Optimum Sample Times for Single-Injection, Multisample Renal Clearance Methods

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The best choice of sample times for measuring renal function in adults by single-injection multisample plasma clearance methods was determined by Monte Carlo simulation, using a two-compartment model with parameters chosen to fit average values (from published clinical studies) for each of the three radiopharmaceuticals ^{99m}Tc-MAG$_3$, ^{99m}Tc-DTPA and ^{131}I-orthoiodohippurate. Random errors were added and the simulated data were then fit to a two-exponential model using a weighted nonlinear curve fitting method. The calculated clearance values were compared with the original values to determine random and systematic errors for different selections of sample time for each radiopharmaceutical at various levels of renal function. The results show that for research-level accuracy with a GFR agent such as ^{99m}Tc-DTPA, plasma sampling must begin by 10 min after injection and continue at least 3 hr (in adults). With an ERPF agent such as ^{99m}Tc-MAG$_3$ or ^{131}I-OIH, sampling must begin by 5 min and continue for at least 90 min. Six logarithmically distributed samples are sufficient.

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The classical measurements of renal function have been based on continuous-infusion clearance measurements, using inulin to measure glomerular filtration rate (GFR) or para-aminohippurate (PAH) to measure effective renal plasma flow (ERPF). Accurate urine collection required washing the catheterized bladder with saline to ensure complete collection of each sample. Even so, the reproducibility of individual measurements was poor; the standard deviation was 8.9% for normal volunteers in the hands of an expert (*1*). To improve reproducibility, the measurement was routinely performed at least three times and averaged. The classical studies were performed in normal volunteers but in a clinical population, accurate urine collection may be prevented by dilatation or obstruction of the collecting system or by low urine flow rates. Because of these problems, the classical continuous-infusion methods are not often used in humans even for research studies.

Reliable alternative methods are clearly needed. Single-injection clearance methods have generally been considered less reliable than the continuous-infusion techniques described above, but they are far easier to implement in the clinic, and may actually be more reliable when used under typical clinical conditions. However, the optimum technical procedures for single-injection methods have not been well established.

This paper addresses the question of how best to choose the sample times for single-injection clearance measurement. There has been considerable discussion in the literature of how sample times should be selected for simplified one-sample and two-sample methods, but there has been no such discussion for methods that use three or more samples. Here we treat the multisample case by means of computer simulation, using random errors in the data (matching the known errors of measurement) to estimate the error in the calculated clearance for various sampling schemes.

Representative clearance curves at various levels of renal function were generated from a two-compartment model using average values (from published clinical studies) for each of the three radiopharmaceuticals ^{99m}Tc-MAG$_3$, ^{99m}Tc-DTPA and ^{131}I-orthoiodohippurate (^{131}I-OIH) as model parameters. Random errors were added and the simulated data were then fit to a two-exponential model using a weighted nonlinear curve-fitting program. The calculated clearance values were compared with the original values to calculate random and systematic errors for different sampling strategies for each radiopharmaceutical at various levels of renal function. Finally, results were used to select an optimum sampling protocol.

MATERIALS AND METHODS

Compartmental parameters for the two-compartment model were obtained by averaging values calculated from plasma clearance curves in adults. (Data were scaled to the average 70 kg, 170 cm man before averaging) (*2*). The original plasma clearance studies were described in detail elsewhere: 68 ^{131}I-OIH studies (*3*), 40 ^{99m}Tc-DTPA studies (*4*) and 19 ^{99m}Tc-MAG$_3$ studies (*5*). Results are shown in Table 1, in which V_1 is defined as the volume of that compartment into which the dose is injected and from which activity is excreted (and thus includes the plasma compartment), while V_2 represents the rest of the volume of distribution within the body. The fractional rate constants k_1 and k_2 represent the rates at which activity passes from V_1 to V_2 or from V_2 to V_1

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TABLE 1
Compartmental Models for ^{131}I-OIH, ^{99m}Tc-DTPA and ^{99m}Tc-MAG_3*

	V_1, l	k_1, min^{-1}	k_2, min^{-1}
^{131}I-OIH	10.5	0.045	0.040
^{99m}Tc-DTPA	9.9	0.020	0.031
^{99m}Tc-MAG_3	5.5	0.049	0.049

*Average values from prior studies (*3–5*).

respectively, so that k_1 corresponds to Sapirstein's (*6*) α/V_1. The value of V_2 is not independent but can be calculated from the other values in Table 1. Illustrations and detailed theory can be found in the literature (*6–8*).

The counting interval was assumed to be long enough to ensure that the dominant error in the plasma activity arose from laboratory manipulations and not from Poisson counting error, and was thus proportional to the measured activity instead of to its square root. Gaussian random noise was generated using subroutine GASDEV (*9*) and was added both to the time measurement and to the plasma activity to generate simulated plasma time-activity curves. A value of 0.5 min was used for the standard deviation of the measured time, since times are typically rounded to the nearest minute in clinical practice. A value of 3% of the measured activity was used for the standard deviation of the plasma activity, a value determined in our clinic from 100 MAG_3 plasma samples that were pipetted in duplicate (using the fact that the variance of the difference between duplicates is twice that of a single measurement).

The simulated plasma clearance curves were fit using the program ODRPACK (*10*), which is capable both of ordinary nonlinear least-squares and orthogonal distance regression. (In orthogonal distance regression, the least-squares distance from each data point to the fitted curve is measured not in the usual vertical direction, but in the direction of closest approach of curve to datapoint. This will be at right angles or "orthogonal" to the curve.) For this study, the program was used in the orthogonal distance mode, weighted for the known error variances in the time and in the activity. This is the correct model when both variables contain errors, and is particularly appropriate in the case treated here because it takes into account the large relative error in sample time when sampling begins as early as 5 min after injection. We found the results to be fairly insensitive to the model used and to whether it was weighted. It is likely, therefore, that more conventional means of curve-fitting would give results similar to those reported here.

Each simulation consisted of duplicate runs of 100 clearance curves each. The reproducibility between duplicates is shown in Table 2, but subsequent tables show only the pooled results of duplicate runs.

RESULTS

Initial pilot studies led to the selection of a logarithmic sequence of sample times (that is, one in which the sample times form a geometric progression, e.g., 5 min, 10 min, 20 min, etc.) Other alternatives were explored, including the linear (uniform or constant time interval) distribution, the Chebyshev abscissae and the Chebyshev abscissae on a logarithmic scale. Chebyshev abscissae are clustered near

TABLE 2
Effect of Sampling Sequence on Bias and Standard Deviation for Orthoiodohippurate Clearance at Two Levels of Renal Function*

	One-third normal		Normal function	
	bias	s.d.	bias	s.d.
Linear	−1.1	3.0	−1.6	7.0
	−1.0	3.3	0.0	3.9
Logarithmic	−0.6	3.2	−0.8	4.0
	−0.3	2.7	−0.1	2.6
Chebyshev	−1.1	4.2	−4.5	8.4
	−0.5	3.6	−0.7	4.4

*Bias and standard deviation are expressed as % of normal clearance for duplicate runs of 100 simulations each. Conditions: six samples from 5 to 90 min.

the ends of the sampling interval and give best fits to polynomial curves. They were considered here for the double exponential fit on the grounds that the early and late exponential components might be better measured by more frequent sampling at the ends of the interval. Examples are shown in Table 2, which also illustrates the variation between duplicate 100-curve simulations. It can be seen from Table 2 that the logarithmic sampling sequence gave the best average results in each case. Statistical significance is not obvious from the duplicate runs, but was confirmed by application of the squared rank test (*11*) to several examples from Table 2. (The squared rank test compares the precision of the estimates. It is analogous to the F-test but a distribution-free statistical test was required since the distribution of results from the simulation was clearly not Gaussian.) The logarithmic sampling sequence was significantly better ($p < 0.05$) in four of eight patients tested and was inferior in no case so it was employed for all subsequent calculations.

The effect of the sampling interval on the precision of measurement is shown in Figure 1 for all three agents at three levels of renal function. Each data point represents the mean of 200 random data sets, in which nine plasma samples were obtained at logarithmic intervals (forming a geometric progression) over the specified sampling interval. To provide a standard scale for comparing the different agents, the results were expressed as a percent of normal clearance for each radiopharmaceutical. (For simplicity, the following rounded values were chosen as normal: 120 ml/min for ^{99m}Tc-DTPA, 300 ml/min for ^{99m}Tc-MAG_3 and 600 ml/min for ^{131}I-OIH.)

For each plot in Figure 1, the central data represent the longest sampling interval. The effects of stopping data collection earlier are shown on the left side of the graph and the effects of starting data collection later are shown on the right. Curves are shown for three different levels of renal function.

Every case shows that the error increases if the interval begins too late or if it ends too soon. For the glomerular

agent ^{99m}Tc-DTPA, good precision requires prolonging the study to at least 3 hr and preferably 4 hr. For the tubular agents ^{131}I-OIH and ^{99m}Tc-MAG$_3$, precision depends both on starting data collection early enough and on continuing for long enough. The time scales are much shorter for the tubular than for the glomerular agents because of their much faster clearance from the blood. At optimal sample times, comparable precision (relative to the normal value for each agent) was obtained for all three agents.

The effect of sampling interval on the bias of the estimate is shown in Figure 2 for all three agents. Once again the error increases if the sampling interval begins too late or ends too soon. Its magnitude varies with the level of renal function. The bias is in the direction of falsely low values, except when renal function is very poor, when truncation of negative clearances to zero creates a positive bias. Statistical bias (Fig. 2) is small relative to the random errors (Fig. 1), and may also be small relative to systemic bias arising from use of a simple two-compartment model to represent a more complex system.

How many samples are needed was also investigated. Since four parameters must be fit, a theoretical minimum of four samples is needed, or five to obtain an estimate of measurement error. Six samples are suggested as a practical minimum. The effect of reducing the sample size from nine to six is shown in Table 3. The precision was found to be less sensitive to the number of samples than to the sampling time; only for the combination of ^{131}I-OIH with normal function was there much difference between six and nine samples. (Why this case should be different is not clear; it was rerun a second time with similar results.) The difference between six and nine samples for ^{131}I-OIH at one-third the normal function can be attributed to chance. (It is only of borderline significance even by the F-test, which is too strong for this non-Gaussian distribution.) Therefore, six samples appear sufficient under most circumstances. It is better to increase the duration of sampling than to increase the number of samples.

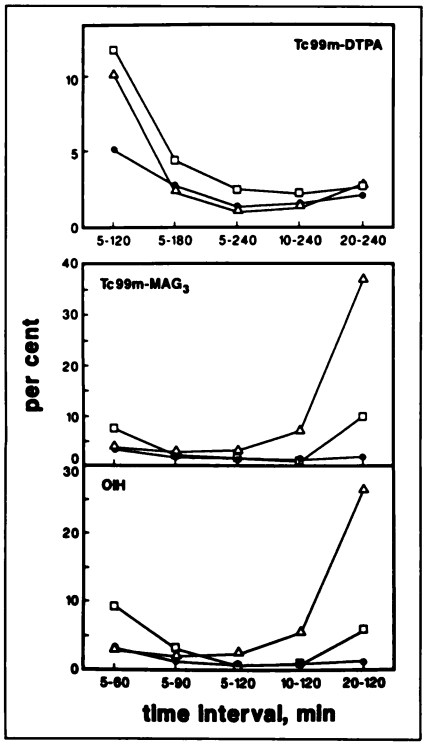

FIGURE 1. Precision of clearance estimate as a function of sampling interval for three radiopharmaceuticals at three levels of renal function (residual standard deviation as % of normal value). Legend: (triangle) normal, (square) one-third normal and (circle) anephric.

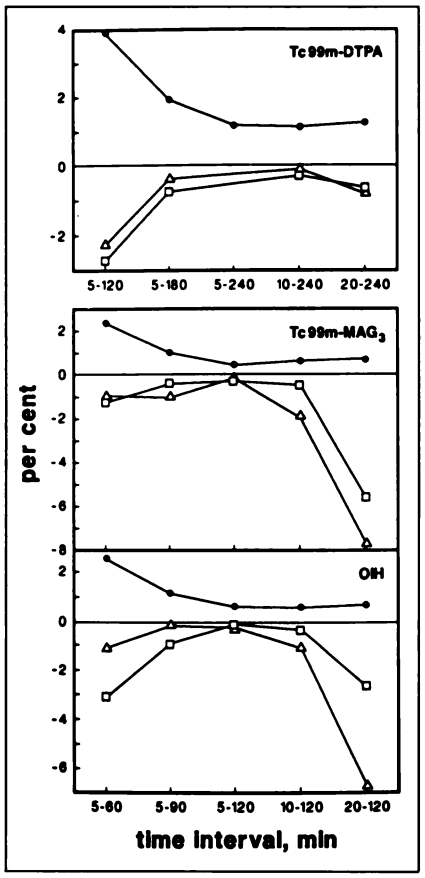

FIGURE 2. Bias of clearance estimate as a function of sampling interval for three radiopharmaceuticals at three levels of renal function (residual standard deviation as % of normal value). Legend: (triangle) normal, (square) one-third normal and (circle) anephric.

DISCUSSION

The renal clearance, for the single-injection urine-free method, is equal to the dose divided by the area under the plasma time-activity curve extrapolated to infinite time (*12–15*). One means of extrapolating and integrating is to fit

TABLE 3
Effect of Changing Number of Samples When the Sampling Interval is Fixed*

	Anephric	One-third normal	Normal
^{131}I-OIH			
6 samples	10.8	17.0	25.4
9 samples	9.4	20.6	13.4
^{99m}Tc-DTPA			
6 samples	1.8	3.4	2.0
9 samples	1.6	2.9	2.0
^{99m}Tc-MAG$_3$			
6 samples	5.2	6.6	10.2
9 samples	4.2	5.6	8.1

*Sampling interval at 5–90 min for ^{131}I-OIH and ^{99m}Tc-MAG$_3$; 5–240 min for ^{99m}Tc-DTPA. Residual standard deviation (ml/min).

TABLE 4
Importance of the Fast Exponential Term in Plasma Clearance: Percent of Total Clearance for ^{131}I-OIH, ^{99m}Tc-DTPA and ^{99m}Tc-MAG$_3$ at Various Levels of Renal Function

	Anephric	One-third normal	Normal
^{131}I-OIH	0%	13%	37%
^{99m}Tc-DTPA	0%	3%	11%
^{99m}Tc-MAG$_3$	0%	10%	31%

the data empirically to the sum of two exponentials, then use the analytic formula for the integral of exponentials. The same mathematical result can be obtained by assuming a two-compartment linear model but no such assumption is necessary, as shown in the cited references. Here we use the two-compartment model simply as an empirical means of generating representative plasma time-activity curves at various levels of renal function for the three agents under study, and of examining the effects of random errors on the clearances calculated from these curves. This is justified by the fact that the two-compartment model closely reproduces the experimental curves. No physiologic significance need be ascribed to the model.

Let the plasma time-activity curve be described by the equation:

$$c = c_1 e^{-\lambda_1 t} + c_2 e^{-\lambda_2 t}, \qquad \text{Eq. 1}$$

where c is the plasma concentration, t the time after injection and c_1, c_2, λ_1 and λ_2 fitted parameters. Integrating to obtain the area under the curve and dividing into the administered dose D gives the clearance F:

$$F = \frac{D}{c_1/\lambda_1 + c_2/\lambda_2}. \qquad \text{Eq. 2}$$

The values of c_1, c_2, λ_1 and λ_2 can be calculated from V_1, k_1 and k_2 as shown by Matthews (*7*); a computer program fragment implementing this calculation has also been published (*2*). The denominator (i.e., the integral of the time-activity curve) contains two terms; one corresponding to the faster of the two exponentials and one to the slower. The relative importance of these terms depends both on the agent used and on the level of renal function, as shown in Table 4. The fast exponential component is more important for the tubular agents than for the glomerular agents. It is most important when function is normal, and shrinks to nothing as the renal function decreases to zero.

From this, one can see why one-compartment models and "terminal slope" methods have persisted for GFR measurement. For the glomerular agent ^{99m}Tc-DTPA, the error introduced by neglecting the fast component is on the order of 11% with normal function and is significantly less when function is impaired. Such errors are acceptable in clinical use although they are unnecessary since more exact methods are available. On the other hand, the fast term cannot be neglected for tubular agents. This may explain why single-injection methods have been more controversial for ERPF measurement with tubular agents than for GFR measurement with glomerular agents. However, Figure 1 shows that there is not much difference in precision between ERPF and GFR when the sampling intervals are chosen correctly.

Figures 1 and 2 can be explained by considering the effects of the slow and fast components. Accurate measurement of the slow component requires measurements long after injection whereas accurate measurement of the fast component requires data soon after injection. The slow component is important for both glomerular and tubular agents so that errors increase if data collection is terminated too early and the curves of Figures 1 and 2 turn up at the left. The fast term is less important for ^{99m}Tc-DTPA than for the tubular agents, so that the upturn on the right is small for ^{99m}Tc-DTPA but large for the tubular agents.

When renal function decreases from normal to anephric, the rate constants λ_1 and λ_2 in Equation 1 both decrease with the slower constant decreasing to zero. With poor function, therefore, the term corresponding to the slower component dominates the denominator of Equation 2. Accurate measurement of the dominant slow component requires measurements long after injection so that one might expect the curves in Figures 1 and 2 to turn up more on the left when function is poor. That they fail to do so is a consequence of the fact that the plots show absolute errors, not relative errors. (If relative errors are plotted, each decrease in function raises the curve, and the relative error approaches infinity as function approaches zero. Urine-free clearance methods should not be used at very low levels of renal function when small percentage changes must be detected.) Poor function, on the other hand, decreases the importance of the fast term (Table 4) so that the curves turn up less on the right when function is poor.

CONCLUSIONS

To calculate clearance from multiple plasma samples, it is more important to cover a wide time interval than to have a large number of samples. When measuring ERPF with ^{99m}Tc-MAG$_3$ or ^{131}I-OIH, it is particularly important to start within 5 min of injection, since the fast component of excretion for these agents contributes significantly to the calculated clearance and can be accurately determined only from early data. When measuring GFR with a filtered agent such as ^{99m}Tc-DTPA, it is particularly important to continue data collection for at least 3 hr and preferably 4 hr, since the slow component is very slow for these agents and contributes most of the calculated clearance. For adults, it is suggested that six or more samples be obtained at sampling times forming a geometric progression between 5 min and 90 min for the ERPF agents and between 10 min and 240 min for the GFR agents.

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