Extracellular Volume Estimation from Ratios of Bromide to Chloride in Urine or Saliva

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Abstract. **Use of either urine or saliva samples to estimate extracellular water volume was investigated in 10 men using nonradioactive bromide (Br) and in seven newborn piglets using radioactive Br (^{82}Br) and chloride (^{36}Cl). The relation of Br to Cl concentrations in urine enabled an estimation of Br dilution volume from human urine (267 ± 42 ml/kg, mean ± SD) that was not significantly different ($P = 1.0$) from the Br dilution volume calculated from plasma Br concentration (268 ± 20 ml/kg). Although the Br dilution volume estimated from saliva was not different from that of plasma, the error in the estimates of Br dilution volume from saliva was too large (mean difference, −36 ± 64 ml/kg) to make its use practical. The data from piglets showed good agreement between ^{82}Br and ^{36}Cl dilution volumes calculated from 4-hr plasma samples (356 ± 14 ml/kg and 347 ± 12 ml/kg; $P > 0.1$) and between ^{82}Br dilution volumes calculated from urine ^{82}Br:^{36}Cl and plasma ^{82}Br (360 ± 31 ml/kg and 356 ± 14 ml/kg; $P > 0.1$). Extracellular water volume can be estimated in both adult and young animals using the Br dilution volume calculated from urine samples. It requires (i) two urine collections: one before and one 4 to 8 hr after administration of Br; (ii) a measurement or estimate of plasma Cl concentration; and (iii) a correction factor that describes the relationship of the ratio of Br to Cl in urine to that ratio in plasma.** [P.S.E.B.M. 1992, Vol 199]

Simultaneous measurements of body fluid volumes, for example, total body water and extracellular water (ECW), are useful in the study of changes in the body composition of growing young animals. Such measurements are also requisite for the quantitative study of fluid shifts between the extra- and intracellular compartments of newborns and older infants. Changes in ECW volume have been reported in the first week after birth (1) and during growth (2). Abnormal states of hydration also occur in malnutrition (3), respiratory distress syndrome (4), and other diseases (2). The impetus to develop a reliable method for the measurement of ECW volume *in vivo* has developed from our desire to (i) understand the growth process more completely, and (ii) manage abnormal states of hydration in the newborn more successfully.

The volume of extracellular space is conventionally defined by the volume distribution of chloride (Cl), with a correction for intracellular Cl (5). It is well established that the distribution volume of bromide (Br) is almost identical to that of Cl (6–11) and that a corrected Br dilution volume should give a close approximation of ECW volume. In the pediatric population, therefore, the Br dilution technique is used most often to estimate extracellular space. Many data indicate that Br space is useful in the estimation of ECW in newborns, older infants, children, and adults (12–15). Br can be administered in a nonradioactive form, and it is easily measured by spectrophotometry (1) and ion chromatography (16); it is, therefore, ideal for clinical use.

A commonly used method to estimate ECW volume is an oral (or intravenous) administration of a solution of Br, followed by timed blood sampling for the calculation of its distribution volume (6, 12–15, 17, 18). Volume distribution of Br can be calculated from extrapolation of multiple time-concentration data to zero time or from a single plasma sample after equili-

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bration has been achieved. Studies have shown that equilibration of Br is achieved in 1 to 4 hr (14, 15, 17, 18). Br dilution volume measurements could be used more often in young infants if multiple blood samplings were not required. Although orally administered Br appears in urine and saliva (9, 11, 19), the concentration of Br ([Br]) in these biological fluids differs significantly from that in plasma. Thus ECW volume cannot be estimated from [Br] in any of these fluids.

The differences between plasma [Br] and urine or saliva [Br] are a result of differences in the secretion or absorption rates of Br relative to those of water by the respective organs (19). If it is assumed, however, that the rate of transport of Br is equal, or has a constant relationship, to that of Cl, then the urine or saliva [Br] to [Cl] ratios should be equal, or have a constant relationship, to the plasma [Br] to [Cl] ratio. This relationship should permit the calculation of the volume distribution of Br from either urine or saliva samples and thereby eliminate the need for blood samplings.

Our objective was to investigate the use of either urine or saliva samples to estimate the volume distribution of Br. Our experiment consisted of two parts. First, adult humans were studied using nonradioactive Br; second, 7- to 10-day-old miniature piglets, whose ECW volume is similar to that of premature infants (16), were studied using radioactive Br. In humans, nonradioactive Br was administered orally and (i) urine and saliva [Br] were compared with plasma [Br], (ii) urine and saliva [Br] to [Cl] ratios were compared with plasma [Br] to [Cl], and (iii) volume distributions of Br calculated from urine and saliva [Br] to [Cl] ratios were compared with those calculated from plasma [Br] using the 4-hr equilibration time point. In piglets, radioactive ^{82}Br and ^{36}Cl were given intravenously, and the urine ^{82}Br to ^{36}Cl activity ratio was used to calculate ECW volume.

Methods

Human Studies. Ten healthy men were studied. The mean (±SD) age was 27.5 ± 3.5 years; weight, 76.7 ± 9.0 kg; and height, 1.790 ± 0.044 m. Each subject consumed his normal diet and performed his normal daily activity during the study. However, no food or drink was taken 45 min before the start of blood, urine, and saliva collections.

After 5-ml baseline samples of blood, urine, and saliva were obtained from each subject, an oral dose of 0.305 mmol Br/kg body wt was administered in a sodium bromide solution (0.625 mmol Br/ml; Fisher Scientific, Pittsburgh, PA) free of bacterial contamination and pyrogens. Two-milliliter samples of blood, urine, and saliva were collected again at hourly intervals between 4 and 8 hr after Br administration. Plasma, urine, and saliva samples were analyzed for Br and Cl concentrations, and the [Br] to [Cl] ratios in each of these fluids were calculated and compared. The 4-hr sample collections were used to calculate Br dilution volumes because we assumed that equilibration would have been achieved by that time (14, 15, 17, 18). The protocol was approved by the Institutional Review Board for Human Research of Baylor College of Medicine (Houston, TX), and informed consent was obtained from all subjects.

Concentrations of Br in plasma samples were measured by ion chromatography (Waters, Milford, MA), as reported by Wong *et al.* (16). Briefly, the plasma samples were diluted (1/10) and passed through an ultrafilter before the Br concentration was measured. Detection was set at an ultraviolet wavelength of 210 nm. Urine and saliva samples were analyzed as above, with modifications in the ion chromatography procedure: samples were filtered through a Sep-Pak C_{18} (Waters), and a different column (Waters IC-PAK anion column, 4.6 mm × 150 mm), a higher temperature (60°C), and a longer run time (60 min per sample) were used.

Chloride concentrations in all samples were measured on a Buchler chloridometer (Saddle Brook, NJ). Water contents of plasma, urine, and saliva were determined by desiccation, and the concentrations of Br and Cl in plasma, urine, and saliva were expressed as μmol/g of water.

Animal Studies. Seven 7- to 10-day-old piglets (Pitman Moore/Hanford strain; Bastrop Farms, Bastrop, TX) were studied. The body weights ranged from 1.7 kg to 2.5 kg. On the morning of the experiment, the piglets were allowed to suckle until 1 hr before anesthetization. They were anesthetized with a combination of ketamine (20 mg/kg im) and pentobarbital-sodium (10 mg/kg ip) and maintained under anesthesia with a maintenance dose of pentobarbital-sodium (5 mg/kg iv). A central catheter was inserted via the jugular vein for collection of blood samples (2 ml), and a Foley catheter was inserted through an abdominal incision into the bladder for continuous urine collection. After 5-ml baseline samples of blood and urine were collected, a single bolus (2.5 ml/kg) of each of the radiotracers, ^{82}Br (5 μCi/kg body wt; NEN Products, Wilmington, DE) and ^{36}Cl (5 μCi/kg; NEN Products, Wilmington, DE), was injected through the venous catheter. The catheter was then flushed with 2 ml of saline. Blood and urine samples (2 ml) were taken 2 hr after injection of the radiotracers, then hourly over the next 6 hr. The volume of blood withdrawn was replaced with saline. After the final blood and urine samples were collected (at 8 hr), the piglets were sacrificed with an overdose of pentobarbital-sodium. The γ- and β-activities of ^{82}Br and ^{36}Cl in plasma and urine were determined and the ratios of [Br] to [Cl] were compared. The 4-hr sample collections were used to calculate Br dilution volume. The protocol was approved by

the Animal Protocol Review Committee of Baylor College of Medicine.

The γ-activities of ^{82}Br in known weights of plasma and urine samples were measured using a gamma spectrometer (Packard, Laguna Hills, CA) and the activities (cpm/g) were corrected for decay (half-life, $t_{1/2}$ = 35.5 hr) back to the time of injection. The β-activities of ^{36}Cl ($t_{1/2} = 10^5$ years) in known weights of plasma and urine (dpm/g) were measured using a liquid scintillation spectrometer (TM Analytic, Elk Grove Village, IL). The water content of all plasma and urine samples was determined by desiccation; γ- and β-activities in plasma and urine are expressed as cpm/g water or dpm/g water.

Calculations. *Human studies.* Plasma, urine, and saliva [Br] to [Cl] ratios ($[Br]_p$:$[Cl]_p$, $[Br]_u$:$[Cl]_u$, and $[Br]_s$:$[Cl]_s$) were calculated and compared at each time point. Values for K_u were calculated from $[Br]_u$:$[Cl]_u$ divided by $[Br]_p$:$[Cl]_p$ and values for K_s from $[Br]_s$:$[Cl]_s$ divided by $[Br]_p$:$[Cl]_p$.

The 4-hr dilution volume of Br was calculated from the amount of Br given less urinary Br loss and divided the amount by the 4-hr $[Br]_p$ (1):

$$\text{Br dilution volume (g/kg)} = \frac{\{(V_i \times [Br]_i) - (V_u \times [Br]_u)\} \times 0.95}{[Br]_p} \div \text{body wt (kg)} \quad \text{[Eq. 1]}$$

where V_i is the volume (g); $[Br]_i$, the concentration of the Br solution ingested (μmol/g); V_u, urinary volume (g); and $[Br]_u$, the concentration of urinary Br (μmol/g). The equation was multiplied by 0.95 to correct for the Gibbs-Donnan factor (1).

Equation 2 was derived from Eq. 1 to enable calculation of Br dilution volume using urine samples. In Eq. 2, $[Br]_u$ was used instead of $[Br]_p$. As $[Br]_u$ depends greatly on the concentration of urine, calculation of $[Br]_u$:$[Cl]_u$ would eliminate the variability of $[Br]_u$ that would result from conservation or elimination of water by the kidneys. The amount of Br given was divided by $[Cl]_p$ to provide a common denominator of [Cl] rather than water content in the equation.

$$\text{Br dilution volume (g/kg)} = \frac{\{(V_i \times [Br]_i{:}[Cl]_p) - (V_u \times [Br]_u)\} \times 0.95}{[Br]_u{:}[Cl]_u} \div \text{body wt (kg)} \quad \text{[Eq. 2]}$$

The corrected 4-hr dilution volume of Br from urine samples was calculated using Eq. 2 multiplied by the factor K_u, which corrects for the differences in Br and Cl transport by the kidneys.

The 4-hr dilution volume of Br was calculated from saliva samples using Eq. 2, then Eq. 2 multiplied by K_s, and substituting saliva concentrations for all urine concentrations.

Piglet studies. The value K_u is calculated from the ratio of ^{82}Br to ^{36}Cl activity in urine divided by the ratio of ^{82}Br to ^{36}Cl activity in plasma at each time point.

The 4-hr dilution volume of ^{36}Cl was calculated from plasma samples using a modification of Eq. 1 in which all Br concentrations were substituted by ^{36}Cl activities. The 4-hr dilution volume of ^{82}Br was calculated from urine samples using Eq. 2, then using Eq. 2 multiplied by K_u. In these calculations, all Br and Cl activities were expressed as either cpm/g or dpm/g.

Statistical Analysis. Changes over time in plasma, urine, and saliva [Br] were plotted semilogarithmically and lines of best fit were calculated by linear regression for each individual. The slopes of all individual regressions were averaged, and this average was compared with zero using a t test. The process was repeated for each of the three obtained body fluids. Differences among [Br] to [Cl] ratios in plasma, urine, and saliva were tested by analyses of variance for repeated measures. When a significant difference was detected, the data were further analyzed by paired t test using the Bonferroni correction for multiple comparison. Similar statistical analyses were made to compare differences among various dilution volumes. In addition, agreement between two dilution methods was tested by plotting the difference between two dilution volumes against the mean of the dilution volumes, and a regression analysis was performed to determine whether the differences depended on the volume. The mean difference was analyzed with a paired t test, and the variability among the individual differences was described (20).

Results

Human Studies. In all subjects, $[Br]_p$ consistently decreased over time ($P < 0.05$). Urine and saliva [Br] showed large, inconsistent fluctuations over the study period; the directions and the extent of the fluctuations differed among individuals. Both $[Cl]_p$ and $[Cl]_s$, but not $[Cl]_u$, remained relatively stable throughout the study period for each individual. Over the study period, $[Cl]_u$ showed fluctuations for each individual in the same direction as those of $[Br]_u$.

When $[Br]_u$ was divided by $[Cl]_u$ for each individual, the ratio of $[Br]_u$ to $[Cl]_u$ showed a consistent pattern of decrease over time that was similar to those of $[Br]_p$ to $[Cl]_p$ ($P < 0.05$). The ratio of $[Br]_s$ to $[Cl]_s$ showed no consistent pattern over the study period and differed among individuals. Figure 1 shows $[Br]_p$, $[Br]_u$, and $[Br]_s$ from one of the subjects at 4 to 8 hr after Br administration. Figure 2 shows [Br] to [Cl] ratios in plasma, urine, and saliva for the same subject whose data were used for Figure 1. As seen in Figure 2, $[Br]_u$:$[Cl]_u$ showed a consistent pattern of decrease over

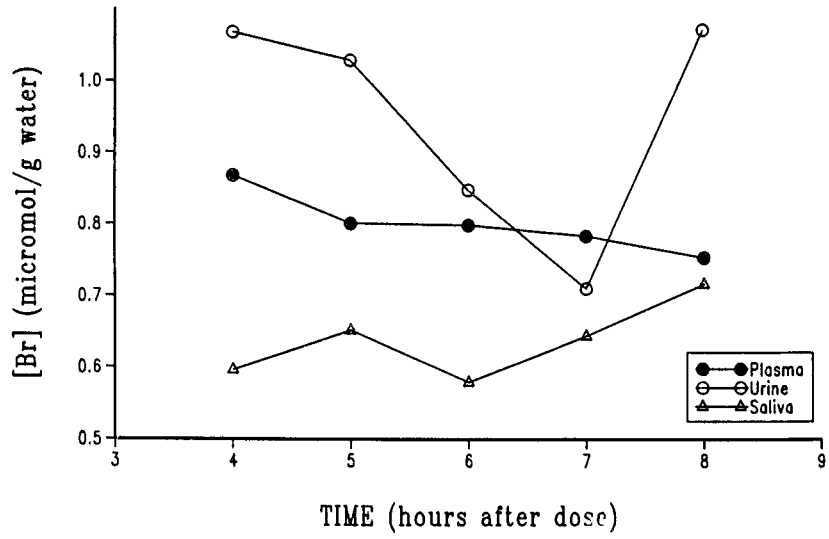

Figure 1. [Br] in samples of plasma, urine, and saliva collected from one person at hourly intervals between 4 and 8 hr after an oral dose of Br. Plasma [Br] decreased over time ($P < 0.05$).

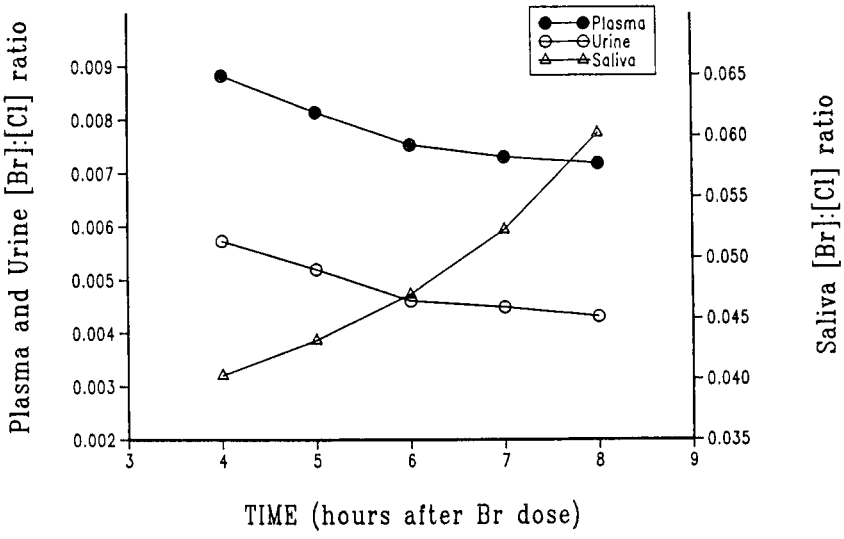

Figure 2. [Br] to [Cl] ratio in samples of plasma, urine, and saliva collected from same person described in Figure 1. Ratios of [Br] to [Cl] decreased over time in plasma and urine and increased in saliva ($P < 0.05$).

time. Mean [Br] to [Cl] ratios for plasma, urine, and saliva for the 10 subjects are shown in Table I. The mean K_u was 0.7 ± 0.01 (±SD) and the mean K_s was 6.1 ± 0.3. Both values were significantly different from zero and from each other ($P < 0.01$; Table I).

The dilution volumes of Br calculated from the 4-hr samples showed significant differences between those estimated from $[Br]_u$:$[Cl]_u$ and plasma [Br] ($P < 0.01$), and between those estimated from $[Br]_s$:$[Cl]_s$ and plasma [Br] (Table II). However, when the mean K_u of 0.7 was used to correct for the difference between $[Br]_u$:$[Cl]_u$ and $[Br]_p$:$[Cl]_p$, the difference between the two calculated Br dilution volumes was eliminated ($P = 1.0$) (Table II). Similarly, when the mean K_s of 6.1 was used as a correction factor, there was no statistical difference between Br dilution volumes calculated from saliva [Br]:[Cl] and plasma [Br] ($P = 0.2$) (Table II).

Piglet Studies. In piglets, the ratio of ^{82}Br to ^{36}Cl activities in urine was also significantly lower than that in plasma. The mean K_u over the 2- to 8-hr period was 0.84 for seven piglets ($P < 0.05$) (Table III).

The mean 4-hr dilution volumes calculated from plasma ^{82}Br and ^{36}Cl activities were 356 ± 14 ml/kg body wt and 347 ± 12 ml/kg, respectively, and were not significantly different ($P > 0.05$). The mean 4-hr dilution volume calculated from urine ^{82}Br:^{36}Cl activities was significantly different from those volumes calculated from plasma ^{82}Br activity ($P < 0.05$) (Table II). However, when the dilution volumes calculated from urine ^{82}Br:^{36}Cl activities were multiplied by the mean K_u of 0.84, the mean corrected dilution volume from

Table I. Mean Ratios of Br and Cl Concentrations ([Br]:[Cl]) in Samples of Plasma, Urine, and Saliva in the Human (Mean ± 1 SD, $n = 10$)[a]

Time (hr)	[Br]:[Cl] × 10^{-3}[b]			$\frac{\text{Urine [Br]:[Cl]}}{\text{Plasma [Br]:[Cl]}} = K_u$	$\frac{\text{Saliva [Br]:[Cl]}}{\text{Plasma [Br]:[Cl]}} = K_s$
	Plasma	Urine	Saliva		
0	0.6 ± 0.1	0.7 ± 0.3	3.6 ± 1.1	1.16 ± 0.69	6.3 ± 2.6
4	7.8 ± 0.7	5.4 ± 0.8	43 ± 10	0.70 ± 0.12	5.5 ± 1.0
6	7.3 ± 0.5	5.2 ± 0.9	46 ± 9	0.71 ± 0.12	6.2 ± 1.2
7	7.2 ± 0.5	5.0 ± 0.9	45 ± 10	0.70 ± 0.14	6.3 ± 1.2
8	7.1 ± 0.6	4.8 ± 0.6	44 ± 9	0.68 ± 0.07	6.2 ± 1.2
Mean ± SD[c]				0.70 ± 0.01[d]	6.1 ± 0.3[d]

[a] Br and Cl concentrations measured in μmol/g water.
[b] Ratios of [Br] to [Cl] were different among plasma, urine, and saliva at each time point, $P < 0.001$, except there was no difference between plasma and urine at zero hour.
[c] Mean ± 1 SD was calculated from the values obtained at 4, 6, 7, and 8 hr.
[d] Significantly different from 1 and from each other, $P < 0.01$.

Table II. Mean Br Dilution Volumes Calculated from 4-hr Plasma, Urine, and Saliva Samples (Mean ± 1 SD)

Fluid/calculation method	Br dilution vol (g/kg body wt)	
	Adult humans ($n = 10$)	Piglets ($n = 7$)
Plasma		
Eq. 1[a]	268 ± 20	356 ± 14
Urine		
Eq. 2[b]	381 ± 60[c]	429 ± 37[c]
Eq. 2 × K_u[b,d]	267 ± 42	360 ± 31
Saliva		
Eq. 2[b]	50 ± 12[c]	—
Eq. 2 × K_s[b,e]	300 ± 70	—

[a] Eq. 1:

$$\frac{V_i(g) \times [Br]_i\ (\mu mol/g) \times 0.95}{[Br]_p\ (\mu mol/g)} \div \text{body wt (kg)}$$

[b] Eq. 2:

$$\frac{V_i \times [Br]_i:[Cl]_p \times 0.95}{[Br]_u\ (\text{or}_s):[Cl]_u\ (\text{or}_s)} \div \text{body wt (kg)}$$

[c] Dilution volume was significantly different ($P < 0.05$) from that calculated from 4-hr plasma Br.
[d] Mean value of $K_u = 0.70$ for humans and 0.84 for piglets.
[e] Mean value of $K_s = 6.1$.

Table III. Mean Ratios of ^{82}Br and ^{36}Cl Activity in Simultaneously Taken Samples of Urine and Plasma in the 1-Week-Old Pig (Mean ± 1 SD, $n = 7$)

Time (hr)	^{82}Br:^{36}Cl Activity[a]		$\frac{\text{Urine } ^{82}Br:^{36}Cl}{\text{Plasma } ^{82}Br:^{36}Cl} = K_u$
	Urine	Plasma	
2	0.67 ± 0.15	0.78 ± 0.11	0.86 ± 0.11
3	0.63 ± 0.13	0.77 ± 0.11	0.82 ± 0.09
4	0.63 ± 0.14	0.77 ± 0.11	0.82 ± 0.10
5	0.64 ± 0.14	0.76 ± 0.10	0.84 ± 0.10
6	0.65 ± 0.15	0.76 ± 0.10	0.84 ± 0.11
7	0.65 ± 0.15	0.76 ± 0.10	0.85 ± 0.12
8	0.65 ± 0.15	0.76 ± 0.09	0.85 ± 0.12
Mean ± SD			0.84 ± 0.01[b]

[a] Plasma and urine ^{82}Br and ^{36}Cl activities were measured in cpm/g water and dpm/g water, respectively.
[b] Significantly different from 1, $P < 0.01$.

urine samples was not different from the 4-hr plasma ^{82}Br dilution volume.

Discussion

The measurement of ECW in very young infants is difficult because blood samples are required for the calculation of the distribution volume of a solute. Water labeled with the stable isotopes deuterium or oxygen-18, however, is particularly useful in measuring total body water in infants because of the evidence that, after an oral or intravenous dose of these isotopes, isotopic enrichments of biological fluids such as breath water, saliva, and urine are similar to those of plasma; therefore, the need to take blood samples is circumvented (21, 22). The use of breath water as a sample source for measurement of Br dilution volume was tested without success because ^{82}Br activity was found to be too close to background levels (23). Similarly, the use of urine or saliva to measure Br dilution volume would not be feasible because of the significant differences between the concentration of Br in these biological fluids and the concentration of Br in plasma, as demonstrated in our present study and by other investigators (9, 11, 19).

Because the distribution volume of Br is almost identical to that of Cl (6–11), we hypothesized that the calculation of the volume distribution of Br from either urine or saliva samples might be possible if we used [Br] to [Cl] ratios (rather than [Br]) of these fluids. Although our data indicated that the transport characteristics of Br and Cl were not similar in fluids produced by the kidneys and the salivary glands, a characteristic relationship that was constant for each individual was observed between [Br] to [Cl] ratios in plasma and those in urine and saliva, respectively.

In urine, more Br than Cl was reabsorbed, as demonstrated by the mean urine to plasma ratio for Br

to Cl of 0.70 ± 0.01 for the humans. Similar data were reported by Mason (19) in an adult with a mean urine to plasma ratio for Br to Cl of 0.73 ± 0.04 (±SD). He also reported a mean value of 0.7 for the same relationship in three dogs (19). Gamble *et al.* (9) calculated an average value of 0.76 for the urine to plasma ratio of ^{82}Br to ^{38}Cl in six adult humans. Our experiment confirmed that in both adult humans and very young piglets the urine to plasma Br to Cl ratios remained constant over 4 to 8 hr after administration of Br. In piglets, however, the mean urine to plasma ratio of 0.84 ± 0.01 (±SD) was higher ($P < 0.05$) than the value of 0.7 for humans, perhaps because of differences in species, age, or both.

Bromide dilution volumes calculated from urine and saliva [Br] to [Cl] ratios were significantly different ($P < 0.01$) from those calculated from plasma [Br] for both adult humans and piglets. The dilution volumes from urine [Br] to [Cl] ratios were 42% higher for humans and 21% higher for piglets. These differences were eliminated when the appropriate K_u values were used to correct for the differences between the [Br] to [Cl] ratios of urine and those of plasma. After corrections with K_u, mean differences between dilution volumes calculated from urine samples and those calculated from plasma [Br] were 1 ± 40 ml/kg for adult humans and 4 ± 34 ml/kg for piglets.

The mean Br dilution volume calculated from the saliva [Br] to [Cl] ratio was only 19% of that calculated from plasma [Br] (50 ± 12 ml/kg, compared with 268 ± 20). The large difference was eliminated, however, after the use of the K_s value of 6.1 to correct for the differences between the [Br] to [Cl] ratios in saliva and those in plasma. The variation in individual Br dilution volumes for saliva compared with those for plasma [Br] (mean difference, −36 ± 64 ml/kg) was greater than the variation obtained from comparing urine Br dilution volumes with plasma [Br]. The relatively larger error in the calculations from saliva probably resulted from the fact that the saliva collected was a mixture of secretions from different salivary glands. Mason (19) reported that the Br to Cl ratio in the parotid saliva of dogs was similar to that of plasma, but a mixed saliva Br to Cl ratio was higher than that of plasma. In our human experiment, the Br to Cl ratio in mixed saliva was 6.1:1 compared with that in plasma; one or more of the human salivary glands must, therefore, preferentially secrete Br.

Our study indicates the feasibility of using urine samples for the determination of Br dilution volumes and, therefore, ECW volumes, in adult males and young animals. The calculation of Br dilution volume from urine samples requires (i) the collection of two urine samples, one at zero time and one 4 to 8 hr after administration of Br; (ii) a measurement or estimate of plasma chloride concentration; and (iii) a correction factor describing the relationship of urine to plasma ratios of [Br] to [Cl], which can be determined in advance for the population to be studied. The major advantages in using this technique to determine ECW volumes are that fasting is not required and that little or no blood is taken.

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