A Comparison of Chloride, Bromide, and Sucrose Dilution Volumes in Neonatal Pigs[1] (43199)

J. CHRISTIAN MØRKEBERG, HWAI-PING SHENG,[2] AND RUSSELL A. HUGGINS

Unites States Department of Agriculture, Agricultural Research Service, Children's Nutrition Research Center, Department of Pediatrics and Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

Abstract. **Use of ^{36}Cl, ^{82}Br, and [^{3}H]sucrose to estimate extracellular water volume was evaluated in 14 piglets (7–14 days old). ^{36}Cl and ^{82}Br were distributed in approximately the same volume, but a period of 5–6 hr after injection was required to reach equilibrium in the neonatal pig. Dilution volumes calculated before equilibration (2–5 hr) for ^{36}Cl (326 ± 11 ml/kg) and ^{82}Br (328 ± 13 ml/kg) were different from equilibration (6–8 hr) phase volumes (356 ± 13 ml/kg and 355 ± 13 ml/kg, respectively; $P < 0.001$). A 3-hr sample estimated the same volume distribution calculated by extrapolation of the 6- to 8-hr period because of the relationship between the two slopes of the plasma clearance curves. After the ^{82}Br and ^{36}Cl had achieved equilibration, each was distributed in a volume equivalent to total body chloride space (362 ± 29 ml/kg) measured by neutron activation; no statistical differences were found ($P = 0.6$). The early equilibration phase measured a 10% smaller, faster exchangeable fraction of total body Cl. Sucrose dilution volume (332 ± 19 ml/kg) required multiple plasma samples for extrapolation and measured a dilution volume 7% smaller ($P < 0.05$) than total body chloride space.**

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Estimates of extracellular water (ECW) volume in an individual can differ significantly, depending on the substance used in the measurement. Some of these indicator substances enter cells, others are distributed in plasma and other fluids—interstitial and cerebrospinal—at different rates; their rate of penetration into interstitial fluid in connective tissue, cartilage, and bone may also differ (1–3). In addition, significantly different volumes of distribution will result depending on the time at which plasma samples are taken in relation to the time of equilibrium of the indicator (2, 4).

Most investigations into the time required for equilibration of the indicator substances used to estimate ECW have been made on adult animals, including humans (1, 2, 4–8). Equilibration times for Br and Cl ranged between 1 and 6 hr. More recently, studies in neonates used a single timed plasma sample collected between 1 and 3 hr for the calculation of dilution volume of Br (9, 10). This time interval was chosen based on limited data on neonates, which showed that the times required for equilibration of Cl and Br were between 2 and 3 hr after injection (11–13), a range considerably narrower than that reported for adults. The difference in the reported ranges between adults and neonates may be the consequence of the limited amount of data collected over the period after injection. Other explanations, however, might be the effect of growth on the volume distribution of substances used to estimate ECW (14) or the effect of growth and size on rates of distribution of these substances (15).

Because of the revival of interest in the use of sucrose to estimate ECW in infants [sucrose does not enter cells and consequently requires no correction factor (16, 17)], an experiment was designed to compare its volume distribution with those of Cl and Br.

The objectives of the present study were (i) to establish the time required after injection for Cl, Br, and sucrose to reach equilibrium in neonatal pigs; (ii)

[1] This work is a publication of the United States Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

[2] To whom requests for reprints should be addressed at 1100 Bates Street, Houston, TX 77030.

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to compare the volume distribution of Br, Cl, and sucrose calculated from a single plasma sample at 3 hr with the volumes calculated from extrapolation of multiple time-concentration data to zero time; and (iii) to compare these results with those calculated from total body Cl measured by total body neutron activation analysis (TBNAA). The neonatal pig was selected for this experiment because of the similarity of its body composition to that of a newborn infant (18).

Materials and Methods

Experimental Design. Fourteen 7- to 14-day-old Pitman Moore/Hanford piglets (Bastrop Farms, Bastrop, TX) were studied. The body weights ranged from 1.7 to 3.0 kg. The piglets were anesthetized with a combination of ketamine (20 mg/kg) and pentobarbital-sodium (10 mg/kg). A central venous catheter was inserted via the jugular vein for collection of blood samples, and a Foley catheter for continuous urine collection was inserted into the bladder through an abdominal incision. After baseline samples of blood and urine were collected, a single bolus of each of the radiotracers was injected through the venous catheter. The exact amount of radiotracer injected was determined by weighing the syringe before and after injection. Seven piglets (Group 1) received ^{82}Br (5 μCi/kg body wt; NEN Products, Wilmington, DE) and ^{36}Cl (5 μCi/kg; NEN). The remaining seven piglets (Group 2) received ^{82}Br (5 μCi/kg) and [^{3}H]sucrose (15 μCi/kg; NEN Products). The catheter was flushed with 2 ml of saline after injection of the radiotracers. In Group 1, blood (2 ml) and urine samples were taken 2 hr after injection of the radiotracers, then hourly over the next 6 hr. In Group 2, blood (2 ml) and urine samples were collected 1 hr after injection of radiotracers, then every 30 min for the next 4 hr. A shorter study time was chosen for the second group because [^{3}H]sucrose activity in plasma was close to background levels at 5 hr after injection. At the end of the study, the piglets were killed with an overdose of pentobarbital-sodium, and the carcasses were frozen. Total body chloride was measured by TBNAA after the γ activities of ^{82}Br in the carcasses had decayed to baseline values (approximately 4 weeks, $>10 \cdot t_{1/2}$, where $t_{1/2}$ is half-life). The protocol was approved by the Animal Protocol Review Committee of Baylor College of Medicine.

Analytical Procedures. Plasma Cl concentrations were analyzed using a Buchler chloridometer (Saddle Brook, NJ) on the last plasma samples obtained before the piglets were killed. Water content of all plasma and urine samples was determined by desiccation so that measurements of plasma chloride concentrations and γ and β activities in plasma and urine could be adjusted for water content.

The γ activities of ^{82}Br in known volumes of plasma and urine samples were measured using a gamma spectrometer (Packard, Laguna Hills, CA) and the activities (cpm/g) corrected for decay ($t_{1/2}$ = 35.5 hr) back to the time of injection. The β activities of ^{36}Cl ($t_{1/2}$ = 10^5 years) and [^{3}H]sucrose ($t_{1/2}$ = 12.3 years) in known volumes of plasma and urine samples (dpm/g) were measured using a liquid scintillation spectrometer (TM Analytic, Elk Grove Village, IL). Total body chloride was measured by TBNAA using the facility and procedures described by Evans *et al.* (19) and Sheng *et al.* (20): a plastic cylinder containing the piglet was rotated at 6 rpm and was irradiated with neutrons for 15 min. The neutron source was 2.2 mg of ^{252}Cf, and each piglet received a radiation dose of 16 rem. The γ-rays emitted from ^{38}Cl were detected by two NaI crystal detectors (292-mm diameter × 102-mm thick). Each piglet was monitored for 1500 sec, and the integrated counts for the Cl peaks, at 1643 and 2167 keV, were determined. Phantoms of appropriate sizes, each containing a known concentration of Cl, were used to calibrate the instrument.

Calculations

The volumes of distribution of the radiotracers were calculated with the commonly used dilution method (21):

$$\text{Dilution volume (g)} = \frac{\text{Total activity injected (cpm or dpm)}}{\text{Plasma activity (cpm or dpm/g plasma water)}} \times 0.95$$

Extrapolation Method for Plasma Activities. When volumes of distribution were calculated from the extrapolation method, plasma clearance of either γ or β activities of ^{82}Br, ^{36}Cl, or [^{3}H]sucrose with time was plotted semilogarithmically. Lines of best fit were calculated by linear regression for each individual and the slopes extrapolated back to injection (time zero). Two separate slopes were calculated for each data set from the 2- to 5-hr and 6- to 8-hr periods. If the slopes differed significantly, two extrapolations to time zero were calculated. The y-intercept values for the plasma activities of ^{82}Br, ^{36}Cl, or [^{3}H]sucrose clearance curves were used to calculate the dilution volumes of these radioisotopes (2). The equation for dilution volume was multiplied by 0.95 to account for the Gibbs-Donnan factor for ^{82}Br and ^{36}Cl (21).

Single, Timed Plasma Sample. The volumes of distribution of the radiotracers at 3- and 6-hr time points were calculated using plasma activities for those time periods. In these calculations, the total amount of activity lost in urine (over the 3- or 6-hr period) was subtracted from the total activity injected. The equation was multiplied by 0.95 to account for the Gibbs-Donnan factor for ^{82}Br and ^{36}Cl.

Body Chloride. Total chloride space (distribution

volume) was calculated from total body chloride measured by TBNAA and plasma chloride concentration (22):

$$\text{Cl space (g)} = \frac{\text{Total body chloride (mmol)}}{\text{Plasma Cl concentration (mmol/g plasma water)}} \times 0.95$$

The Gibbs-Donnan correction factor of 0.95 was used.

Statistical Analysis

Differences between the rates of clearance of various isotopes at different time intervals were tested using a paired t test on the slopes obtained by regression analysis. Differences among various dilution volumes were tested by analysis 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. Also, agreement between two dilution volumes was assessed by a pairwise comparison, which showed the relative bias (mean difference) and the limits of agreement (mean difference ± 2 SD of the differences) between the two dilution volumes. The differences between the two dilution volumes were plotted against the mean of the two dilution volumes for each animal (23). Regression analysis was used to test for any significant relationship between the differences and the mean dilution volumes. The difference between the two dilution volumes was tested for significance using a paired t test.

Results

Plasma Clearance. Both ^{36}Cl and ^{82}Br showed two different rates of clearance: an early phase between 2 and 5 hr after injection, and a later phase between 6 and 8 hr after injection (Fig. 1). The rates of clearance for the early phase were −0.0028 ± 0.0004% dose/hr for ^{36}Cl ($P < 0.001$) and −0.0036 ± 0.0008% dose/hr for ^{82}Br ($P < 0.001$). The clearance rates for the later phase were −0.0003 ± 0.0005% dose/hr for ^{36}Cl ($P = 0.3$) and −0.0009 ± 0.0010% dose/hr for ^{82}Br ($P = 0.06$). There was no difference between clearance rates for ^{36}Cl and ^{82}Br ($P > 0.5$) for both the early and late phases.

A single regression slope was calculated for the plasma clearance curves for ^{82}Br and [^{3}H]sucrose over the 4-hr experimental period (Fig. 2). The 5-hr time point was excluded in the analysis because the [^{3}H]sucrose activity was close to background level. The clearance rate for [^{3}H]sucrose (−0.0168 ± 0.0022% dose/hr) differed significantly ($P < 0.001$) from ^{82}Br (−0.0030 ± 0.0010% dose/hr). Both regression slopes differed from zero ($P < 0.001$).

Urinary Loss of Isotopes. The losses of ^{82}Br and ^{36}Cl activity in urine over the 8-hr period were small and did not differ significantly from each other: 1.4% ± 0.9% of the injected amount for ^{82}Br and 1.7% ± 1.3% for ^{36}Cl ($P = 0.1$). However, the urinary excretion of [^{3}H]sucrose was significantly larger than that of ^{36}Cl and ^{82}Br: 87.2 ± 5.1% of the injected amount over the 5-hr study period ($P < 0.001$).

Dilution Volumes. The dilution volumes calculated from the 2- to 5-hr period were significantly smaller than those calculated from the 6- to 8-hr period ($P < 0.001$). The mean differences were 27 ± 7 ml/kg for ^{82}Br (mean ± 1 SD) and 30 ± 7 ml/kg for ^{36}Cl, a mean percentage difference of 8–9%. There were no significant differences ($P = 0.8$) between ^{82}Br and ^{36}Cl dilution volumes from the extrapolation method for either the 2- to 5-hr or 6- to 8-hr period (Table I).

There was no difference statistically between Cl space determined from whole body Cl using neutron activation and ^{36}Cl and ^{82}Br dilution volumes determined by extrapolation of samples from 6- to 8-hr to zero ($P = 0.6$; Table I). However, the ^{82}Br and ^{36}Cl dilution volumes calculated from the 2- to 5-hr samples were significantly less than the Cl dilution volume determined by neutron activation ($P < 0.01$; Table I): the mean difference was 36 ± 26 ml/kg for ^{36}Cl (Fig. 3) and 34 ± 30 ml/kg for ^{82}Br, a mean percentage difference of 9–10%.

Mean dilution volumes for ^{36}Cl and ^{82}Br calculated from the 3-hr samples or from the 6-hr samples with a correction for urine loss of tracer did not differ significantly from those calculated from the 6- to 8-hr extrapolation method or from Cl space determined by neutron activation ($P > 0.05$) with one exception: when calculated from a single 6-hr sample, the dilution volume of ^{82}Br was significantly larger than that calculated by the extrapolation method ($P < 0.05$, mean difference = 22 ± 12 ml/kg; Table I).

Mean [^{3}H]sucrose dilution volume calculated from a single 3-hr plasma sample with a correction for urinary excretion was significantly larger than that calculated from extrapolation (mean difference = 96 ± 41 ml/kg, $P < 0.001$; Table II) and also larger than the 3-hr ^{82}Br dilution volume (mean difference = 86 ± 38 ml/kg; $P < 0.001$).

Significant differences ($P < 0.05$) also existed for both [^{3}H]sucrose dilution volume and ^{82}Br dilution volume calculated by extrapolation to time zero using the 1- to 4-hr samples when compared with Cl space determined from whole body Cl (Table II). Mean differences were 29 ± 23 ml/kg for [^{3}H]sucrose dilution volume and 32 ± 25 ml/kg for ^{82}Br dilution volume.

Discussion

Our data are in agreement with those of previous investigators (3, 6, 7, 13): after equilibration, both Br and Cl were distributed in approximately the same volume. In our experiment, however, equilibration of

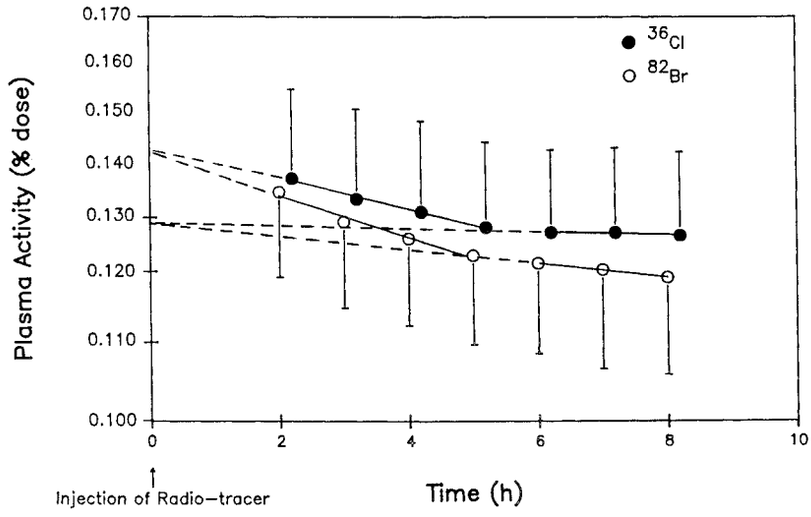

Figure 1. Semilogarithmic x, y plot of the clearance of ^{82}Br and ^{36}Cl from plasma in seven animals. Plasma activity (cpm/g plasma water for ^{82}Br and dpm/g plasma water for ^{36}Cl) is expressed as a percentage of the injected dose. The dashed lines represent extrapolation of plasma activity to time of injection by linear regression. The vertical bars indicate 1 SD. The rate of clearance from plasma was significantly different between the 2- to 5-hr and 6- to 8-hr periods ($P < 0.001$) for both ^{82}Br and for ^{36}Cl.

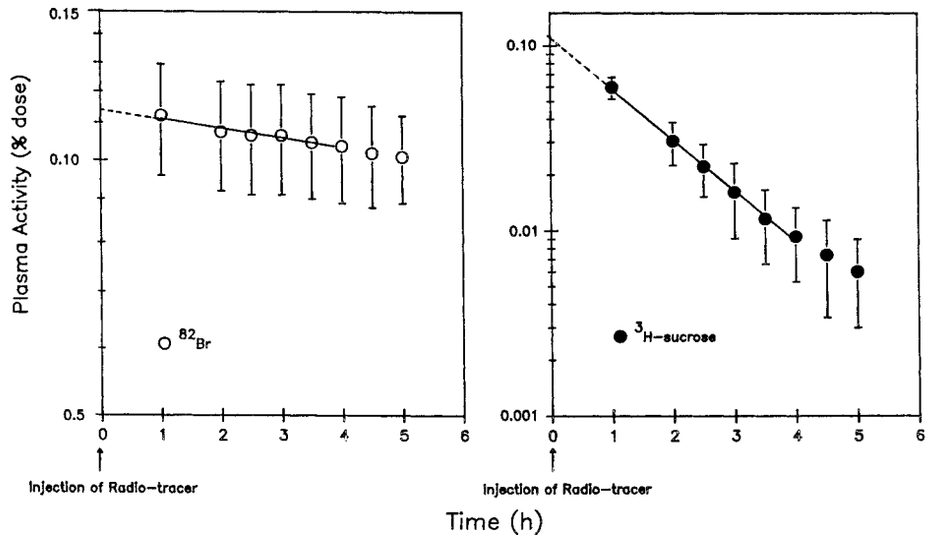

Figure 2. Semilogarithmic plots of the clearance of ^{82}Br and [^{3}H]sucrose from plasma in seven animals. Plasma activity (cpm/g plasma water for ^{82}Br and dpm/g plasma water for [^{3}H] sucrose) is expressed as a percentage of the injected dose. The dashed lines represent extrapolation of plasma activity to time of injection by linear regression. The vertical bars indicate 1 SD. The rate of clearance from plasma was significantly different for ^{82}Br and [^{3}H]sucrose ($P < 0.001$).

both radioisotopes required 5–6 hr after injection. Equilibration was confirmed by the fact that the 6- to 8-hr extrapolated dilution volumes of both isotopes were similar to total chloride space calculated from TBNAA and thus reflected total body distribution. The 5- to 6-hr period was longer than the 1–3 hr reported for Br in young animals and infants (9, 10), and for Cl and Br in adult dogs and humans by some investigators (1, 5, 6, 8, 21, 24); it was, however, in agreement with the time period reported for adult rats and humans by others (2, 4, 7). Factors that may contribute to the differing results between investigations in the time required to reach equilibrium include: the frequency of plasma samplings, the duration of the study, and the definition of the term "equilibration" or "equilibrium." Most investigators assume that equilibration is attained when the concentration-time course of the substance in plasma approaches a straight line, usually within 1–2 hr after injection of Cl or Br (2, 5, 6, 8, 11, 21). Therefore, if relatively few data points collected over a 1- to 24-hr period give plasma concentration values similar to those at 1–2 hr, these investigators assume that equilibration has occurred.

Our data on piglets support the conclusion that

Table I. ^{82}Br and ^{36}Cl Dilution Volumes and Cl Space Obtained by TBNAA[a]

Space (ml/kg)	Extrapolation[b]		Single sample[c]	
	2–5 hr	6–8 hr	3 hr	6 hr
^{82}Br	328 ± 13a[d]	355 ± 13	356 ± 14	372 ± 15b[e]
^{36}Cl	326 ± 11a	356 ± 13	347 ± 12	358 ± 8
Cl (TBNAA)[f]				362 ± 29

[a] Values (in ml/kg) are given as the mean ± SD of data from seven animals.
[b] ^{82}Br and ^{36}Cl dilution volumes were determined from plasma concentrations obtained by using extrapolation to time of injection of the 2- to 5-hr and 6- to 8-hr samples, respectively.
[c] ^{82}Br and ^{36}Cl dilution volumes were determined from a single plasma sample collected at either 3 or 6 hr and correction was made for amount of tracer excreted in urine.
[d] Significantly different from other dilution volumes without same letter, $P < 0.01$.
[e] Significantly different from other dilution volumes without same letter except Cl (TBNAA), $P < 0.05$.
[f] Cl space calculated from total body Cl determined by TBNAA.

equilibration, as indicated by a constant rate change in the plasma, is attained in 1 hr. However, a more detailed analysis of these data demonstrated two significantly different rates of decay from plasma for both ^{82}Br and ^{36}Cl. The initial and faster rate occurred between 1 and 5 hr after injection and the second, slower rate between 6 and 8 hr. These results agree with those from adult rats in which equilibration was achieved only after 5 hr (2). Because of the existence of two different rate changes, two different volumes of distribution were calculated for each of the radioisotopes. For both ^{36}Cl and ^{82}Br, the dilution volumes calculated from the 2- to 5-hr period were significantly smaller (by 8–9%) than those calculated from the 6- to 8-hr period. It can be inferred from other studies (2) that the initial phase of equilibration (1–5 hr after injection) indicates that equilibrium of isotopic distribution had occurred in the fluids of a majority of well-perfused tissues and organs. Thus, in most studies, particularly clinical studies in which an estimate of ECW is of prime interest, the early equilibration phase should be used. The second phase of equilibration (after 5 hr) indicates a steady state after the isotopes have distributed in the fluids of less well-perfused tissues and organs. This second phase would include complete exchange of the isotopes with intracellular Cl, which has been calculated to be approximately 10% of total body chloride (25). This phase reflects the total body chloride pool.

The conclusions from data on piglets and adult rats (2) that final equilibration was attained after 5 hr differed from an earlier study by Cheek and West (13) on relatively immature rats (body weight range, 74–435 g). Cheek and West (13) found no difference between Br dilution volume calculated from 3-hr plasma samples and Cl space obtained by carcass analysis and concluded that a 3-hr period for equilibration of Br was sufficient. The apparent difference between the equilibration times in the two studies [Pierson *et al.* (2) and Cheek and West (13)] may be attributed to a difference in the age of the rats. Examination of data from both studies, however, showed that in mature rats, Br space was 27.5% body wt at 2 hr, 32.9% at 5 hr, and 33.6% at 28 hr. Therefore, Br dilution volume calculated from the period between 2 and 5 hr reached 82–98% of its maximum value at equilibrium. Further examination of the data from 33 immature rats in the study by Cheek and West (13) showed that, although there was no difference between mean Br space and mean Cl

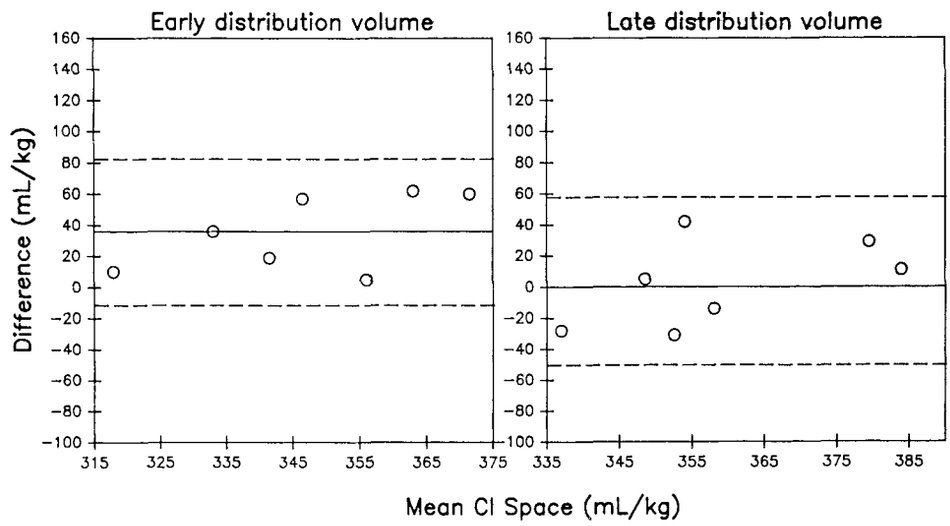

Figure 3. Comparison of Cl space calculated from total body Cl determined by neutron activation to dilution volume of ^{36}Cl calculated by extrapolation from the 2- to 5-hr period and the 6- to 8-hr period after injection, respectively [(23), $n = 7$]. The solid line represents the mean difference between two measurements and the dashed lines represent the upper and the lower limits of agreement (mean difference ± 2 SD of the differences). O, Individual differences between the two measurements.

Table II. ^{82}Br and [^{3}H]Sucrose Dilution Volumes and Cl Space Measured by TBNAA[a]

Space (ml/kg)	Extrapolation, 2–4 hr[b]	Single sample, 3 hr[c]
[^{3}H]Sucrose	332 ± 19a[d]	428 ± 41b[e]
^{82}Br	324 ± 12a	347 ± 9
Cl (TBNAA)[f]		356 ± 18

[a] Values (in ml/kg) are given as mean ± SD of data from seven animals.
[b] ^{82}Br and [^{3}H]sucrose dilution volumes were determined from plasma concentrations obtained by using extrapolation to time of injection of the 2- to 4-hr samples.
[c] ^{82}Br and [^{3}H]sucrose dilution volumes were determined from a single plasma sample collected at 3 hr; correction was made for amount of tracer excreted in urine.
[d] Significantly different from other dilution volumes without same letter, $P < 0.05$.
[e] Significantly different from other dilution volumes without same letter, $P < 0.05$.
[f] Cl space calculated from total body Cl determined by TBNAA.

space at 3 hr, Br space ranged from 11.1% less to 16.5% more than the Cl space. This relatively large variability between Br and Cl spaces in the study by Cheek and West (13) may explain the differences between their reported equilibration times and those of Pierson *et al.* (2). The large variability may also explain the reported differences in equilibration time between the Cheek and West (13) study and our piglet study, in which we found a mean difference of 8–9% between dilution volumes calculated from the early (2- to 5-hr) period and those calculated from the late (6- to 8-hr) period, for both Br and Cl.

Interest has recently been renewed in sucrose as a substance for use in the measurement of ECW, because it is not metabolized in humans and it does not enter cells, cerebrospinal fluid, or gastric juices, as do Cl and Br (7, 26). However, other indirect evidence in humans suggests that the use of sucrose may contribute to an overestimation of ECW because of extrarenal losses of sucrose as a result of metabolism or sequestration in some tissues (27). Our findings in piglets showed that dilution volumes of sucrose extrapolated from the 1- to 4-hr period were similar to those occurring in the early phases of Br and Cl, but were 7% less than those calculated from total body chloride; these results confirm the usefulness of sucrose to measure ECW. In contrast to our findings in piglets, mean volume distribution of sucrose calculated from extrapolation of blood samples obtained before 6 hr was approximately 30% smaller than those of chloride in adult nephrectomized dogs (28). This finding was unexpected because it has been reported that sucrose is secreted into the gastric lumen and metabolized in the dog (28). Even the relative nonpenetration of sucrose into the cerebrospinal fluid does not explain why the sucrose distribution volume was smaller than that of chloride.

The estimation of the dilution volumes of Br, Cl, and sucrose by the extrapolation method requires multiple blood samples. In most studies, however, particularly those of the neonate, only a single blood sample is used to estimate ECW; the sample is usually taken at 3 hr (9). However, because of the high disappearance rate of sucrose from the circulation, multiple samples must be used for extrapolation of the data. The mean dilution volume for sucrose from the 3-hr sample was approximately 29% larger than those calculated from extrapolation, even though sucrose excreted in the urine was included in the calculation. It is possible, however, that sucrose was metabolized and secreted into the gastric lumen by the piglets, a phenomenon reported in rats (2), dogs (28), and possibly humans (27).

Dilution volumes of ^{82}Br estimated from a single 3-hr plasma sample were approximately 9% larger than the volume calculated from the 2- to 5-hr extrapolation, but similar to the volume calculated from the 6- to 8-hr extrapolation. This disparity arises from the fact that ^{82}Br activity in the plasma decreased at two different rates: an initial faster rate and a later slower rate. Extrapolation of the slope derived from 6 to 8 hr (which reflects the total body chloride pool) gave a dilution volume similar to the 3-hr sample. An estimate of the ECW should be obtainable by adjusting the dilution volume from the 3-hr sample (by a factor of 0.9) to account for the amount of intracellular Cl (25). Therefore, based on the results of our piglet study, it is feasible to estimate ECW from a Br dilution volume that is calculated from a 3-hr plasma sample and adjusted to account for intracellular Cl.

We conclude from these data that ECW can be estimated from the dilution volumes of both Cl and Br using the 2- to 5-hr extrapolation period. A dilution volume calculated from the 6- to 8-hr extrapolation period measures the total body chloride pool and is approximately 9% larger than that calculated from the 2- to 5-hr extrapolation period. Dilution volume of Br calculated from the 3-hr plasma sample can be used to estimate ECW after correction for intracellular chloride content. The estimation of ECW from sucrose dilution volume, however, requires multiple blood sampling because the 3-hr sample gave results approximately 29% larger than ECW estimated from Br.

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