A single-injection method for measuring glomerular filtration rate

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HALL, JOHN E., ARTHUR C. GUYTON, AND BARRY M. FARR. A single-injection method for measuring glomerular filtration rate. Am. J. Physiol. 232(1): F72-F76, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. (1): F72-F76, 1977. A method for estimating glomerular filtration rate (GFR) has been developed that is based on an analysis of the total area under the plasma radioactivity-time curve after a single intravenous injection of \(^{125}\text{I} \text{iothalamate. Glomerular filtration rates obtained by this method (method A) and those obtained with two widely used single-injection techniques, the slope-intercept method (method B), and the two-compartment method (method C), were compared with GFRs obtained by standard inulin clearance techniques in 14 dogs. Method B consistently overestimated inulin clearances more than 30%. Method C also overestimated inulin clearance considerably in dogs with an increased extracellular fluid volume, but was fairly reliable in normal dogs. Glomerular filtration rates obtained by the new method (method A) were in excellent agreement with inulin clearances in all dogs, regardless of the state of body hydration. The mean inulin clearance for all 14 experiments was 72.7 ± 6.0 SE ml/min, while GFRs obtained by method A averaged 75.1 ± 6.0 ml/min. The data from this study suggest that method A is a reliable means for estimating GFR that is especially useful in chronic experiments. [\(^{125}\text{I} \text{iothalamate clearances; single-injection clearances]}

STANDARD CLEARANCE METHODS for estimating glomerular filtration rate (GFR) are tedious and often impractical in chronic experiments. These methods require bladder catheterization and accurate collection of urine samples, as well as continuous intravenous infusions; the repeated bladder catheterizations that are necessary for sequential GFR measurements in long-term experiments often cause bladder infections and pyelonephritis. Furthermore, during oliguria, methods for measuring GFR that require urine collection are unreliable.

To obviate some of these problems associated with standard clearance methods, investigators have developed various single-injection techniques for estimating GFR (1, 2, 6, 8). The two most widely used single-injection methods are the slope-intercept (2, 6, 7) and the two-compartment methods (1, 2, 8). Using the slope-intercept method, it is assumed that after intravenous injection, the indicator is rapidly distributed in a single compartment and that after a given period of time (60-80 min) the indicator is in equilibrium between the plasma and extravascular extracellular fluid. The renal clearance is then derived from the final slope (\(k_1\)) of the plasma disappearance curve and the zero-time intercept concentration (A) of the indicator (2, 6, 7):

\[
GFR = Q k_1/A
\]

where Q is the total amount of indicator injected.

With the two-compartment method, the plasma disappearance curve of the indicator is assumed to approximate a two-component exponential function of the form:

\[
C(t) = Ae^{-k_1 t} + Be^{-k_2 t}
\]

where \(C(t)\) is the plasma concentration of the indicator at a given time (t), A is the intercept of the slow component (at \(t = 0\)), \(k_1\) is the rate constant of the slow component, B is the intercept of the rapid component (at \(t = 0\)), and \(k_2\) is the rate constant of the rapid component. These values are then substituted in the formula developed by Sapirstein et al. (8) to obtain the renal clearance:

\[
GFR = (Q k_1 k_2)/(Ak_2 + Bk_1)
\]

Both the slope-intercept and the two-compartment single-injection methods have been used with success by different investigators (1, 7). However, in animals with expanded extracellular fluid volume or ascites, distribution of the indicator in the extracellular fluid is slow and the plasma disappearance curve often does not fit a simple two-component exponential function. In these animals, both the slope-intercept and the two-compartment single-injection methods tend to overestimate the GFR as determined by inulin clearance (2).

The present study was therefore undertaken to develop a single-injection method for estimating GFR that is reliable regardless of the extracellular fluid...
volume state of the animal. Unlike the slope-intercept and the two-compartment single-injection methods, the method that is described in the present study requires no assumption regarding either the number of compartments in which the indicator is distributed, or the general form of the plasma disappearance curve.

METHODS

In order for any indicator to be useful in measuring GFR, it must satisfy the following criteria: 1) it must not be metabolized or removed from the circulation by any organ other than the kidney; 2) it must be freely filtered through the glomerular capillary membranes (i.e., not bound to plasma proteins or sieved in the process of ultrafiltration); 3) it must not be reabsorbed or secreted by the renal tubules; 4) it must be nontoxic; and it must not alter renal function. Several studies indicate that [125I]iothalamate satisfies all of these criteria (1, 4, 5, 9).

Since iothalamate is excreted only by the kidneys and is neither secreted nor reabsorbed by the renal tubules, the total excretion must equal the total renal clearance. Therefore:

\[ \frac{dQ}{dt} = \text{GFR} \times C(t) \]  

where Q is the total radioactivity of the body at any instant (cpm), and C(t) is the radioactivity per unit of plasma (cpm/ml) at any given time (t). Integration of equation 1 gives

\[ Q = \text{GFR} \int_0^\infty C(t) \, dt \]  

When equation 2 is rearranged, the following expression for GFR is obtained:

\[ \text{GFR} = \frac{Q}{\int_0^\infty C(t) \, dt} \]  

Glomerular filtration rate is determined from the total amount of [125I]iothalamate injected (Q) and from the total area under the plasma radioactivity-time curve from t₀ to t₁. The total area can be divided into two parts (Fig. 1). One part (A₁) corresponds to the total area from t₀ to the time of the last plasma sample (t₁). This area can be determined easily by graphical techniques (e.g., planimeter or counting of squares) or by numerical integration with a computer. Assuming that plasma sampling has been continued long enough for complete distribution of the iothalamate in the extracellular fluid, the decline in plasma radioactivity will be monoeponential and due only to renal excretion. Therefore, when \( t \geq t₁ \), the plasma concentration-time curve can be approximated as:

\[ C(t) = C(t₁)e^{-kt(t-t₁)} \]  

Area A₂ can be calculated by integrating the area under the monoeponential curve from t₁ to t₂:

\[ A₂ = \int_{t₁}^{t₂} C(t) e^{-kt(t-t₁)} \, dt \]  

where k is the rate constant of the monoeponential part of the curve.

An accurate estimate of A₂ can be made only if plasma sampling is continued until the decline in plasma radioactivity is monoeponential. However, since A₁ normally represents the major portion of the total area under the curve, small errors in estimating A₂ will not greatly alter the total area calculated. These small errors in estimating A₂ can be further minimized by prolonging the sampling period. Since

\[ \text{GFR} = \frac{Q}{(A₁ + A₂)} \]  

then

\[ \text{GFR} = \frac{Q}{(A₁ + C(t₁)/k)} \]  

Fig. 1. Plasma radioactivity-time curve obtained after a single intravenous injection of [125I]iothalamate. Area A₁ is determined graphically and area A₂ is determined as:

\[ A₂ = \int_{t₁}^{t₂} C(t₁)e^{-kt(t-t₁)} \, dt = C(t₁)/k \]

In this experiment, last plasma sample was taken at \( t₁ = 140 \) min.

The solution of this equation is:

\[ A₂ = C(t₁)/k \]  

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then

\[ \text{GFR} = \frac{Q}{(A₁ + C(t₁)/k)} \]  

This method, and the two-compartment and slope-intercept single-injection methods, were evaluated in 11 normal dogs which were anesthetized with sodium pentobarbital (30 mg/kg, iv) and in one conscious dog which had a greatly expanded extracellular fluid volume due to a chronic thoracic inferior vena caval constriction. In two additional anesthetized dogs, extracellular fluid volume was increased by intraperitoneal injection of approximately 1.5 liters of isosmotic mannitol.

For comparison with the single-injection methods, glomerular filtration rates were determined simultaneously using the standard inulin clearance method. A priming dose of inulin (50 mg/kg in 50 ml of isotonic saline) was given followed by a continuous intravenous infusion of inulin in isotonic saline (1 ml/min) to establish a plasma concentration of inulin appropriate for measuring GFR. After a 45- to 60-min equilibration
period, approximately 0.5–0.8 μCi/kg body wt of
\([^{125}I]\)iothalamate (Glofil; Abbott Laboratories) in 20 ml
of isotonic saline was injected intravenously and radio-
activity was measured in duplicate arterial plasma
samples (1.0 ml) taken at 1, 5, 10, 20, 40, and 60 min
after injection of the iothalamate, and thereafter at 20-
to 40-min intervals. Duplicate 10-μl samples were also
taken from the \([^{125}I]\)iothalamate injectate to determine
the total amount of radioactivity injected. All radioac-
tivity measurements were carried out on a Searle auto-
matic gamma-well counter (series 1185). Five or six 20-
to 30-min inulin clearances were also obtained after
injection of the iothalamate, and plasma and urine
inulin concentrations were determined by the an-
throne method (3). Glomerular filtration rates ob-
tained by the three different single-injection methods
were compared with the average of the five or six
inulin clearances obtained in each animal.

Standard least-squares regression analysis was used
to compare the single injection GFRs with inulin clear-
ances. In calculating the regression lines, it was as-
sumed that the standard inulin clearance method was
not subject to errors of measurement.

RESULTS

A plasma radioactivity-time curve obtained in a nor-
mally hydrated dog is illustrated in Fig. 2. Distribu-
tion of the iothalamate in the extracellular fluid was
virtually complete within 60 min after injection, and
thereafter the plasma radioactivity curve was monoex-
ponential. In this experiment the plasma disappear-
ance curve was fairly well represented by a two-compo-
nent exponential function. However, in several normal
dogs, and especially in dogs with an increased extracel-
lar fluid volume due to chronic thoracic inferior vena
cava constriction, or because of intraperitoneal injec-
tions of isosmotic mannitol, the plasma disappearance
curve did not fit a simple two-component exponential
function (Fig. 3); in these dogs glomerular filtration
rate was consistently overestimated by the slope-inter-
cept method, and by the two-compartment method.
Glomerular filtration rates obtained by analyzing the
total area under the plasma radioactivity-time curve
were similar to those obtained by inulin clearance even
in the volume expanded animals.

A comparison of the GFRs obtained by the slope-
intercept method with those obtained by inulin clear-
ances is shown in Fig. 4. The slope-intercept method
consistently overestimated the inulin clearance by
more than 30% even in the normal dogs. For all 14
experiments, the average inulin clearance was 72.7 ±
6.0 SE ml/min, while the mean GFR obtained using

![Fig. 2. Semilogarithmic plot of plasma radioactivity-time curve obtained after a single intravenous injection of \([^{125}I]\)iothalamate in a normal dog. Solid dots represent measured plasma radioactivity and circles represent resultant plasma radioactivity obtained by subtracting slow component of curve. Glomerular filtration rates obtained by slope-intercept method, two-compartment method, and single-injection method based on an analysis of total area under curve were 120.6, 93.3 and 89.2 ml/min, respectively. Inulin clearance averaged 90.9 ± 9.3 SD ml/min.](http://ajprenal.physiology.org/)

![Fig. 3. Semilogarithmic plot of plasma radioactivity-time curve obtained after a single intravenous injection of \([^{125}I]\)iothalamate in a dog with ascites due to a chronic thoracic inferior vena caval constriction. Solid dots represent measured plasma radioactivity and circles represent resultant plasma radioactivity obtained by subtracting slow component of curve. Glomerular filtration rates obtained by slope intercept method, two compartment method, and single injection method based on an analysis of total area under curve were 79.2, 69.6, and 56.0 ml/min, respectively. Inulin clearance averaged 50.5 ± 7.7 SD ml/min.](http://ajprenal.physiology.org/)

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SINGLE-INJECTION CLEARANCES

With the slope-intercept method, it is necessary to assume that the indicator is distributed in a single compartment and that mixing is instantaneous. As Chantler et al. (2) have pointed out, this method overestimates the renal clearance by approximately 25%. In the present study, glomerular filtration rate was overestimated by more than 30% when the slope-intercept method was used.

With the two-compartment single-injection method,

the slope-intercept method was $101.5 \pm 8.1\, \text{ml/min}$.

There was fairly good correlation in normal dogs between the GFRs obtained by the two-compartment analysis and by inulin clearance, although the two-compartment method tended to overestimate inulin clearance slightly (Fig. 5). However, in the three dogs with expanded extracellular fluid volume the two-compartment method overestimated the inulin clearance considerably. In the three volume expanded dogs, inulin clearance averaged $67.2 \pm 8.5\, \text{SE ml/min}$, while GFR obtained by the two-compartment method averaged $90.7 \pm 11.0\, \text{SE ml/min}$.

There was excellent agreement between inulin clearances and GFRs obtained by the single-injection method based upon an analysis of the total area under the plasma radioactivity-time curve in normal dogs as well as in dogs with expanded extracellular fluid volume (Fig. 6). For all 14 experiments, the mean inulin clearance was $72.7 \pm 6.0\, \text{SE ml/min}$, and the mean GFR obtained using this single-injection method was $75.1 \pm 6.0\, \text{SE ml/min}$.

DISCUSSION

A method for estimating GFR that is based on an analysis of the total area under the plasma radioactivity-time curve after a single intravenous injection of $[^{125}]$iodothyramate has been described. This method appears to offer a reliable and convenient means of estimating GFR that is superior (especially in animals with an expanded extracellular fluid volume) to the slope-intercept method or methods that utilize a two-compartment analysis of the distribution of the indicator in the extracellular fluid.

With the two-compartment single-injection method,
it is assumed that mixing of the indicator is very rapid and that the plasma disappearance curve of the indicator is approximated by a two-component exponential function. In normal animals, this method appears to be a fairly reliable means of estimating GFR (1, 2). However, in the present study, the plasma disappearance curve of $^{[125]}$iothalamate did not fit a simple two-component exponential function in dogs with an expanded extracellular fluid volume, in which there is a slow mixing of the indicator; in these dogs, the two-compartment analysis consistently overestimated the inulin clearance.

A major difficulty with both the slope-intercept and two-compartment methods is that they assume a fixed number of homogeneous compartments in which the indicator is distributed. This assumption is of course theoretically incorrect although in actual practice the plasma disappearance curve may sometimes be adequately fitted by a given number of exponential functions. Often, however, it is not possible to accurately describe the distribution and excretion of the indicator by the sum of two or even three exponential functions, especially when the extracellular fluid volume is expanded and mixing of the indicator is slow.

The single-injection method described in the present study is based on an analysis of the total area under the plasma radioactivity-time curve after a single intravenous injection of $^{[125]}$iothalamate and requires no assumption regarding the shape of this curve. This approach, which has been used to estimate blood flow through various circulations (10), does not depend on the number of compartments in which the indicator is distributed or whether the various compartments are in equilibrium. Theoretically, this technique should provide an absolute means of measuring GFR provided that 1) enough plasma samples are taken to define the early part of the disappearance curve, 2) sampling is continued until the decline in plasma radioactivity is due only to renal excretion, 3) GFR is relatively constant during the sampling period, and 4) the substance injected is suitable for measuring GFR. Either inulin or radiolabeled iothalamate could be used as an indicator for measuring GFR with this method, but the use of radiolabeled iothalamate requires considerably less laboratory time and is not complicated by interfering substances (i.e., glucose) as with inulin.

The single-injection method is particularly advantageous for chronic experiments since it does not require constant intravenous infusions, bladder catheterization, or urine collection, thus allowing GFR to be measured under relatively undisturbed conditions and eliminating the risk of urinary tract infections in long-term studies. Another advantage of the single-injection method is that it allows accurate measurements of GFR even in oliguric animals, in which standard clearance methods are unsuitable.

This method for calculating GFR, which is based on an analysis of the total area under the plasma disappearance curve of the indicator, may also be useful for determining effective renal plasma flow if para-aminohippuric acid (or some radiolabeled indicator such as [ortho-$^{[125]}$]iodohippurate) is used as the indicator.

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