A single-injection method for measuring glomerular filtration rate

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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)l: 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 [125I]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.

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 (k1) 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:

\[
C(t) = Ae^{-k_1t} + Be^{-k_2t}
\]

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), k1 is the rate constant of the slow component, B is the intercept of the rapid component (at t = 0), and k2 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:

\[
GFR = \frac{Q k_1 k_2}{A(k_1 + Bk_1)}
\]

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
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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 $^{[125]}$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:

$$\frac{dQ}{dt} = GFR \times C(t)$$  \hspace{1cm} (1)

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

$$Q = GFR \int_{0}^{\infty} C(t) \, dt$$  \hspace{1cm} (2)

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

$$GFR = \frac{Q}{\int_{0}^{\infty} C(t) \, dt}$$  \hspace{1cm} (3)

Glomerular filtration rate is determined from the total amount of $^{[125]}$iothalamate injected ($Q$) and from the total area under the plasma radioactivity-time curve from $t_0$ to $t_\infty$. The total area can be divided into two parts (Fig. 1). One part ($A_1$) corresponds to the total area from $t_0$ to the time of the last plasma sample ($t_1$). 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 $t \geq t_1$, the plasma concentration-time curve can be approximated as:

$$C(t) = C(t_1)e^{-kt}$$  \hspace{1cm} (4)

Area $A_2$ can be calculated by integrating the area under the monoeponential curve from $t_1$ to $t_\infty$:

$$A_2 = \int_{t_1}^{\infty} C(t_1)e^{-kt} \, dt$$  \hspace{1cm} (5)

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

![FIG. 1. Plasma radioactivity-time curve obtained after a single intravenous injection of $^{[125]}$iothalamate. Area $A_1$ is determined graphically and area $A_2$ is determined as:

$$A_2 = \int_{t_1}^{\infty} C(t_1)e^{-kt} \, dt = \frac{C(t_1)}{k}$$

In this experiment, last plasma sample was taken at $t_1 = 140$ min.

The solution of this equation is:

$$A_2 = \frac{C(t_1)}{k}$$  \hspace{1cm} (6)

An accurate estimate of $A_2$ can be made only if plasma sampling is continued until the decline in plasma radioactivity is monoeponential. However, since $A_1$ normally represents the major portion of the total area under the curve, small errors in estimating $A_2$ will not greatly alter the total area calculated. These small errors in estimating $A_2$ can be further minimized by prolonging the sampling period. Since

$$GFR = \frac{Q}{A_1 + A_2}$$  \hspace{1cm} (7)

then

$$GFR = \frac{Q}{[A_1 + C(t_1)/k]}$$  \hspace{1cm} (8)

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 cava 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 [125I]iothalamate (Glofil; Abbott Laboratories) in 20 ml of isotonic saline was injected intravenously and radioactivity 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 [125I]iothalamate injectate to determine the total amount of radioactivity injected. All radioactivity measurements were carried out on a Searle automatic 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 anthrone method (3). Glomerular filtration rates obtained 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 clearances. In calculating the regression lines, it was assumed that the standard inulin clearance method was not subject to errors of measurement.

RESULTS

A plasma radioactivity-time curve obtained in a normally hydrated dog is illustrated in Fig. 2. Distribution of the iothalamate in the extracellular fluid was virtually complete within 60 min after injection, and thereafter the plasma radioactivity curve was monoexponential. In this experiment the plasma disappearance curve was fairly well represented by a two-component exponential function. However, in several normal dogs, and especially in dogs with an increased extracellular fluid volume due to chronic thoracic inferior vena cava constriction, or because of intraperitoneal injections 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-intercept 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 clearances 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
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**FIG. 4.** Comparison of GFRs obtained by inulin clearance (abscissa) and slope-intercept single-injection method (ordinate). Solid dots represent data obtained in normal dogs, circles represent data obtained in dogs that had received 1.5 liters of isosmotic mannitol intraperitoneally, and triangle represents data obtained in a dog with a chronic thoracic inferior vena caval constriction. Solid line is line of identity. $y = 1.32x + 5.35$, $r = 0.95$.

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 $101.5 \pm 8.1$ 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 $67.2 \pm 8.5$ SE ml/min, while GFR obtained by the two-compartment method averaged $90.7 \pm 11.0$ 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 $72.7 \pm 6.0$ SE ml/min, and the mean GFR obtained using this single-injection method was $75.1 \pm 6.0$ 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 $^{125}$Iiothalamate 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.

**FIG. 5.** Comparison of GFRs obtained by inulin clearance (abscissa) and two-compartment single-injection method (ordinate). Symbols are same as in Fig. 4. $y = 0.99x + 9.56$, $r = 0.92$.

**FIG. 6.** Comparison of GFRs obtained by inulin clearance (abscissa) and by single-injection method which is based on an analysis of total area under plasma radioactivity-time curve ($GFR = \frac{Q}{\int_0^t c(t)dt}$; ordinate). Symbols are same as in Fig. 4. $y = 0.97x + 4.81$, $r = 0.98$. 
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 \[^{131}I\]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 adequately 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 \[^{[125]}I\]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 inthalamate could be used as an indicator for measuring GFR with this method, but the use of radiolabeled inthalamate 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 \(\text{para-aminoohippuric acid (or some radiolabeled indicator such as [ortho-\(^{125}\)]iodohippuric}}\) is used as the indicator.

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