Volumes of Distribution and Clearances of Intravenously Injected Creatinine in the Dog

LEO A. SAPIRSTEIN, DONALD G. VIDT, MORRIS J. MANDEL AND GORDON HANUSEK

From the Department of Physiology, The Ohio State University, Columbus, Ohio

In a recent study it was reported by Greenberg et al. (1) that the volume of distribution of creatinine in the dog was of the order of 40–60% of the body weight (average 48.5%; range 37.9–59.7%). This estimate was made by dividing the equilibrium concentration of exogenous creatinine in the plasma at the end of a constant infusion into the exogenous creatinine balance in the body fluids. The latter was estimated by determining creatinine excretion after discontinuing the infusion and subtracting the ‘dead space’ creatinine and basal creatinine excretion. The creatinine space obtained was considerably lower than that previously reported for the volume of distribution of creatinine in the dog by Dominguez, Goldblatt and Pomerene (2). These authors, studying the disappearance curve of creatinine after single intravenous injection estimated the volume of distribution of creatinine to be 76% of the body weight, a value which they recognized to be greater than the body water.

In their analysis of the disappearance curve of creatinine, Dominguez et al. originally made the basic assumption that with the passage of time creatinine became homogeneously distributed throughout its entire volume of distribution; that is to say, that the ‘tissue’ creatinine concentration became identical with the ‘plasma’ concentration after sufficient time had elapsed. That this assumption is invalid for two compartment systems with excretory and intercompartmental clearances can be demonstrated readily with physical models. It has also been shown (3) that after single intravenous injections of inulin in dogs and men true equilibrium between the plasma and extracellular fluid is never attained, equal plasma and extracellular fluid concentrations, existing at one moment and one moment only. Although the volumes of inulin distribution are not identical with those for creatinine, the kinetic situations with respect to the two substances are precisely analogous. In a later analysis Dominguez et al. (4) corrected their earlier assumption, and derived new equations for the determination of the creatinine space, which gave values somewhat lower than those they had previously obtained.

In the present studies we have attempted to determine the volume of distribution of creatinine in the dog by analysis of the plasma disappearance curve of this substance after a single intravenous injection. The analysis was based on the assumption that creatinine was distributed between two compartments and moved between the first and second in proportion to the concentration difference between them, while being excreted from the first in proportion to its concentration there. A more detailed consideration of the assumptions will be presented in the discussion. The results indicate that these assumptions are useful and that sufficient information is contained in the disappearance curve to permit estimation of the volumes of the two creatinine compartments, the bladder clearance of creatinine and the intercompartmental clearance.

METHODS

Ten dogs were used in these experiments. The animals were anesthetized with sodium pentobarbital 30 mg/kg by the intravenous route. An indwelling Courand needle in the femoral artery served for blood sampling. After taking a sample for blank determinations of mannitol and creatinine, a single rapid injection of 2.0 gm of mannitol and 2.0 gm of creatinine in 50 ml of saline was made into a foreleg vein. Blood samples were taken in heparinized tubes at 5-minute intervals for 60 minutes. The plasma creatinine was determined by the method of Bonsnes and Taussky (5) and the

Received for publication October 22, 1954.

This work was supported in part by a contract between the Ohio State University Research Foundation and the USAF School of Aviation Medicine, Randolph Field, Tex. Support was also received from the American Heart Association.
plasma mannitol by the method of Corcoran and Page (6). Standards for both creatinine and mannitol were made in every experiment from an aliquot of the solution injected.

The plasma disappearance curves for both substances were plotted semilogarithmically against time. The mannitol disappearance curve was used for the estimation of the extracellular fluid volume and the glomerular filtration rate by a method previously described (7), the 'mixing' portion of the curve being neglected. The creatinine disappearance curve was resolved into the sum of two exponential functions by constructing an initial linear fit to the late portion of the curve, and then rotating and translating this line until subtraction of its value from the observed curve generated the most nearly linear set of points. The double exponential fit so achieved was usually in excellent agreement with the experimental data. In the case of one animal (dog 1), however, no satisfactory double fit could be achieved; in two other animals (dogs 5 and 6) although a double fit could be accomplished, the scatter of data was such that it was uncertain whether the best fits had been made. We wish to stress at this time that the ability to make a double exponential fit suggests but does not prove the double exponential character of the process being fitted. This point will be considered in more detail later.

The intercept on the ordinate of the steep member of the pair of lines constructed was designated $A$; that of the shallow member $B$. The slopes of the two lines were determined by dividing the natural logarithm of the ratio of the zero to the 10-minute value on either line by 10. The slope of the steep line was designated $\gamma_1$, that of the shallow line $\gamma_2$.

The equations used in the calculations of the volumes of distribution and the excretory and intercompartmental clearances are as follows: (For derivation see the appendix.)

$$G = \frac{I_1 \gamma_1}{A \gamma_2 + B \gamma_1} \quad (1)$$

where $G$ is the glomerular filtration rate in milliliters per minute, $I$ is the dose of creatinine in milligrams injected and the other terms have the significance already noted.

$$V_1 = \frac{I}{A + B} \quad (2)$$

where $V_1$ is the initial volume of distribution of creatinine. It is evident of course, that the 'initial' volume of distribution of creatinine is dependent on multiple mixing processes through the circulation and the extracellular fluid. Since sampling was not begun for 5 minutes, the greatest part, if not all, of these processes would not be apparent in the disappearance curve. Thus, a more precise definition of $V_1$ should be that it measures the volume from which the creatinine clearance at the end of 5 minutes is negligibly small compared to its rate of mixing. Although this definition of $V_1$ may appear unnecessarily clumsy, it is important that it be kept in mind, for circumstances may arise when because of faulty early mixing $V_1$ may become a functional rather than an anatomical space.

$$\alpha = \frac{V_1 [A \gamma_1 + B \gamma_2]}{A + B} \quad (3)$$

$\alpha$ is defined as the intercompartmental clearance. It has the dimensions of milliliters per minute of fluid containing creatinine virtually transferred from the first compartment ($V_1$) to the second ($V_2$) and vice versa. It is considered that this fluid always contains creatinine at the existing concentration of creatinine in the compartment from which it is derived. It is evident from this definition that the movement of creatinine from one compartment to the other will be the product of the creatinine concentration difference between the two compartments and $\alpha$.

$$V_2 = \frac{\alpha G}{V_1 \gamma_1 \gamma_2} \quad (4)$$

$V_2$ is the second volume of distribution of creatinine. The sum of $V_1$ and $V_2$ is, of course, the total volume of creatinine distribution.

RESULTS

The results in the ten dogs studied are given in table 1. Figure 1 shows the results of a typical experiment.

The volume of distribution of mannitol shows a very constant relationship to the body weight, the average value being 20.3% with a standard deviation of 2.2%. As we have noted, the mannitol disappearance curve is treated as if it were a single exponential, the initial mixing being neglected. We have done this because mixing with mannitol was complete within 10 minutes in all the animals used in this study, and the use of the more complicated formulas developed for the two compartment system gives essentially the same results as the simple calculation based on the single exponential decay curve. We have shown elsewhere that a positive error of about 4% is introduced in the mannitol space determination by this method of calculation.

The initial volume of creatinine distribution showed a correspondence with the volume of mannitol distribution which was only fair in individual comparisons. The average values, however, were in quite good agreement, the $V_1$, for mannitol being about 9% greater than that for creatinine. When allowance is made for the fact that the mannitol estimate of the extracellular fluid is 4% high, the $V_1$ for creatinine appears to be nearly identical with the extracellular fluid volume.

With three exceptions (dogs 1, 5, and 6) the
value of G calculated from the creatinine disappearance curve is in excellent agreement with that obtained from the mannitol disappearance curve. In the case of dog 1, the creatinine disappearance curve could not be resolved into a double exponential function, and a number of early points were omitted from the 'fit.' The data on this dog, which was the only one of this series of this type, were not included in the averages. In dogs 5 and 6, although it was possible to make a double exponential fit to a smooth curve drawn through the experimental data, the scatter of the values was sufficiently great to impair our confidence in the validity of the construction. In the remaining seven dogs, the fitting of the double exponential was accomplished with no difficulty, and the deviation of the experimental points from the smooth curve drawn through them was negligibly small.

The value for \( \alpha \) varied from 105–359 ml/min. and did not appear to be related to the other quantities measured, or to the body weight.

\( V_2 \) varied from 2.48–4.06 l/dog, and bore no obvious relationship to any of the other quantities measured or body weight. The small value for \( V_2 \) in dog 1 is, as has been noted, probably in error.

The ratio of \( V_1 + V_2 \) to the body weight, i.e., relation between creatinine space and the body weight varied from 29.1–45.3%. The average of 36.8% had a standard deviation of 5.7%.

**DISCUSSION**

The formulas derived in the appendix and employed above indicate the manner in which the volumes of distribution of creatinine and its excretory and intercompartmental clearances may be calculated from the coefficients \( A \) and \( B \) and the exponents \( \gamma_1 \) and \( \gamma_2 \), which describe the disappearance curve of creatinine from the plasma. Self-evidently, the validity of these formulas depends on the extent to which the model employed in their derivation resembles the physiological reality in the body. It will be advantageous at this time to consider the assumptions made in setting up the mathematical model and the criteria upon which the adequacy of the model is judged.

The assumptions made are the following: a) it is assumed that upon its introduction into the blood stream creatinine is homogeneously mixed through a uniform compartment. It is assumed further that mixing through this compartment is extremely rapid in relation to removal from this compartment. The boundary of this compartment may be identified as the limit of that volume where further increases in volume occur at a rate significantly reduced in comparison with the filling of that volume.

b) It is assumed that the compartments penetrated by creatinine are arranged in series rather than in parallel. That is to say, that penetration of the second compartment can occur only by way of the first. This is an important and perhaps invalid assumption; its implication is that the entire first volume of
creatinine distribution becomes homogeneously mixed before the second is significantly penetrated.

c) It is assumed that creatinine leaves its first volume of distribution for the bladder at a rate which is proportional to its concentration in that volume of distribution; it is further assumed that creatinine leaves this volume for its second volume of distribution at a rate proportional to the difference in its concentrations between the first and second volumes of distribution. It is assumed also that creatinine is homogeneously distributed in its second volume of distribution.

d) It is assumed that exogenous creatinine is not metabolized to any significant extent.

e) It is assumed that throughout the period of observation the bladder and intracompartamental clearances of creatinine are stable.

The criteria employed to validate these assumptions are two: first, that the creatinine disappearance curve must be resolvable into a double exponential process (as would be expected from a system which conformed to the above assumptions); second that the quantities predicted by formulas based on these assumptions must be in agreement with the same quantities measured by other methods.

So far as the first criterion is concerned, an unmistakable double exponential fit was obtained in 7 of 10 experiments, all the experimental data being included by such a fit within the limits of analytical accuracy; in two experiments the experimental data were scattered on either side of the fit, but no consistent deviation from the fit was noted; in one experiment, no double exponential fit could be made to include the data. Although no explanation is at hand for the failure of the fit in this experiment, we believe that the fact that in 9 of 10 experiments creatinine disappearance corresponded to a double exponential process is strong evidence in favor of the assumptions on which such a process is predicted.

The criterion that the measurable quantities predicted from the equations based on the assumptions must agree with the same quantities based on other methods is unfortunately limited in application to the quantities \( G \) and \( V_1 \). Neither intercompartmental clearance nor \( V_2 \) can be measured by any other technique which we know. The sum of \( V_1 \) and \( V_2 \) (total creatinine space) could presumably be measured by the technique of Greenberg et al.; we will, however, present arguments below to indicate that this method may not be valid.

We have already noted that the agreement between the two values of \( G \) was remarkably good. In 7 of the 10 experiments, the two values differed from each other by less than 10%. In the other three experiments (exps. 1, 5, 6) the construction of double exponential fits was impossible (dog 1) or uncertain (dogs 5 and 6).

The correspondence between the initial volume of distribution of creatinine and mannitol space was poor in any one dog, the value \( V_1/V_{mannitol} \) ranging from 0.67 to 1.53. Yet the averages of these values were in very good agreement with each other, the initial creatinine space being only 9% less than the mannitol space which, as we have pointed out, is about 4% higher than it would be if the correction for mannitol mixing were considered.

The total creatinine space which we have obtained is considerably smaller than that re-
ported by others. Our average value of 36.8% of the body weight is less than half of that reported by Dominguez et al., and about 75% of that found by Greenberg et al.

As we have noted previously, we believe that the very high creatinine spaces reported by Dominguez et al. in their original study (2) can be attributed to their erroneous assumption that the concentrations of creatinine in its two volumes of distribution approach each other with time. Even when these authors revised their assumptions (4), the calculated spaces were higher than those which we have found. We have recalculated the total creatinine space from their data and we have found the same values as they. We believe that the discrepancy between our findings and theirs results from the fact that we have more completely characterized the earlier portion of the disappearance curve, which permits in effect a more accurate estimation of the 'mixing' losses.

It may be noted that Dominguez et al. reported a $V_1$ of 28% of the body weight in contrast with our 17.7%, a finding which suggests that losses in the mixing period unrecognized in their curve fitting may have occurred.

The difference between our value for the total creatinine space and that obtained by Greenberg et al. is somewhat more difficult to explain. We believe, however, that these authors may have overlooked a significant source of error in estimating the creatinine contained in the renal dead space. They multiplied the 'appearance time' of creatinine by the rate of urinary elimination of creatinine at the time of discontinuing the infusion to obtain the dead space creatinine. Since the appearance time was estimated at 100 seconds this, in effect, states that the volume of the dead space is no more than one and two-thirds times the minute volume at all rates of urine flow encountered in their experiments. In other words, at urine flows of 1.5 ml/min., the dead space calculated in this manner would appear to be only 2.5 ml. This value appears to us to be impossibly small; Smith states that the pelvis of each dog kidney contains a 6-ml dead space. It is interesting to note that if the total dead space of both kidneys in a large dog is 20 ml and the urine flow 1.5 ml/min. while the U/P ratio for creatinine is 50-1, then the creatinine content of the dead space is equivalent to 1 liter of plasma. The 'appearance time' calculation would indicate that the dead space was equivalent to only 125 ml plasma. Clearly, large errors can be introduced by the use of the 'appearance time' correction for the amount of a substance contained in the dead space, especially when U/P ratios are high and $V$ is low. These errors would tend to increase the apparent space, and may account in part for the discrepancy between our results and those of Greenberg et al.

At the present time, the validity of our formulation rest on the similarity between the values of $G$ and $V_1$ obtained with creatinine and mannitol, and on the double exponential character of the creatinine disappearance curve. Further validation awaits the development of a satisfactory alternate method for determination of the total creatinine space.

The value for total creatinine space which we have obtained is in close agreement with the thiocyanate space of normal dogs reported by Gaudino and Levitt (8) who found the thiocyanate space to compose 33.8% of the body weight. In our experience similar values for thiocyanate space have been obtained in normal dogs. Whether the similarity of the volumes of thiocyanate and creatinine indicates the existence of a true compartment presumably including the extracellular and a restricted portion of the intracellular fluid, or is an accidental circumstance cannot be determined at the present time.

We presume that the intercompartmental clearance $\alpha$ is related to the permeability of the barrier between $V_1$ and $V_s$. Since $V_1$ appears to correspond to the extracellular fluid, it seems probable that $\alpha$ is related to the permeability of the cell surface. Its significance in changing physiological circumstances, however, remains to be assessed.

**SUMMARY**

Equations have been derived for the analysis of the disappearance curve of an injected material from a two compartment system, one compartment of which eliminates the injected material to the outside at a rate proportional to its existing concentration and transfers it to the second compartment at a rate proportional to the existing concentration difference between the first and second compartments.
The disappearance curves predicted from these equations are double exponential curves. Formulas are given for converting the intercepts and slopes of such double exponential curves into values for the excretory and intercompartmental clearances and the first and second volumes of distribution.

The disappearance curve of creatinine corresponded to a double exponential process in 9 of 10 experiments in dogs. The creatinine clearance calculated from the creatinine disappearance curve was in good agreement with the mannitol clearance simultaneously determined. The volume of rapid distribution of creatinine corresponded roughly to the mannitol space (extracellular fluid) but may not always yield a valid measure of a true anatomical space. The intercompartmental clearance ranged from 100–359 ml/min. The total volume of creatinine distribution was 36.8% of the body weight, a figure considerably smaller than any previously reported. The reasons for the discrepancy are discussed.

**APPENDIX**

Let it be assumed that two compartments $V_1$ and $V_2$ are related to each other as indicated in the text and figure 2, and that the intercompartmental clearance is $\alpha$ and that the excretory clearance in compartment $V_1$ is $G$. Let $I$ mg of a substance be injected into $V_1$ at time 0.

![Diagram](image)

**Fig. 2.** System for which the equations of the appendix are derived. The excretory clearance is designated $G$, and is the virtual (not the actual) volume leaving $V_1$ for the outside.

The rate at which the material leaves compartment $V_1$ depends on $G$, $\alpha$, $C_1$ and $C_2$, (the concentrations in $V_1$ and $V_2$, respectively, as follows):

$$V_1 \frac{dC_1}{dt} = -GC_1 - \alpha[C_1 - C_2] \tag{1}$$

The amount injected, $I$, can be expressed as the sum of the amounts in $V_1$ and $V_2$ and the amount excreted at time $t$ ($L_t$).

$$I = C_1 V_1 + C_2 V_2 + L_t \tag{2}$$

$L_t$ is the product of the excretory clearance and the integrated concentration of the substance in $V_1$ or

$$L_t = G \int_0^t C_1 \, dt \tag{3}$$

Substituting this value of $L_t$ in equation 3 and solving for $C_2$ we obtain

$$C_2 = \frac{I - G \int_0^t C_1 \, dt - C_1 V_1}{V_2} \tag{4}$$

This value of $C_2$ may be substituted in equation 1, giving

$$\frac{dC_1}{V_2} = -GC_1 - \alpha C_1$$

$$+ \frac{\alpha I}{V_2} - \frac{\alpha}{V_2} \int_0^t GC_1 \, dt - \frac{\alpha C_1 V_1}{V_2} \tag{5}$$

Differentiating equation 5 with respect to time removes the integral on the right.

$$V_1 \frac{d^2 C_1}{dt^2} = -G \frac{dC_1}{dt} - \frac{\alpha dC_1}{V_2} - \frac{\alpha GC_1}{V_2} - \frac{\alpha V_1 dC_1}{V_2} \tag{6}$$

Rearranging terms, and dividing by $V_1$

$$\frac{d^2 C_1}{dt^2} + \left[ \left( \frac{G}{V_1} + \frac{\alpha}{V_2} \right) \frac{dC_1}{dt} + \frac{\alpha GC_1}{V_1 V_2} = 0 \right] \tag{7}$$

This is a linear differential equation of the second order with constant coefficients and has the general solution:

$$C_1 = Ae^{-\gamma_1 t} + Be^{-\gamma_2 t} \tag{8}$$

where

$$\gamma_1 \gamma_2 = \frac{\alpha G}{V_1 V_2} \tag{9}$$

and

$$\gamma_1 + \gamma_2 = \frac{G + \alpha}{V_1} + \frac{\alpha}{V_2} \tag{10}$$

The two boundary conditions are:

at $t = 0$ \hspace{1cm} $C_0 = \frac{I}{V_1} \tag{11}$

at $t = 0$ \hspace{1cm} $C_2 = 0 \tag{12}$

From equations 11 and 8

$$\frac{I}{V_1} = A + B = C_0 \tag{13}$$

$$V_1 = \frac{I}{A + B} \tag{14}$$
From equations 12 and 1

\[ \frac{dC_1}{dt} = -[\alpha + G]C_0 \]  

(15)

But from equation 8 at \( t = 0 \)

\[ \frac{dC_1}{dt} = -A_1 \gamma_1 - B \gamma_2 \]  

(16)

From equations 15 and 16

\[ -\left[ \frac{\alpha + G}{V_1} \right] C_0 = -A_1 \gamma_1 - B \gamma_2 \]  

(17)

From equations 10 and 17

\[ \frac{\alpha + G}{V_1} = \gamma_1 + \gamma_2 - \frac{\alpha}{V_0} = \frac{A_1 \gamma_1 + B \gamma_2}{A + B} \]  

(18)

Solving equation 18 for \( \alpha/V_2 \)

\[ \frac{\alpha}{V_2} = \gamma_1 + \gamma_2 - \frac{A_1 \gamma_1 + B \gamma_2}{A + B} \]  

(19)

From equation 9

\[ G = \frac{\gamma_1 \gamma_2 V_1}{\alpha/V_2} \]  

(20)

Substituting the value of \( V_1 \) from equation 14 and \( \alpha/V_2 \) from equation 19, \( G \) is found to be

\[ G = \frac{\gamma_1 \gamma_2 I}{A_1 \gamma_1 + B \gamma_1} \]  

(21)

From equations 17 and 13

\[ \alpha = \frac{[A_1 \gamma_1 + B \gamma_2]V_1}{A + B} - G \]  

(22)

From equation 9

\[ V_2 = \frac{\alpha G}{V_1 \gamma_1 \gamma_2} \]  

(23)

(Note: inspection of equations 21 and 23 reveals that errors in the estimation of \( \gamma_2 \) are reflected and may be magnified in the determination of \( G \), \( \alpha \), and \( V_2 \). Since \( \gamma_2 \) is the slope of a shallow line it is important that it be estimated with the greatest precision. It is suggested that when \( \gamma_2 \) is very small the above equations be employed only when \( \alpha \), \( G \), or \( V_2 \) can be independently estimated. The estimation of any of these can be used in the calculation of \( \gamma_2 \), and the subsequent determination of the others.)

The derivation reported here is a solution of the special case with one infinite compartment of a general closed steady state system described by Sheppard and Householder (9). The notations are compared in the following:

<table>
<thead>
<tr>
<th>Sheppard and</th>
<th>Sapirstein et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_0 )</td>
<td>( C_1/C_0 )</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>( C_2/C_0 )</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>( A_1/V_1 )</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>( A/V_2 )</td>
</tr>
<tr>
<td>( A_1 )</td>
<td>( G/V_1 )</td>
</tr>
<tr>
<td>( X_1 )</td>
<td>( A/C_0 )</td>
</tr>
<tr>
<td>( X_2 )</td>
<td>( B/C_0 )</td>
</tr>
<tr>
<td>( \lambda_1 )</td>
<td>( \gamma_1 )</td>
</tr>
<tr>
<td>( \lambda_2 )</td>
<td>( \gamma_2 )</td>
</tr>
</tbody>
</table>

REFERENCES