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 1 1/2 hours.

What are the volumes of distribution of creatinine and urea? The reasonable calculated dilution values obtained from its equilibrium concentration after infusion (1) suggest that urea is distributed very nearly in total body water. The evidence for the distribution of creatinine, even in nephrectomized dogs, is conflicting, however (2-6). The rapid renal excretion of administered creatinine presents experimental difficulties and seems to account in part for the differing values reported. With one recent exception (6), the volumes of distribution of neither of these substances have been related to body water as determined by isotopic water or other standard method. Edwards (6) recently reported body water distribution of creatinine in ureter-ligated rabbits with direct measurement of water by antipyrine dilution and by desiccation and presented additional evidence, in 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.

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 μg 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, Tcnns.) 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.
during the 30-minute constant infusion (excepting dog 2) and for 30 minutes thereafter, at 10-minute intervals for the next hour; and at 20-minute intervals for the following 3 hours, with a total infusion-sampling time of 5 hours. In subsequent studies, samples were taken after infusions for periods up to 20 hours.

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-brombenzene 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 (PPO), in toluene and 6.0 ml of 0.01% 1,4-di(2-(5 phenyloxazolyl))benzene (POPOP) 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 volumes 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 tritiated 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
of creatinine dilution was 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. 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 concentrations 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 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).
concentration to be half that in plasma water. These volume discrepancies which involve small fractions of total body water, apparently were within the limits of error of the measurement techniques employed.

The average values for total body water in these 14 nephrectomized dogs are shown in Table 1 and approximate 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 concentration without correction for the precinfusion 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 antipyrine 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 disappearance 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 standard 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 distribution 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 concentrations 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 nephrectomized 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 creatinine 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 extracellular 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 secretions, 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 - molecular 'units' including the various regions having specialized 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 substance in the blood will reach these many units at different 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 secretion 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-
The disappearance 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.

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REFERENCES