Total body water distribution of creatinine and urea in nephrectomized dogs

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SCHLOERB, PAUL R. Total body water distribution of creatinine and urea in nephrectomized dogs. Am. J. Physiol. 199(4): 661-665, 1960.—It was the purpose of this study to measure and to compare the volumes of distribution of creatinine, urea and tritiated water in nephrectomized dogs. After bilateral nephrectomy a solution containing known amounts of these was infused intravenously and at a constant rate in some studies. Frequent arterial blood samples were taken during and following infusion for periods up to 20 hours and were analyzed for water and the infused substances. The respective volumes of distribution and rates of dilution were calculated. Isotopic water and urea were distributed in 95% and creatinine in 61% of their final volumes of distribution at the end of a 30-minute infusion. Final equilibrium occurred in about 90 minutes with water and urea and in about 4 hours with creatinine. Creatinine became distributed in 99 ± 3% of the tritiated water volume and in 101 ± 5% of the urea volume. Urea distributed in 98 ± 4% of the tritiated water volume. It is concluded that administered creatinine is distributed in total body water of nephrectomized dogs with an equilibrium time of about 4 hours and that urea is similarly distributed within 4 hours.

METHODS

Adult dogs of both sexes, weighing 10.45-15.96 kg were used. Bilateral nephrectomy, through a mid-line abdominal incision under sodium pentobarbital anesthesia (30 mg/kg), was performed usually 1 day prior to infusion studies to avoid postoperative hemorrhage due to the systemic heparinization employed for collection of successive arterial blood samples. Following nephrectomy, 500 ml of 5% glucose in water were given intravenously and further fluid and food were withheld until the infusion on the next day. After the same anesthetic dose, the right femoral artery was cannulated with polyethylene tubing; heparin (5 mg/kg) was administered intravenously; and the infusion solution was given into the external jugular vein from an infusion burette. A constant infusion pump was not employed, but in the first four studies the rate was maintained at 6 ml/min. by adjusting the volume each 30 seconds during a 30-minute infusion.

The infusion solution consisted of about 500 mM of urea, 15 mM of creatinine and usually 500 μc of tritiated water in a volume of 75-180 ml of 0.85% sodium chloride. These amounts of urea and creatinine were selected to simulate final plasma concentrations observed in moderate uremia (blood urea nitrogen, 200 mg %; creatinine 20 mg %). The tritiated water (Abbott Laboratories, Oak Ridge, Tcmn.) had a specific activity of 4500 μc/gm of water or 81 μc/mM of water. In dogs 1, 2, 3 and 4 arterial blood samples (5 ml) were taken initially, at carefully timed 5-minute intervals

Received for publication January 18, 1960.

1 This study was supported by Research Grant H-2363 from the National Heart Institute.

patients with renal failure, that creatinine is distributed in the body water phase. It was the purpose of this investigation to measure and to compare, in nephrectomized animals, the rates and volumes of distribution of tritiated water, urea and creatinine as well as the possible independent effects of each of the infused substances upon the volumes of distribution of the other two. The data confirm that both urea (1) and creatinine (6) are distributed in total body water.
did not occur. From the observed urea and creatinine standards of less than 1% of the mean. It was determined that mutual interference of urea and creatinine had standard deviations of reproducibility of a series of samples.

The second four dogs (5, 6, 7, 8) were given tritiated water and creatinine without urea. To investigate further the possible osmotic effects of urea upon the creatinine volume of distribution, two dogs (9, 10) were given tritiated water and creatinine followed by urea 16 hours later; two dogs (11, 12) were given tritiated water and creatinine followed by urea 4 hours later; and two dogs (13, 14) were given tritiated water and urea followed by creatinine 2 hours later. All blood samples, which were collected in heparin tubes, were centrifuged and the plasma was analyzed for tritium, urea, creatinine and for water in the first six studies. The specific gravity of the plasma was determined by the falling drop method of Barbour and Hamilton (7), using a xylene-benzene medium and potassium sulfate standards of known density. From the specific gravity, the plasma water and protein were derived from the tables compiled by Sunderman (8). Because plasma water was found to be quite constantly about 95%, this value was assumed for later studies. All data were expressed as concentrations in plasma water.

Tritium assay was done as follows: About 1 ml of plasma was vacuum distilled from the frozen state by a method described for deuterium oxide analysis (9). To a 20-ml optically clear glass counting vial (Wheaton Glass Co.) 0.5 ml of the distillate, 7.5 ml of absolute alcohol, 6.0 ml of 0.4% 2,5 diphenyloxazole (POPOP), in toluene and 6.0 ml of 0.01% 1,4-di(2-(5 phenyloxazolyl) benzene (POPPOP) in toluene were added, and the contents were mixed by gentle agitation after capping the vial. These samples together with appropriate standards made in triplicate from each infusion solution, were counted at -15°C in a Tri-carb liquid scintillation counter after at least 1 hour of dark and temperature adaptation in the freezing compartment. Counting efficiency was about 5%. Samples were counted a sufficient time to assure a standard deviation of less than 2% of the mean. The observed counting data were calculated to counts per minute per milliliter plasma water.

Urea was determined by the dioxime reaction using the Technicon autoanalyzer with appropriate standards made in triplicate from each infusion solution. Creatinine was also determined with the autoanalyzer using the alkaline picrate reaction by a method developed at this institution (10), and triplicate standards from each of the infusion solutions were analyzed similarly and were interposed frequently with the unknown plasma samples.

Both the urea and creatinine autoanalyzer methods had standard deviations of reproducibility of a series of standards of less than 1% of the mean. It was determined that mutual interference of urea and creatinine did not occur. From the observed urea and creatinine concentrations were subtracted the initial plasma concentrations before infusion. Small corrections for the hourly increases of urea nitrogen (2.1 mg%/hr, and creatinine (0.14 mg%/hr) in these nephrectomized dogs were derived from observations of the daily increases of these plasma concentrations in 34 nephrectomized dogs employed in another study (unpublished observations). Corrections for molecular values of urea and creatinine in standards and plasma samples were made.

The tritium, urea and creatinine plasma water concentrations were plotted against time and by inspection smoothed curves were drawn for each. The equilibrium concentrations of tritium water, urea and creatinine were calculated from the average of the observed points which characterized the equilibrium portion of the disappearance curve following the end of the infusion. For tritium and urea these points included those after 70 minutes following the completion of the infusion; for creatinine the values after 3 hours were used.

From the respective equilibrium concentrations and the amounts infused, the final volumes of distribution were calculated. From these calculated volumes, the amounts of water given during infusions were subtracted to obtain the corrected volumes existing prior to infusions. For dogs 1, 3 and 4, the volumes of distribution at each of the times during and after infusion were also calculated from the known amounts infused and the concentrations at these times. These volumes of distribution were then divided by the respective final volumes of distribution to calculate the percentage distribution in the final volume of dilution of each substance at each time for each dog. All of these values at each time for each of these three experiments were averaged; the standard deviations were calculated, and the results were plotted (figs. 1-3). By the method of least squares the regression lines for tritium, urea and creatinine concentrations during the 30-minute constant infusion periods were calculated. An attempt was made to calculate in three dogs the apparent volumes of distribution for each of the substances during this interval by dividing the infusion rate (in radioactivity or milligrams per minute) by the slopes of the regression lines (in radioactivity or milligrams per liter per minute), as adapted from the method of Hlad et al. (11) for the volume of distribution of radiosodium.

RESULTS

From figure 1, figure 2 and table 1 it is apparent that for dogs 1, 2, 3 and 4 the rates of dilution of tritiated water and urea were not measurably different and that both approximated their final equilibrium concentrations about 90 minutes after the mid-times of the intravenous infusions.

Figure 3 shows that in these same animals creatinine distributed itself more slowly, but the final volumes of dilution were essentially the same as those of tritium and urea (table 1). When infused without urea, the volume...
BODY WATER DISTRIBUTION OF CREATININE AND UREA

Fig. 1-3. Rates of dilution of tritiated water, urea and creatinine. Each point is the average value for 3 dogs during infusion and for 4 dogs thereafter. Ordinates do not begin at zero. Vertical bars indicate 1 standard deviation. Solid bar at lower left represents the infusion period. Broken line represents extrapolation of the regression line calculated by least squares from those points falling on the straight lines. Rates of dilution of tritiated water and urea are not measurably different and reach equilibrium about 90 min. after infusion, while creatinine is distributed in about 4 hr. within nearly the same volume (table 1).

The rates of dilution of tritiated water, urea and creatinine were about the same as tritiated water in dogs 5, 6, 7 and 8 (table 1), indicating that the osmotic effect of urea did not influence measurably creatinine body water exchange.

The results of further evaluation of the possible effects of urea and creatinine on the rates of dilution of each other are also summarized in table 1. When two dogs (9, 10) were given tritiated water and creatinine, the 16-hour volumes of dilution were nearly the same as that for urea which was given at that time. Considerable extrapolation for correction due to endogenous creatinine production was necessary for this calculation, however. These values suggest that the volume of dilution of creatinine at 16 hours is not measurably greater than that at 4 hours. In another group (dogs 11, 12) the almost identical volumes of distribution, at 4 hours, of infused tritiated water and creatinine were not changed significantly by infusion of urea at the end of this time. Considerable extrapolation for correction due to endogenous creatinine production was necessary for this calculation, however. These values suggest that the volume of dilution of creatinine at 16 hours is not measurably greater than that at 4 hours. In another group (dogs 13, 14) the almost identical volumes of distribution, at 4 hours, of infused tritiated water and creatinine were not changed significantly by infusion of urea at the end of this time and the volumes of distribution of urea corresponded favorably with the volumes of the other two, suggesting that urea infusion did not enhance mixing of creatinine with body water. In the final study (dogs 15, 16), tritiated water and urea were infused. After equilibrium, samples were obtained at 2 hours; creatinine was given intravenously; and its volume of dilution was obtained at 5 hours. Again, the volumes of dilution of the three substances were nearly the same. In all of these studies, the concentrations of all of the first substances infused diminished during the subsequent infusion but returned to within 1% of their values before the second infusion, when corrected for the small additional load of water added to body water by the second infusion. Calculations of the volumes of dilution at the end of infusion in three dogs by dividing the infusion rate by the slope of the linear increase in plasma concentrations of each of the three substances resulted in unacceptable variations, probably because of small changes in the infusion rate which produced relatively large alterations in the concentration slope. This difficulty might be obviated by use of a constant infusion pump. The volumes of dilution for dogs 11, 13 and 14 at the end of infusion, expressed as percentage of their final volumes of dilution were, respectively, for tritiated water 96, 102 and 95%; for urea 100, 107 and 106%; and for creatinine 73, 63, 65%. These data only confirm that creatinine was distributed more slowly than either tritiated water or urea but do not afford expressions sufficiently precise to predict the final volumes of dilutions from that percentage distributed at the end of infusion.

Evidence that some areas of body water do not reach the same creatinine and urea concentrations as plasma after equilibrium was afforded by the study of dog 11 which was pregnant and near-term. Although the tritiated water, urea and creatinine volumes of distribution were nearly identical at equilibrium, amniotic fluid had a creatinine concentration (13.2 mg %) about half of that in the plasma (24.0 mg %) 1 day earlier, and the corresponding urea nitrogen volumes were 177 and 226 mg %, respectively. The tritium concentrations were nearly the same. While cerebrospinal fluid creatinine was not measured in these animals, it is probable that this small area of body water did not reach the same concentration as that of plasma because observations in uremic patients have shown the spinal fluid creatinine...
concentration to be half that in plasma water. These
current discrepancies which involve small fractions of
total body water, apparently were within the limits of
equal error of the measurement techniques employed.
The average values for total body water in these 14
nephrectomized dogs are shown in Table 1 and approxi-
mate those reported for normal human beings (12, 13).

**DISCUSSION**

The findings of this study confirm that creatinine
is distributed in total body water (6) and using the more
direct evidence of tritium oxide dilution, agree with
Painter’s observation that urea is similarly distributed
(1). In normal dogs, it has been reported that nearly all
administered creatinine appears in the urine within 24
hours (2) suggesting that this is a true metabolic end-
product, and no available evidence indicates body
creatinine sequestration.

If endogenous creatinine is distributed in body water
it should not be necessary to apply a base line correction
to each sample concentration. Total body water is then
equal to the sum of exogenous and endogenous creatinine
divided by the equilibrium plasma water creatinine
distribution without correction for the preinfusion value.

The data presented in this study confirm observations
by Edwards (6) indicating that creatinine is distributed
in body water. Their observed ratio of creatinine space
to antipyrene space of 0.99 ± 0.06 is comparable to the
ratio of creatinine volume to tritiated water volume of
0.99 ± 0.03 observed in this study, although our disap-
ppearance curves suggest equilibrium somewhat earlier,
at 4 hours, in nephrectomized dogs compared to the
6-hour value found by Edwards in ureter-ligated rabbits
and anuric patients.

The findings in this study and those of Edwards (6) are
in conflict with previous reports, none of which have
included measurement of total body water by any stand-
ard method. Dominguez et al. (3) recalculated creatinine
distribution data from their previous paper (2) using a
newer equation and found this volume to be 63% of
body weight in normal dogs. They have assumed the
existence of two body fluid compartments. Greenberg
et al. (4), using normal dogs, found the volume of distribu-
tion of creatinine to average 48.5% of body weight.
The duration of their equilibrating infusions was 161+177
minutes, and the volumes of dilution were calculated
from the known amounts of creatinine given minus that
excreted in the urine divided by the plasma concentra-
tions at the end of the infusions. Sapirstein et al. (5) have
offered the objection to this study that renal dead space
estimations were too low and that the dilution volumes
observed by Greenberg and associates were therefore
too high.

The volume of dilution for creatinine in nephrecto-
mized dogs found by Sapirstein and associates averaged
36.8% of body weight, the lowest value reported (5).
These investigators analyzed the 1st hour of the creat-
ine arterial disappearance curve and derived formulas
from the changing slope of the curve at this time to
calculate the volume of distribution. A fundamental
assumption in their interpretations is that there are two
body water compartments and that a substance must
penetrate the first before it can enter the second. While
this assumption is in accord with the fact that cells are
surrounded by extracellular fluid, it is probably not
justified to assume that transfers from blood or extra-
cellular fluid into all cells occur at equal rates. Marked
differences in visceral, muscle and other organ blood
flow rates, different flow rates within individual organs
and organ systems, and differing fates of transported
substances in various organ systems (gastrointestinal
secrections, renal excretion, cerebrospinal fluid, etc.) are
present. These factors make it necessary to recognize that
the body is composed of an almost infinite number of
intravascular - extracellular - cellular - subcellular - mo-
lecular 'units' including the various regions having speci-
cialized secretory mechanisms and areas of normal fluid
sequestration. Because each of these small areas of almost
infinite number are exposed to differing blood flow rates,
it follows that the changing concentration of a sub-
stance in the blood will reach these many units at differ-
tent times and therefore at different concentrations since
areas having more rapid exchange rates will decrease
the plasma concentration proportionately more. It may
be reasoned further that under these circumstances, the
intracellular concentration of an infused substance may
be higher in one area than the plasma or extracellular
concentration in another. Indeed it has been shown in
an earlier study (12) that in a normal human subject, 10
minutes following an intravenous infusion of deuterium
oxide, the gastric juice concentration of the heavy water
was greater than the concentration in venous blood,
indicating, in this example, a more rapid rate of secre-
tion of heavy water into the stomach than its over-all
equilibrium rate of dilution in body water.

It seems reasonable to assume that the plasma disap-

### Table 1. Volumes of Distribution

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<th>Dog No.</th>
<th>Sex</th>
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<th>HTO Vol. (L.)</th>
<th>HTO % Wt.</th>
<th>Urea Vol. (L.)</th>
<th>Urea % Wt.</th>
<th>Creat. V.</th>
<th>Creat. % Wt.</th>
<th>Creat. % V.</th>
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appearance curve of creatinine, urea, isotopic water or any other substance must be a composite, representing at any given moment in its decreasing concentration the average result of an almost infinite number of exchanges, transfers, and mixings, rather than just those among a small finite number of compartments.

Rapid excretion of creatinine by the kidneys precludes its usefulness at present for the measurement of total body water when renal excretory function exists. Additional studies of tritiated water and creatinine infusions in four normal dogs with serial measurement of blood and urine have shown smooth double exponential disappearance curves for creatinine as previously reported (2, 4, 5).

Attempts to analyze the creatinine disappearance curves by formulas proposed by Dominguez et al. (2, 3) have, however, resulted in creatinine spaces differing widely from the corresponding tritiated water volumes. It seems possible, as Edwards has mentioned (6), that a constant creatinine infusion technique may be applicable to the problem of finding a simpler method for the measurement of total body water.

It is concluded from this study that creatinine is distributed in total body water, with an equilibrium time of about 4 hours, and that urea is distributed similarly within 90 minutes.

The capable technical assistance of Mrs. Sandra Scholes and Christopher H. Miller is gratefully acknowledged.

REFERENCES