A simple method for measuring glomerular filtration rate in dogs

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SUMMARY

A technique for measuring glomerular filtration rate (by six-sample plasma clearance) and extracellular fluid volume is described. The results obtained for glomerular filtration rate per extracellular fluid volume with a three blood sample technique are also presented. Concurrently with the blood sample techniques measuring plasma clearance, glomerular filtration rate per extracellular fluid volume was also measured using an external radiation detector and no blood samples. The validity of expressing glomerular filtration rate per extracellular fluid volume (rather than per body weight or surface area) and the clinical utility of the results obtained with the external radiation detector are discussed.

GLOMERULAR filtration rate (GFR) is generally regarded as the most informative indicator of renal function, despite its minor imperfections in chronic renal failure (Levey 1990), but it has not been easy to measure it accurately and conveniently. The renal clearance of inulin has been used as the gold standard; inulin is a small molecule freely filtered at the glomerulus and neither secreted nor reabsorbed by the renal tubule and so fulfils the theoretical criteria for a GFR marker. Nevertheless, it has practical limitations in clinical cases, including the need for a constant intravenous infusion and a timed urine collection during a period of stable plasma inulin. Furthermore, it is not easy to obtain, sterilise or analyse.

Creatinine clearances have been used for the clinical assessment of GFR, especially in dogs (Finco et al 1981). Since creatinine is a normal plasma constituent, its clearance may be measured endogenously or, more accurately, with exogenous creatinine given as a single dose. This measurement still has the disadvantage of requiring a timed and complete urine sample, involving the use of metabolism cages and catheterisation and rinsing out of the bladder. An incomplete collection of urine seriously affects the accuracy of the results. This problem can be overcome by using an index of GFR, the plasma disappearance of injected creatinine, instead of a true measurement (Labato and Ross 1991). An even more indirect index of GFR during progressive renal disease is the rate of change of reciprocal creatinine concentrations (Allen et al 1987) but they are insensitive and imprecise in detecting early changes and in monitoring the progress of the disease (Walser et al 1989, Levey 1990). Plasma creatinine concentration only rises significantly in the advanced stages of renal disease.

In human beings, such tests have been largely superseded by tests using radiolabelled filtration markers which are very easy to measure. Technetium-99m diethylene-triamine-pentaaetic acid (99mTc-DTPA) is a well established marker for the measurement of human GFR. Kits are available for the preparation of 99mTc-DTPA and their widespread use suggests that they can be used to measure GFR accurately (Sampson and Keegan 1985). The short half-life of 99mTc (six hours), makes it relatively safe for routine use in out-patients. It has been shown to give equivalent results to inulin clearances in dogs (McAfee et al 1981) and human beings (Perrone 1992). Like inulin, DTPA becomes distributed into a volume (‘inulin space’, ‘DTPA space’) which approximates to that of the extracellular fluid (ECF). 99mTc-DTPA has also been used to measure GFR in dogs, cats and horses by using data derived either from a renogram (Krawiec et al 1986, 1988, Uribe et al 1992, Walsh and Royal 1992) or from plasma disappearance rates (Rogers et al 1991). A renogram requires a gamma-camera and in human beings the method is generally considered to be less accurate than plasma clearances (Rehling et al 1984, Mulligan et al 1990).

The clearance of intravenously injected 99mTc-DTPA from plasma approximates to a bi-exponential curve (Cohen 1974). The first component corresponds to the distribution of the marker throughout the ECF and the second component corresponds to renal clearance, as has been shown in a variety of species (Robbins 1984, Walsh and Royal 1992). Groth et al (1983) and Peter (1992) have shown that the slope of the second exponential component is approximately equal to GFR relative to ECF volume (ECFV). This relationship is strong at low values of GFR but becomes weaker at higher values of GFR. For clinical purposes, this is advantageous because the measurement of GFR is most important when it is low. ECF volume (DTPA space) can also be calculated.

A major advantage of using the ratio GFR/ECFV is the comparatively simple mathematics involved in deriving it. Only three blood samples are needed and the dose of tracer does not need to be measured. It is also possible to derive GFR/ECFV without taking blood samples by relying on the external monitoring of the disappearance of 99mTc-DTPA from extrarenal tissues. Although GFR has generally been normalised to bodyweight in dogs, and to body surface area in man, there are large practical and theoretical advantages to normalisation to ECFV (White and Strydom 1991).

The aims of this study were to assess the value of external monitoring of 99mTc-DTPA activity for measuring GFR and to examine the use of GFR/ECFV as an expression of renal function.
MATERIALS AND METHODS

GFR was measured in 120 dogs by giving an intravenous injection of 50 to 200 MBq of $^{99m}$Tc-DTPA. Dogs with known or suspected renal disease were examined in an effort either to confirm a diagnosis of renal insufficiency or to monitor the progression of renal disease. The dogs were all fasted overnight (to avoid the post prandial rise in GFR) but allowed free access to water during the procedure. Heparinised venous blood samples were taken at 10, 20 and 30 minutes and two, three and four hours after the injection. The dose was measured and a standard prepared with a measured aliquot of the $^{99m}$Tc-DTPA which was diluted and counted at the same time as the blood samples. The blood samples were centrifuged and between 0.1 and 0.25 ml of plasma was counted for one minute in a gamma-counter (Canberra Packard). The activity was expressed in counts ml$^{-1}$.

Between one and four hours after the injection of $^{99m}$Tc-DTPA, an external radiation detector (Scintillation Detector, Oakfields Instruments) was used to measure the disappearance of radioactivity from the dog (resulting from both renal clearance and the radioactive decay) by external counting. The detector was placed over the following three regions at 30-minute intervals between one-and-a-half and four hours after the administration of the $^{99m}$Tc-DTPA (Fig 1). Over the head, the detector was first centred over the middle of the dog's forehead, mid-way between the eyes and ears; it was directed downwards and the dog's neck was extended so that only the head was being counted. It was then centred over the back of the dog's head and directed caudocranially. Over the heart, the detector was centred over the apex beat and directed laterally. These areas were chosen first because the counts were reproducible, and secondly to avoid counts from the kidneys and bladder, thus avoiding the radioactivity which accumulates in the urine during the procedure.

The dogs were taken for a walk in a designated area to encourage them to urinate during the procedure and before they were discharged, and they were sent home about one half-life after the administration of the isotope. The procedure was not performed on dogs which were not house trained and the owners were instructed to take their dogs outside to urinate regularly during the evening.

The curve of the disappearance of the $^{99m}$Tc-DTPA from the plasma was fitted to a biexponential equation:

$$P(t) = A e^{-\alpha_1 t} + B e^{-\alpha_2 t}$$

where $P$ = plasma concentration of $^{99m}$Tc-DTPA at time $t$, $A$ is the intercept and $\alpha_1$ the rate constant for the first exponential component (mainly a reflection of the distribution of the isotope through the ECFV), and $B$ is the intercept and $\alpha_2$ the rate constant of the second exponential component (mainly a reflection of the renal clearance of the isotope).

By using the following equations, the absolute GFR, the ratio GFR/ECFV and the ECFV were calculated. Following the measurement of absolute GFR, the GFR bodyweight$^{-1}$ and body surface area$^{-1}$ were derived.

$$\text{GFR} = \frac{\text{Dose}}{A/\alpha_1 + B/\alpha_2} \quad (1)$$

$$t = \text{The transit time of a filtration marker}$$

$$\text{ECFV} = \frac{\text{Volume of distribution}}{\text{rate of clearance}}$$

$$t = \frac{A/(\alpha_1)^2 + B/(\alpha_2)^2}{A/\alpha_1 + B/\alpha_2} \quad (4)$$

$$\frac{\text{GFR}}{\text{ECFV}} = \frac{A/\alpha_1 + B/\alpha_2}{A/(\alpha_1)^2 + B/(\alpha_2)^2} \quad (5)$$

If the concentration of the marker in plasma is the same as in the extracellular fluid, then the transit time is inversely proportional to the rate constant of the second exponential:

$$t = \frac{1}{\alpha_2}$$

Therefore,

$$\frac{\text{GFR}}{\text{ECFV}} = \alpha_2 \quad (6)$$
Linear regression analysis was applied to the tracer concentrations in the last three blood samples (two, three and four hours) to obtain the rate constant (α₂) of the second exponential, which represents the ratio GFR/ECFV. Linear regression was also applied to the readings of the external detector to obtain an independent estimate of the rate constant of the second exponential.

The relationship between GFR/ECFV and α₂ (from the blood samples or external counting) was non-linear; the relationships between GFR/ECFV and the various measurements of α₂ were therefore fitted to second order polynomials (Brochner-Mortensen 1972, Peters 1992). The ratio of GFR/ECFV to α₂ as a function of GFR/ECFV was plotted to assess the divergence of the latter from α₂ at higher levels of renal function which results from an increasing difference between the concentrations of the tracer in plasma and interstitial fluid. This relationship was fitted with an exponential function (Fig 6).

In all the analyses, correction factors for the radioactive decay of ⁹⁹mTc were included because significant physical decay occurs during the procedure.

Plasma creatinine concentrations were measure on a Gilford Selective Batch Analyser (SBA 300) by the Jaffe method.

### TABLE 1: Polynomial equations describing the relationships between the values of α₂ determined from the last three blood samples and from the external detector and GFR/ECFV (ml min⁻¹ litre⁻¹) determined from six blood samples

<table>
<thead>
<tr>
<th>α₂ derived from</th>
<th>Equation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three blood samples</td>
<td>(-0.326 + 1.146 x + 0.0020 x^2)</td>
<td>0.984</td>
</tr>
<tr>
<td>External detector</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head 1</td>
<td>(-0.335 + 1.090 x -0.0191 x^2)</td>
<td>0.943</td>
</tr>
<tr>
<td>Head 2</td>
<td>(-0.14 + 1.068 x -0.0172 x^2)</td>
<td>0.938</td>
</tr>
<tr>
<td>Heart</td>
<td>(+0.142 + 0.899 x -0.0010 x^2)</td>
<td>0.896</td>
</tr>
</tbody>
</table>

x = GFR/ECFV
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RESULTS

When the plasma creatinine concentration was plotted against the GFR (expressed as GFR/ECFV) increases were observed only at very low levels of GFR (Fig 2). Bearing in mind that the ECF volume is approximately 20 per cent of bodyweight, a GFR/ECFV of 10 ml min⁻¹ litre⁻¹ corresponds to 2 ml min⁻¹ kg⁻¹. The mean (SD) value obtained for the ECFV from the DTPA space in these experiments was 18.9 (0.4) per cent when expressed as a percentage of bodyweight.

The plot of GFR/ECFV against α₂ obtained from the last three blood samples shows a strong but non-linear relationship (Fig 3, Table 1). The relationships between GFR/ECFV (six blood samples) and the α₂ derived from the external detector were also non-linear (Fig 4) and were satisfactorily fitted to a second order polynomial (Table 1). The relationship between the value of α₂ derived from the external detector and the value of α₂ obtained from the last three blood samples was linear and highly correlated (Fig 5, Table 2). This level of correlation was highly significant and shows that the results derived from the external detector gave a clinically satisfactory measurement of α₂. By using the polynomial regression equation, the GFR/ECFV can be derived from α₂ at any level of renal function. A plot of the ratio of GFR/ECFV to α₂ against GFR/ECFV shows that the ratio was close to unity at subnormal levels of GFR/ECFV, when α₂ approximated to GFR/ECFV, but that the values diverged at high levels of GFR/ECFV (Fig 6). The equation of the exponential curve that fits the relationship between GFR/ECFV and α₂ in dogs, human adults and children (Peters et al 1994), as shown by the constants of the exponential increase in GFR/ECFV to α₂ against GFR/ECFV (0-161, 0-147, 0-146 min × 1000) shown in Table 3; the units reflect the expression of ECFV in litres and GFR in ml min⁻¹.

The relationships between the results obtained with the external detector and GFR/ECFV were also well fitted by a second order polynomial (Table 4). Using the polynomial equations given in Tables 1 to 4, the absolute GFR/ECFV can

![Graphs showing linear relationships between values of α₂ and GFR/ECFV for different positions of the external detector.](image-url)

![Table 2: Linear equations describing the relationships between the values of α₂ determined from the last three blood samples and from the external detector.](table-url)

<table>
<thead>
<tr>
<th>Detector</th>
<th>α₂ equation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head 1</td>
<td>0.0225 + 0.936α₂</td>
<td>0.926</td>
</tr>
<tr>
<td>Head 2</td>
<td>0.210 + 0.942α₂</td>
<td>0.918</td>
</tr>
<tr>
<td>Heart</td>
<td>0.114 + 0.868α₂</td>
<td>0.874</td>
</tr>
</tbody>
</table>

![Graph showing relationship between GFR/ECFV and α₂.](image-url)

![Table 3: Relationships between the ratio of GFR/ECFV to α₂ in dogs, human adults and children.](table-url)

<table>
<thead>
<tr>
<th>Species</th>
<th>Relationship</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>GFR/ECFV -1 = 0.0166α₂ GFR/ECFV</td>
<td>0.883</td>
</tr>
<tr>
<td>Adult</td>
<td>GFR/ECFV -1 = 0.01735α₂ GFR/ECFV</td>
<td>0.664</td>
</tr>
<tr>
<td>Child</td>
<td>GFR/ECFV -1 = 0.296α₂ GFR/ECFV</td>
<td>0.621</td>
</tr>
</tbody>
</table>
TABLE 4: Polynomial equations describing the relationship between GFR/ECFV and the value of $\alpha_2$ derived from the external detector from which GFR/ECFV can be derived (ml min$^{-1}$ litre$^{-1}$).

<table>
<thead>
<tr>
<th>$\alpha_2$ derived from</th>
<th>Equation</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last three blood samples</td>
<td>GFR/ECFV = 0.217 + 0.882$\alpha_2$ + 0.0237$\alpha_2^2$ + 0.075</td>
<td></td>
</tr>
<tr>
<td>Head 1</td>
<td>GFR/ECFV = -0.454 + 1.40$\alpha_2$ -0.014$\alpha_2^2$ + 0.034</td>
<td>0.934</td>
</tr>
<tr>
<td>Head 2</td>
<td>GFR/ECFV = -0.132 + 1.32$\alpha_2$ -0.0127$\alpha_2^2$ + 0.031</td>
<td>0.931</td>
</tr>
<tr>
<td>Heart</td>
<td>GFR/ECFV = -0.566 + 1.66$\alpha_2$ -0.0237$\alpha_2^2$ + 0.092</td>
<td>0.902</td>
</tr>
</tbody>
</table>

be obtained from the values of $\alpha_2$ derived either from the three last blood samples or from the external detector at any level of renal function. The correlation between the value of $\alpha_2$ derived from three blood samples and the external detector from which GFR/ECFV was 0.975 (Table 4).

**DISCUSSION**

Routine tests for the detection and evaluation of the severity of canine renal disease include the measurement of blood levels of urea and creatinine. However, the non-linear relationship between plasma creatinine and GFR means that increases in creatinine above the normal range do not occur until much renal function has been lost (Fig 2). In the early stages of renal failure, large changes in function are accompanied by only small changes in plasma creatinine, and as a result renal failure cannot be detected early. Normal plasma creatinine concentration is also affected by an animal’s muscle mass which varies between individuals; moreover, in advanced renal disease, there are changes in the metabolism and renal handling of creatinine, although the extent of these changes in dogs remains uncertain (Robinson et al 1974, Walser et al 1989, Levey 1990).

The relationship between plasma creatinine and GFR/ECFV (Fig 2) confirmed how little change in plasma creatinine occurs until after there has been a significant fall in GFR (Biewenga and Van Den Brom 1981, Levey et al 1988). Although this is well known among nephrologists, its importance is often insufficiently appreciated by practitioners. A creatinine in the high-normal range, ie 140 $\mu$M, may be normal in some dogs but may be associated with a marked reduction in renal function in others. Blood urea concentration is even less satisfactory as an indicator of renal function because its concentration is affected by factors such as pyrexia, liver function, hydration and dietary protein content (Epstein et al 1984).

Although isotopic plasma clearance techniques have been described previously in dogs (Van Den Brum and Biewenga 1981), they have used eight or more blood samples. The present results demonstrate the adequacy of a method based on only three blood samples and the potential value of a method relying solely on external monitoring. GFR has been measured by using an external detector in a small group of cats (Rogers et al 1991), but a blood sample was also required.

The choice of the position of the external detector is particularly important for the accuracy of the measurement of GFR. The correlation coefficients between the values of $\alpha_2$ derived from the three blood samples and the external detector were best when the detector was placed on the head, and these results were much more consistent than those obtained when the detector was directed towards the heart. This is probably because the positions on the head were more reproducible; it is likely that the probe was angled slightly differently towards the heart for each measurement. Furthermore, in very small dogs, it is difficult when directing the detector towards the heart to avoid the kidneys and bladder, which would generate erroneously high counts by including counts from urine as well as plasma.

This method is non-invasive, simple and accurate. Because it uses radio-isotopes, it is unlikely to be widely used in general practice in the foreseeable future, but it would be possible in a referral centre. The measurement would be ideally used as a quantitative assessment of renal function in dogs in which renal disease has been suspected from the result of some other suitable screening test.

The technique presupposes that the ratio GFR/ECFV is a valid way of normalising GFR for body size. This is particularly important in dogs because of the wide range of body sizes in different breeds, ranging from the chihuahua to the St Bernard. In dogs the GFR has by convention been expressed per kg, but in human beings GFR values are usually normalised with reference to the body surface area. In the present experiments, normalising the GFR to the ECFV has the major advantage that the GFR can then be measured directly with the external detector. It also simplifies the mathematics in calculating GFR/ECFV compared with that in calculating GFR kg$^{-1}$. Many methods have been described for calculating the absolute GFR in human beings by using one to three blood samples taken two to four hours after the injection of TC-DTPA; it is not considered clinically practical to take more blood samples (Mulligan et al 1990). It has been shown that the error in using $\alpha_2$ to represent GFR/ECFV is less than the error in calculating GFR absolutely from two to three blood samples taken between two and four hours after an injection of DTPA (Peters 1992).

In addition to these considerations, there is a strong theoretical reason why the GFR normalised with respect to ECFV may be a more suitable way of expressing GFR (McCance and Widdowson 1952, White and Strydom 1991), for it is the role of the kidney to filter the extracellular fluid and regulate its composition and volume (Brochner-Mortensen 1980). Barratt (1985) concluded that the ‘normalisation’ of renal function to body surface area has little theoretical foundation and that the ECFV may be more logical. In human beings the GFR per body surface area indicates that GFR is higher in men than women but expressed per ECFV there is no gender difference (Brochner-Mortensen 1982). In human beings with acromegaly, the absolute GFR is increased but so is the ECFV and as a result the GFR/ECFV remains constant (Peters 1992, 1994). In addition, the ECFV is more closely correlated with lean body mass than with weight (Boer 1984). Thus normalisation with respect to ECFV may be more physiologically correct, in addition to simplifying the procedure. The changes in ECFV which are within the clinically probable range are unlikely to affect the GFR/ECFV measurement grossly unless there is oedema or ascites.

This technique allows renal dysfunction to be diagnosed at an early stage, which may be particularly helpful with familial nephropathies, and it should facilitate the assessment of the progression of renal disease. Such an assessment is essential to the evaluation of the effects of therapeutic regimens or dietary measures on the disease, and such evaluations will be of interest for comparative medicine as well as improving the management of renal disease in companion animals.
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