ratio	Minimum–maximum
Urine inulin	1.01 ± 0.09	0.84–1.16
<sup>99m</sup> Tc-pentetate-13 sample	1.02 ± 0.06	0.91–1.12
<sup>99m</sup> Tc-pentetate-2 sample	1.03 ± 0.11	0.87–1.27
<sup>99m</sup> Tc-pentetate-1 sample	1.01 ± 0.16	0.69–1.24
Creatinine-13 sample	1.02 ± 0.13	0.88–1.34
Creatinine-5 sample	0.89 ± 0.10	0.71–1.05
Creatinine-2 sample	1.00 ± 0.13	0.84–1.34

\*Inulin clearance = 1.01 to 4.29 in the 12 dogs (See Figure 10).  
Ratio = First clearance value/second clearance value (± SD of ratios). The second clearance measurement was made at least 1 week after the first.  
See Table 2 for remainder of key.

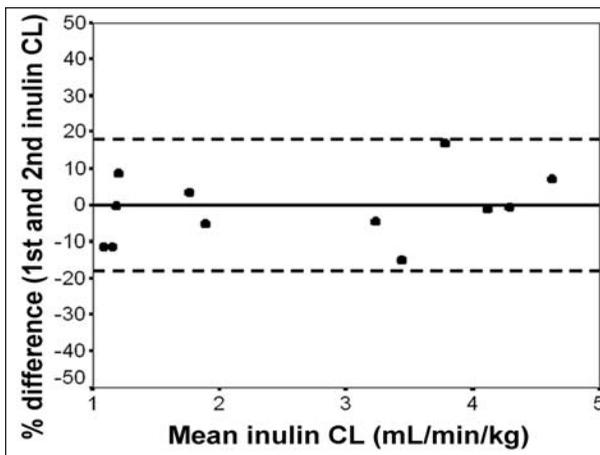


Figure 10—Reproducibility of urine inulin CL values obtained in 12 dogs on different days with different commercial sources of carboxyl <sup>14</sup>C-inulin. Solid and dotted lines represent mean ± 2 SDs of the percentage difference between tests.

## Discussion

Urinary clearance of inulin is the most widely used standard against which other measures of GFR are judged.<sup>2,3</sup> Inulin is metabolically inert, traverses the glomerular barrier as freely as water, and is neither absorbed nor secreted by renal tubules.<sup>10</sup> For accurate measurements of UCI, plasma inulin concentration must be known for each instant of time during which urine is collected. This requirement is met if plasma inulin concentration is kept constant during all urine collections; then only 1 plasma determination would be required for clearance computations. In practice, this ideal can be approached but it cannot be perfectly attained. Practices used to approach the ideal include obtaining more than 1 plasma inulin measurement during each collection period to better estimate plasma concentration and making several urine collections to compare results from each dog for reproducibility.

A potential error in UCI measurements exists because of the dead space represented by the volume of renal pelvis and ureters. Dye injected IV in dogs appears in bladder urine approximately 2.5 minutes later,<sup>10</sup> so inulin collected in bladder urine at any instant represents material filtered a few minutes earlier. Abrupt changes either in plasma concentration of inulin or in urine flow rate could affect accuracy of clearance measurements. Potential dead-space error is minimized by the use of an experimental protocol that

avoids fluctuations in plasma inulin concentration and urine flow rate and by the use of long time periods for urine collection so that dead-space time is a small percentage of collection time.

Technical error in urine collection is often cited as a disadvantage of urine clearance methods. This error occurs when inexperienced personnel perform bladder rinsing or when voluntary voiding without bladder rinsing is used for urine collection.

In the present study, potential errors in urine clearance procedures were minimized or avoided. Plasma inulin concentration in each dog was quite constant, and individual clearance periods were bracketed with measurement of plasma inulin concentration. Urine was collected in a manner that minimized error, and results from consecutive clearance periods were in good agreement. For repeated studies on 12 dogs, better reproducibility occurred for UCI than for all but Tc-13 of the plasma methods. These procedures and results support the author's contention that UCI measurements made in the present study were valid measures of GFR and were a reliable standard by which to judge the plasma clearance methods.

In dogs, some fluctuation in GFR may occur because of several factors, such as state of hydration, food intake, and adrenergic stimulation.<sup>10-12</sup> These fluctuations may mask clearance differences between test materials. Simultaneous measurement of clearance on test materials eliminates time-related factors, so this technique is recommended as a discriminating method for detecting clearance differences between materials.<sup>10,13</sup> In the present study, UCI was measured over a 2.5-hour period coincident with plasma clearance methods. However, plasma samples were collected as much as 85 minutes before and 360 minutes after UCI measurements for Tc-13 and Cr-13 decay curves. One Tc-2 sample and 4 Cr-5 samples also were obtained outside the time of UCI measurements. It could be argued that GFR changes before and after UCI measurements related to changes in hydration state or other factors contributed to clearance differences found between UCI and these plasma methods.

Regarding state of hydration, the present study was conducted over a period of more than 10 hours and potential for dehydration existed. Dogs initially were given water by gavage, received fluids by bolus injection and constant infusion of test materials, and had water available ad libitum between 2.5 hours and the end of the experiment. However, measurement of water intake and urine output, respiratory losses, and insensible skin losses were not conducted, so the possibility of altered fluid balance during the course of the experiment cannot be excluded.

The compound <sup>99m</sup>Tc-pentetate is reported to be a reliable marker for estimation of GFR in humans, dogs, cats, and horses,<sup>14</sup> and it is available for such use at several veterinary institutions.<sup>15</sup> However, despite seemingly wide acceptance, review of supporting literature leads to questions about the validity of its endorsement. In 1 study,<sup>16</sup> renal clearance was measured by determining differences in aortic blood concentration and renal venous blood concentration of <sup>99m</sup>Tc-pentetate. The authors concluded that <sup>99m</sup>Tc-pentetate was

excreted by glomerular filtration because clearance values were similar to those that other authors had reported for inulin.

In another study<sup>17</sup> conducted on 12 dogs, GFR was measured by determining UCI, and within 72 hours, nuclear imaging was performed to determine renal uptake of <sup>99m</sup>Tc-pentetate. Regression analysis of UCI versus percentage uptake ( $R^2 = 0.88$ ) was used to devise a formula for computation of GFR by use of nuclear imaging.

In a study<sup>18</sup> of 7 dogs, UCI was measured simultaneously via plasma clearance of <sup>99m</sup>Tc-pentetate (8 sample, 2-compartment model) and via percentage renal uptake of <sup>99m</sup>Tc-pentetate as measured by use of scintigraphy. The authors reported that UCI correlated better with plasma <sup>99m</sup>Tc-pentetate clearance than with renal <sup>99m</sup>Tc-pentetate imaging but concluded that their results should be considered preliminary because of the small number of dogs studied.

One study<sup>19</sup> in which UCI and urine <sup>99m</sup>Tc-pentetate clearance were measured simultaneously in 4 dogs revealed no significant difference in clearance of the 2 compounds. However, in another study<sup>20</sup> of 8 dogs, urinary clearance of exogenous creatinine and <sup>99m</sup>Tc-pentetate was determined simultaneously and <sup>99m</sup>Tc-pentetate clearance was 87.6% of creatinine clearance, which was significantly different. In another study<sup>21</sup> of 8 dogs, urinary clearance of <sup>99m</sup>Tc-pentetate was  $27 \pm 3$  mL/min, compared with  $33 \pm 4$  mL/min for simultaneously determined UCI.

Studies comparing urinary clearance of test compounds do not address the matter of extrarenal clearance, which must be considered when plasma clearance methods are used. For a material fulfilling requirements for measuring GFR by use of urinary clearance, plasma clearance exceeds GFR by the amount of extrarenal clearance. The error increases as GFR decreases because extrarenal clearance becomes a progressively larger percentage of total clearance. The author is unaware of studies in which extrarenal clearance of <sup>99m</sup>Tc-pentetate has been evaluated in dogs.

Others researchers have performed studies on the basis of suspicion that <sup>99m</sup>Tc-pentetate clearance differed from that of other markers of GFR. In 2 studies,<sup>22,23</sup> the authors concluded that deterioration of the contents of some brands of commercially available <sup>99m</sup>Tc-pentetate kits caused reduced clearance values. In another study,<sup>24</sup> plasma protein binding of <sup>99m</sup>Tc-pentetate and clearance of both <sup>99m</sup>Tc-pentetate and another marker of GFR were measured. Lower clearance values for <sup>99m</sup>Tc-pentetate were obtained, but the values were equivalent when results were corrected for protein binding. In a study<sup>25</sup> published in 2001, it was reported that 9.25% to 11.12% of <sup>99m</sup>Tc-pentetate was bound to human plasma proteins and this amount of binding was similar to plasma protein binding of chromium 51 EDTA and iodine 125 iothalamate. An article<sup>15</sup> that reviewed GFR in dogs included reference to several studies in which <sup>99m</sup>Tc-pentetate, chromium 51 EDTA, and iodine 125 iothalamate have been used as markers of GFR. It is unclear how results from these studies were affected by plasma protein binding of the markers.

In the present study, absolute and relative (percentage difference) comparisons of UCI with Tc-13

were not consistent over the range of clearance values studied. Lower values for Tc-13 at higher clearance values could have been caused by nonfiltration of protein-bound  $^{99m}\text{Tc}$ -pentetate. The changing relationship between UCI and Tc-13 as clearance declined could be attributable to extrarenal clearance of  $^{99m}\text{Tc}$ -pentetate. Simultaneous measurement of inulin and  $^{99m}\text{Tc}$ -pentetate under conditions of constant infusion of both markers in dogs with normal and compromised renal function will be required to resolve this issue. The single-injection technique used in the present study precluded accurate measurement of urinary clearance of  $^{99m}\text{Tc}$ -pentetate because rapidly decreasing plasma concentrations of  $^{99m}\text{Tc}$ -pentetate concentration violated acceptable standards for measuring urinary clearance.

Creatinine has been used for more than 50 years for measurement of GFR with urinary clearance methods in dogs,<sup>10</sup> but certain shortcomings have been reported. Creatinine is freely filtered, but weak tubular secretion in male dogs was detected by use of the stop-flow technique.<sup>26,27</sup> However, UCI and urine exogenous creatinine clearance were the same, even in dogs with reduced GFR, and it was concluded that under free-flow conditions, creatinine was a reliable marker of GFR in dogs of both sexes.<sup>28</sup> The alkaline picrate method of creatinine assay gives spuriously high values for plasma creatinine concentration because several noncreatinine materials react positively.<sup>4</sup> These materials are not excreted in urine, so urine clearance calculations underestimate GFR.<sup>4</sup> Error attributable to analytical methods was resolved initially by use of exogenous creatinine to reduce nonspecific plasma reactants to a negligible percentage of total reactants.<sup>10</sup> More recently, when an enzymatic method of creatinine analysis was used, UCI and urinary endogenous creatinine clearance values in dogs were found to be in good agreement.<sup>29</sup>

For urinary clearance of creatinine to be identical to plasma clearance, extrarenal creatinine clearance must be negligible. In 1 study,<sup>6</sup> 83% to 113% of an IV bolus of creatinine was recovered in urine and these data were interpreted to indicate that nonrenal excretion of creatinine was unimportant. Some evidence exists for creatinine degradation by colonic bacteria in human patients with renal failure,<sup>30</sup> but enteric loss apparently has not been examined in dogs with azotemia.

In the present study, Cr-13 values were somewhat less than UCI. The large percentage of AUC determined by extrapolation from terminal plasma creatinine measurements is a potential source of error in Cr-13 clearance calculations and is a possible explanation for the difference. The stability of creatinine solutions used for clearance determinations also must be considered because degradation over time was found in the present study. Nevertheless, the percentage difference between Cr-13 and UCI was fairly consistent over the range of clearance values tested, indicating an advantage of Cr-13 determination, compared with Tc-13, as a relative marker of GFR.

For both creatinine and  $^{99m}\text{Tc}$ -pentetate, a highly significant association existed between plasma clearance determined on the basis of 13 samples and plas-

ma clearance determined on the basis of limited plasma samples. However, the same absolute and percentage difference values were not obtained. Undoubtedly, 13 samples is superior to lesser numbers for characterization of the plasma decay curve, so it is not surprising that several limited-sample results were inferior. For Tc-2, deviation from UCI was much greater at low GFR values than with Tc-13. Differences between UCI and Tc-1 plasma clearance values were so large and unpredictable that results of the present study do not support use of Tc-1 even as a relative indicator of renal function. The progressive overestimation of UCI by Cr-5 as clearance declined was likely caused by exclusion of AUC beyond 600 minutes in clearance calculations. Because percentage AUC beyond 600 minutes would be expected to increase as clearance declined, clearance (dose/AUC) would increase relative to UCI. The seemingly better predictability of Cr-2 than Cr-5 for measuring GFR was probably attributable to the choice of a time pair with a low mean  $\pm$  SD difference from UCI. Considering that large differences in clearance values existed between UCI and other time pairs, the reliability of the Cr-2 method should be questioned.

Inulin is distributed within the extracellular fluid compartment, and 1 study<sup>31</sup> in humans revealed that the  $V_{ss}$  of  $^{99m}\text{Tc}$ -pentetate and inulin is similar. Values for  $V_{ss}$  for  $^{99m}\text{Tc}$ -pentetate determined in the present study also were consistent with an extracellular distribution. By contrast, creatinine is known to permeate cell membranes and be distributed in volumes consistent with total body water content.<sup>10</sup> The  $V_{ss}$  for creatinine obtained in the present study was similar to other values reported for dogs.<sup>6</sup> The difference in  $V_{ss}$  of the 2 test substances explained the slower decay curve that existed for creatinine and indicated the need for a longer duration of sampling to minimize extrapolation for determining AUC.

Results from the present study reaffirmed that linear regression analysis may be insensitive for detecting differences between test results. In the present study, a significant association existed between UCI and values for all plasma clearance methods tested. Likewise, comparison of 13-sample computations with limited-sample computations resulted in significant associations. The graphic method of comparison used in the present study was more discriminating for identifying test differences over the range of clearance values studied. In instances in which a uniform difference existed, limits of agreement and confidence intervals for those computations provided a basis for test comparisons. In the present study, several clearance comparisons revealed that a uniform difference between tests did not exist over the range of clearance values tested, detracting from the meaning of limits-of-agreement computations. However, in the present study, even when analyses were restricted to nonazotemic dogs, differences between UCI and plasma clearance values existed.

Regression equations can be used as correction factors to modify results from a new test to comply with a standard test. Such corrections may be valid if identical methods are used as when the formula was generated.

However, subtle technical differences within or between laboratories may invalidate the correction factors. In comparing  $^{99m}\text{Tc}$ -pentetate renal imaging with UCI, others have stated that use of a regression equation established in a different laboratory should be used with caution.<sup>18</sup> The present study provided evidence to extend that caveat to use of limited plasma sampling methods for clearance determinations as well, including results from Cr-2 generated in the present study.

The question of whether agreement between an accepted method of measurement and a new test is sufficient for acceptance of the new test has been stated to be a matter of judgment, depending on the use made of the test.<sup>7,8</sup> One scenario is the use of such tests to provide guidance in the management of individual patients, whether to detect dysfunction or monitor changes in function. The high correlation between UCI and Tc-13, Tc-2, Cr-13, Cr-5, and Cr-2 and their reproducibility suggested that these plasma clearance tests may serve a useful function when results of serial measurements are interpreted in the context of that specific patient. Conversely, the mediocre correlation between UCI and Tc-1 and its less reliable repeatability made it a poor candidate even for use in managing individual clinical patients.

Standards for acceptance of a test as a measure of GFR would presumably be higher for clinical research or basic research, compared with patient management, because quantitative results from 1 study may be extrapolated to others. Results from the present study did not support the reporting of the plasma clearance results as measures of GFR. In reviews reporting GFR values in clinically normal dogs, a wide range of values are documented and 1 conclusion is that GFR is highly variable in dogs. Another conclusion is that highly variable results are attributable to error introduced by different methods of clearance measurement used in different laboratories. It may be inadvisable to indicate that any method measures GFR unless the method has been simultaneously compared with UCI or another equally validated and accepted urinary clearance method.

- a. Athens Isotopes, Athens, Ga.
- b. Anhydrous creatinine (C-4255), Sigma Chemical Co, St Louis, Mo.
- c. Carboxyl  $^{14}\text{C}$ -inulin, Perkins-Elmer Inc, Boston, Mass.
- d. Carboxyl  $^{14}\text{C}$ -inulin, Amersham Biosciences, Piscataway, NJ.
- e. Hitachi 912, Roche Diagnostics Corp, Indianapolis, Ind.
- f. WinNonlin, version 4.1, Pharsight Corp, Mountain View, Calif.
- g. SPSS Base 9.0, SPSS Inc, Chicago, Ill.

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