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Dilution Kinetics of Chemicals Used for Estimation of Water Content of Body Compartments in Perinatal Medicine

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ABSTRACT. Dilution kinetics of markers commonly used for estimation of body water content and distribution in perinatal medicine (*p*-aminohippurate, inulin, antipyrine, $H_2{}^{18}O$, bromide, and T1824) were studied in pregnant and neonatal baboons. Amniotic fluid concentrations of *p*-aminohippurate and inulin decreased exponentially after intraamniotic injection of these markers; from 2–24 h after injection, concentrations decreased linearly on semilogarithmic plot (r = 0.96–1.00). Plasma concentrations of antipyrine decreased exponentially during the first 60 min after intravenous injection, then linearly from 1–5 h (r = 0.92–0.90). Plasma concentrations of ^{18}O decreased linearly from 1–6 h after injection in three or four cases (r = 0.94–0.99). Plasma concentrations of bromide decreased during the first 2 h after injection, then stabilized for at least 3 h. Plasma concentrations of T1824 decreased linearly from 10–60 min after intravenous injection (r = 0.97–1.00). Then the decline became exponential until 5 h. These data allow us to make specific recommendations regarding the optimal time and method of amniotic fluid and blood sampling during body water studies. (*Pediatr Res* 25:377–382, 1989)

Abbreviation

PAH, *p*-aminohippurate

Numerous studies of perinatal physiology, metabolism, nutrition, and pathologic conditions require estimation of the fluid content of various body compartments. As direct volumetric determinations are not possible *in vivo*, less direct methods must be relied upon to obtain an acceptable estimate. The most commonly used techniques involve a principle developed by Fick. Fick's principle states that the fluid content (V) of a given space may be calculated following the administration of a dye or chemical into that space if the exact amount (Q) of substance injected and its concentration (C) in the fluid of the space are known, or V = Q/C. The dye or chemical must have five main properties: It must *1*) be harmless to the organism; *2*) be easily measurable; *3*) diffuse evenly and within a predictable period of time throughout its vol of distribution; *4*) have no effect on fluxes of fluids across membranes; and *5*) neither be metabolized nor leave the space for which the vol is being measured for the duration of the study. This last requirement is seldom met. If, however, the decrease in dye concentration with time follows a straight line, extrapolation of that line to time 0 (y-intercept or C_0) still allows a vol determination to be made, or $V = Q/C_0$. In that case, the rate at which the substance escapes from its vol of distribution, be it by metabolism or excretion, may be also calculated (slope of the concentration *versus* time line).

In cases where all four criteria are met, the marker diffuses as soon as it is administered to reach an even concentration throughout its vol of distribution. In cases where the fourth criterion is not met, two phenomena occur simultaneously as soon as the marker is administered. First, the marker diffuses to reach an even concentration throughout its vol of distribution; second it is being cleared from its assigned space. To interpret the data correctly, it is important to determine at what point in time equilibration is complete and the behavior of the marker concentration thereafter, *i.e.* the kinetics of the marker. Yet kinetic data have often been disregarded or remained buried in the investigators' laboratory data books and are scarce in the relevant literature. Also, in human perinatal medicine, there are ethical and practical limitations on the number and vol of body fluid samples that may be obtained. In this report, we have made use of the baboon (*Papio cynocephalus*), whose fetoplacentouterine unit and neonatal physiology and metabolism are very similar to those of *Homo sapiens*, to determine the kinetics of six markers: PAH (amniotic fluid vol), inulin (amniotic fluid vol), antipyrine (total body water), $H_2{}^{18}O$ (total body water), bromide (extracellular water) and T-1824 (plasma vol). From these kinetics, precise recommendations may be made regarding optimal time and method of amniotic fluid or blood sampling.

MATERIALS AND METHODS

PAH and inulin dilution. The kinetics of PAH and inulin dilution in amniotic fluid were studied in three pregnant baboons (*Papio cynocephalus*) at 176–178 d of gestation (term = 180 days). On the day of the study, each female was anesthetized with 10 mg/kg of ketamine injected intramuscularly, followed by a mixture of nitrous oxide and halothane for maintenance. Positions of the fetus and placenta were located by ultrasonography with a Toshiba model SAL-20A real-time scanner (Toshiba

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America, Wayne, NJ) equipped with a 3.5 MHz linear array transducer. A 60-cm long 16-gauge catheter was inserted into the amniotic cavity. After removal of a 5-mL sample of amniotic fluid, 5 mL of a sterile solution containing 5 g/dL of PAH and 10 mL of a sterile solution containing 10 g/dL of inulin were injected successively through the catheter, followed by 2 mL of 0.9 g/dL sodium chloride as flush. Amniotic fluid samples (1 mL) were obtained through the catheter every 10 min for 4 h after injection. At 4 h, a blood sample was obtained by femoral venipuncture. After these 4 h, the animal was returned to the cage and allowed to wake up. The animals were again lightly anesthetized with 10 mg/kg of ketamine for repeat amniocenteses 8 and 24 h after injection of the markers.

Percutaneous amniocenteses, performed with a 5-cm long 20-gauge cannula, were repeated under brief ketamine anesthesia 8 and 24 h after injection of the markers. All three baboons delivered spontaneously within 3 d of the study, but they were not in labor at the time of the study. A blood sample was obtained from the neonate by femoral venipuncture.

Amniotic fluid samples were centrifuged for 1 min at 13 500 rpm in a microcentrifuge, and the supernatants were stored at −15°C until analysis. Blood samples were likewise centrifuged and the plasma stored. Concentrations of PAH in amniotic fluid and plasma were determined in triplicate by a direct spectrophotometric method (1). Concentrations of inulin were determined in quadruplicate by the colorimetric method of Alving *et al.* (2). The steps removing fermentable carbohydrates and proteins were omitted from the assays of amniotic fluid.

Antipyrine and T-1824 dilution. The kinetics of antipyrine dilution in total body water and of T-1824 dilution in plasma were studied in three normally grown baboon neonates born at term by spontaneous vaginal delivery. At the time of study, their postnatal ages ranged from 13 to 24 d and their wt ranged from 796 to 860 g. Under ketamine anesthesia, a catheter was inserted into a femoral vein. After removal of a blood sample, 4 mL/kg of a sterile solution containing 0.5 g/dL of antipyrine (1-phenyl-2,3 dimethylpyrazolone-one, Lemnon Company, Sellersville, PA) and 11.3 mg/dL of T-1824 or Evans' Blue (Harvey Laboratories, Philadelphia, PA) in 0.9 g/dL sodium chloride were injected intravenously through the catheter, followed by 2 mL of 0.9 g/dL sodium chloride as flush. Heparinized blood samples were obtained through the catheter at 10-min intervals during the first h, at 1½, 2, 3, 4, and 5 h after injection of the marker.

Blood samples were centrifuged at 13 500 rpm in a microcentrifuge, and the supernatant was stored at −15°C until analysis. Concentrations of antipyrine in plasma were determined in triplicate using a microadaptation of Mendelsohn and Levin's technique (3). Concentration of T-1824 in plasma were determined in duplicate by a double-wavelength technique (4–6).

$H_2{}^{18}O$. The kinetics of $H_2{}^{18}O$ dilution in total body water were studied in four baboon neonates born at term by spontaneous vaginal delivery. At the time of study, their postnatal ages ranged from 2 to 6 d and their wt ranged from 586 to 860 g. Under ketamine anesthesia, a catheter was inserted into a femoral vein. After removal of a blood sample, 0.6 g/kg of sterile water containing 11.6% of its oxygen as ^{18}O (Monsanto Research Corporation, Miamisburg, OH) were injected intravenously through the catheter, followed by 2 mL of 0.9 g/dL sodium chloride as flush. Heparinized blood samples were obtained hourly for 6 h after injection of the marker.

Blood samples were centrifuged at 13 500 rpm in a microcentrifuge, and the supernatant was stored at −15°C until analysis. The $\delta^{18}O$/mil was determined by equilibrating a known aliquot of plasma with carbon dioxide (7). The $^{18}O/^{16}O$ isotope ratio after equilibration was measured with a Finnegan Delta E gas isotope ratio mass spectrometer by a commercial laboratory (Global Geochemistry Corporation, Canoga Park, CA).

Bromide dilution. The kinetics of bromide dilution in extracellular water were studied in three normally grown baboon neonates born at term by spontaneous vaginal delivery. At the time of study, their wt ranged from 1000 to 1295 g. Under ketamine anesthesia, a catheter was inserted into a femoral vein. After removal of a blood sample, 4 mL/kg of a sterile solution containing 3.9 g/dL of bromide (sodium salt, Bios Coutelier, Brussels, Belgium) in 0.9 g/dL sodium chloride were injected intravenously through the catheter, followed by 2 mL of 0.9 g/dL sodium chloride as flush. Heparinized blood samples were obtained through the catheter hourly for 5 h after injection of the marker.

Blood samples were centrifuged at 13 500 rpm in a microcentrifuge and the supernatant was stored at −15°C until analysis. Concentrations of bromide in plasma were determined in triplicate using a microadaptation of Wolf and Eadie's technique (7).

Statistics. The relation between the concentration of a marker and time was analyzed by means of regression analysis.

RESULTS

The concentrations of PAH and inulin in amniotic fluid decreased exponentially during the 4 h after their intraamniotic injection ($r = 0.90$–0.99 for PAH and 0.92–0.95 for inulin) (Fig. 1, *upper graphs*). The decline in concentration was particularly steep during the first 60 to 90 min, then slowed down for the remainder of the 4-h period. The curves describing this decrease in concentrations remained exponential when the logarithm of the concentration was plotted against time. From 2 to 24 h after injection, the decrease in concentrations became linear if plotted as the natural logarithm of concentration *versus* time ($r = 1.00$ for PAH and 0.96 to 0.99 for inulin) (Fig. 1, *lower graphs*). Whereas PAH could be detected in maternal plasma obtained 4 h after intraamniotic injection, no inulin could be detected. Neither PAH nor inulin could be detected in neonatal plasma obtained shortly after birth.

The concentration of antipyrine in plasma decreased exponentially during the first 60 min after its intravenous injection. From 1 to 5 h after injection, there was a slight linear ($r = 0.92$–0.98) decrease in concentration of the order of 0.15 to 0.22 mg/dL/h (Fig. 2).

The concentration of ^{18}O represented by $\delta^{18}O$/mil, decreased linearly from 1 to 6 h after injection of water enriched in ^{18}O ($r = 0.94$–0.99) in three of four baboon neonates. In one neonate, there was no change in $\delta^{18}O$ over time (Fig. 3).

The concentration of bromide decreased exponentially during the first 2 h after its intravenous injection (Fig. 4). From 2 to 5 h after injection, the concentrations remained stable.

The concentration of T-1824 in plasma decreased linearly from 10 to 60 min after its intravenous injection ($r = 0.96$–1.00), then the decline slowed down to become exponential from 1 to 5 h postinjection (Fig. 5).

DISCUSSION

Of the various markers that have been used to estimate the vol of amniotic fluid (8–10), PAH has been the most widely used (11–15). Investigators of human pregnancies have mostly used the method of Charles and Jacoby (13), which involved sampling of amniotic fluid before injection of a known amount of PAH and 20 to 40 min after injection. Mixing of PAH was said to be complete and the PAH concentration stable by the time the second sample was obtained, although no supportive data were offered. Our data from normal baboon pregnancies raise serious questions about the validity of Charles and Jacoby's method (16). Not only does the concentration of PAH fail to stabilize within 20 to 40 min, this time period is situated on a portion of the concentration *versus* time curve that shows the sharpest decline. Indeed, as the relationship between concentration and time is exponential, the concentration of PAH never does stabilize. With other markers, it has been shown that mixing time of the marker

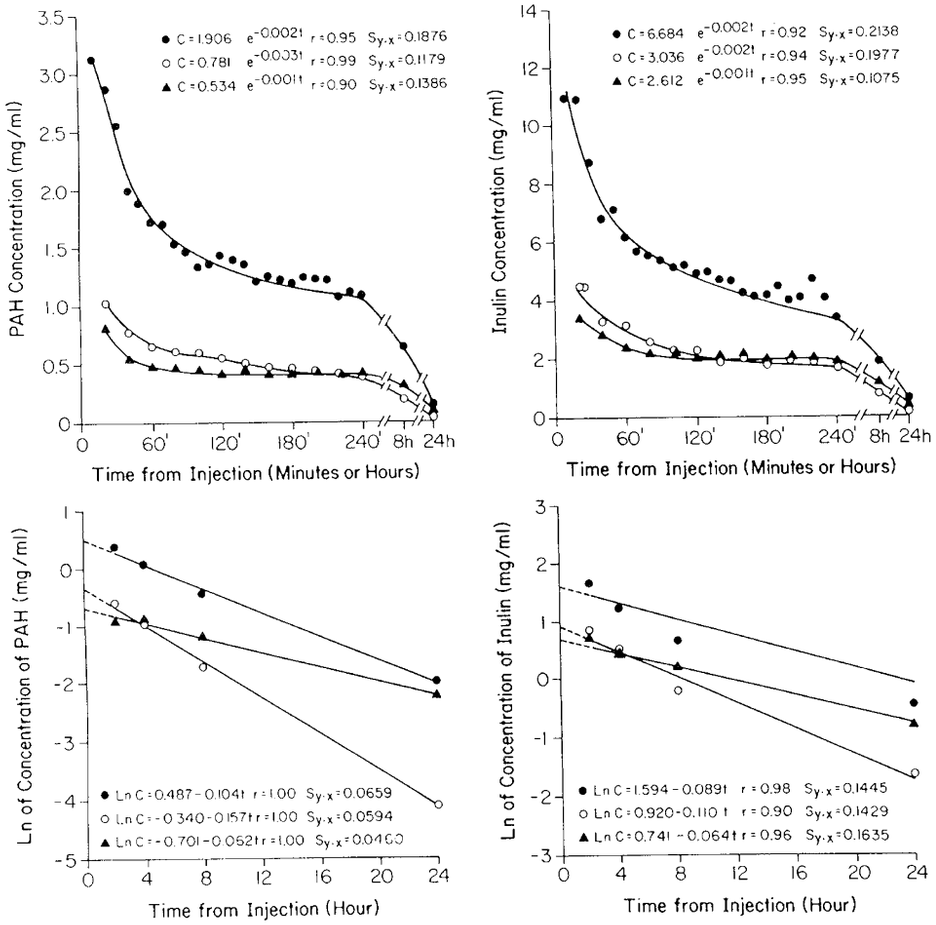

Fig. 1. Concentration *vs.* time kinetics of *p*-aminohippurate and inulin in amniotic fluid of three baboons at term. Regression equations are calculated from the points shown on each graph. (Modified from Brans YW 1988 Amniotic fluid: volume, composition, ingestion and digestion by the fetus. In Brans YW, Kuehl TJ (eds) Nonhuman Primates in Perinatal Research. John Wiley and Sons, New York, pp 201–216; reproduced by permission.)

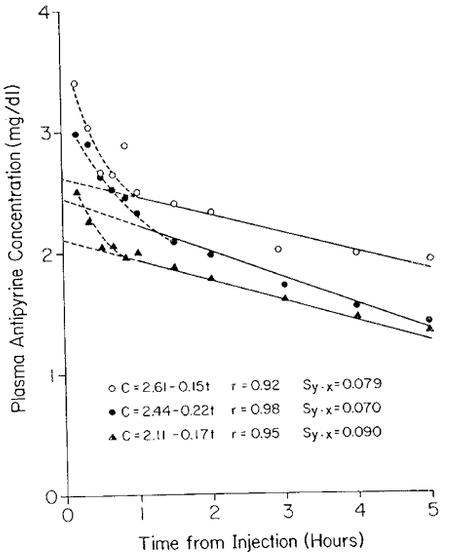

Fig. 2. Concentrations *vs.* time kinetics of antipyrine in plasma of three baboon neonates.

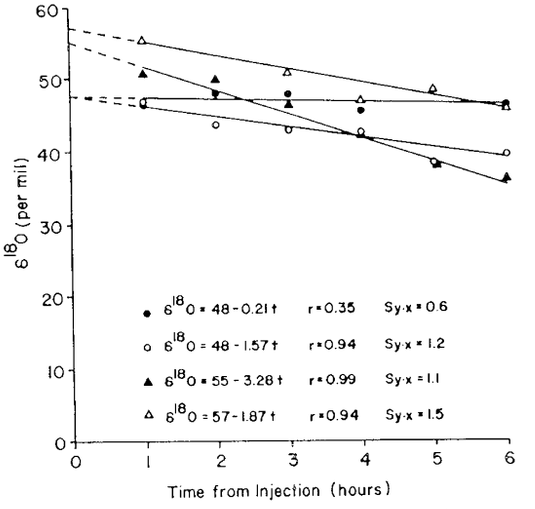

Fig. 3. Concentration ($\delta^{18}O$/mil) *vs.* time kinetics of ^{18}O in plasma of four baboon neonates.

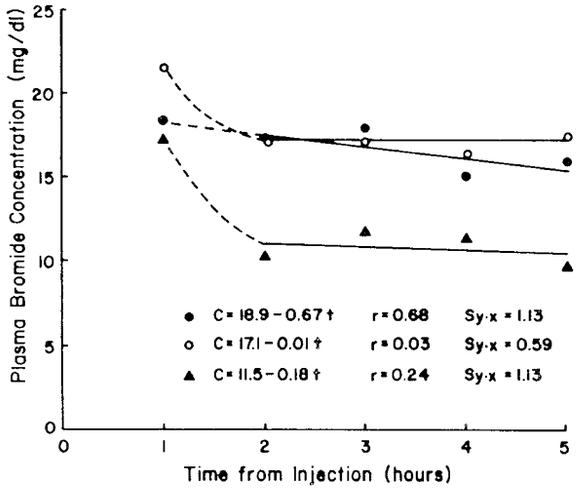

Fig. 4. Concentration *vs.* time kinetics of bromide in plasma of three baboon neonates.

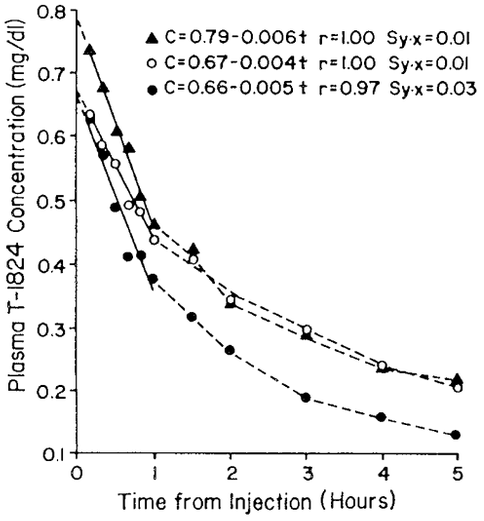

Fig. 5. Concentrations *vs.* time kinetics of T-1824 in plasma of three baboon neonates. The equation reflects the relation between concentration and time (in min) during the first 60 min after injection of T-1824.

varies widely among subjects but is directly proportional to the vol of amniotic fluid ($r = 0.93$) (10). Based on those data, one may predict that in baboons mixing of PAH might be complete in no less than 45 min after injection, and PAH then begins to be cleared by fetal ingestion and/or through the amniotic membranes. For the larger vol of amniotic fluid in humans, 2 h after injection would appear to be an adequate mixing time. Our data suggest that at least three postinjection samples of amniotic fluid must be obtained between 2 and 24 h after injection of a marker to determine the regression line for PAH clearance and to extrapolate it to time 0 to estimate the vol of amniotic fluid. This admittedly curtails seriously the use of marker techniques in human pregnancies. Recently, real-time ultrasonography has been used to assess amniotic fluid vol qualitatively (17–18). It would be well worth attempting to correlate ultrasonographic estimates with chemical dilution techniques.

Estimates of amniotic fluid vol are difficult to perform in baboons without general anesthesia. The effect of ketamine upon the fetus is not known. Fetal limb and breathing movements persist, and it may be assumed that fetal swallowing is not impaired either (16). Nevertheless, our data must be interpreted cautiously until they are confirmed by results obtained without anesthesia in tethered animals.

Inulin behaves essentially the same way as PAH when injected intraamniotically. It has the advantage of being a larger molecule (mol wt 5000 compared to 194 for PAH), which is unlikely to diffuse out of the amniotic cavity or to be absorbed by the fetal gastrointestinal tract. Indeed no inulin can be found in maternal plasma, whereas PAH can be detected in measurable quantities (16). For this reason, we recommend the use of inulin rather than PAH as a marker of amniotic fluid volume.

While studying the fate of antipyrine in adults, Brodie and Axelrod (19) determined that its concentration in the water of various tissues was nearly identical to that in plasma water, that its excretion was negligible, and that it was metabolized slowly. They suggested that antipyrine might be a useful marker to estimate total body water (20). Its average rate of disappearance of 6%/h (range: 1 to 12%/h) made it advisable to obtain serial blood samples and to use the extrapolated time 0 concentration to calculate total body water content. Christian *et al.* (21) were the first to use antipyrine in neonates, and they obtained serial blood samples at 4.5, 6, 7, and 14 h after intravenous injection. MacLaurin (22) obtained blood samples 2, 3, and 5 h after injection. Cassady (23) initially used samples obtained 1, 3, and 5 h after injection, but later reduced sampling to 1 and 3 h (24). None of these investigators provided any data to show that distribution of the marker was complete by the time the first postinjection sample was obtained or that the disappearance rate of antipyrine was linear over the duration of the study. Presumably such data existed but were not published. Murdock *et al.* (25) showed that low birth wt neonates had increased half-lives for antipyrine and decreased disappearance rates (1.2 ± [SEM] 0.1%/h on the first postnatal d, 3.1 ± 0.3%/h on the 4th d) compared to adults. Because, according to our own unpublished data, the decrease in plasma antipyrine was clearly linear between 1 and 5 h post-injection, for convenience we further reduced the timing of blood samples to 1 h and 1.5 to 2 h after injection. Our results were identical to those of the other investigators. The data from the present study suggest that distribution of antipyrine in baboon neonates is indeed complete by the end of the 1st postinjection h and that the disappearance rate remains linear for at least 4 h. The exact timing of blood sampling therefore does not matter so long as it occurs between 1 and 5 h post-injection and that the timing of each sample is known precisely. To improve the accuracy and reproducibility of the technique, it is advisable, whenever possible, to obtain three postinjection blood samples. If circumstances make it difficult to justify multiple blood samples and prolongation of the study, a single postinjection sample may yield adequate data, especially when one is concerned only with comparisons between groups of neonates studied in an identical manner. Our data suggest that using the concentration of antipyrine in plasma obtained 1 h after its injection rather than the time-0-extrapolated concentration introduces an error of only 4 to 5%, which is within the accuracy of the chemical assay.

It has been stated that the ratio of $^{18}O/^{16}O$ in plasma reaches an equilibrium by 4 h after administration of $H_2{}^{18}O$ and maintains that equilibrium for at least 2 h (26). Our data suggest that this is not true in neonates where the ratio begins to return toward normal within minutes of enrichment with ^{18}O. It is therefore necessary to obtain at least two and preferably three blood samples at known times in relation to injection of $H_2{}^{18}O$ to calculate the regression line and extrapolate it back to time 0. Sampling times between 0 and 6 h after intravenous injection appear to be appropriate. Published results to-date, based on a single determination of $\delta^{18}O$, probably overestimate the amount of total body weight.

In human adults, bromide is excreted very slowly by the kidneys, at an average rate of 0.3%/h during the first 24 h after administration of an oral dose (27), and the plasma concentra-

tions stabilize by 2.5 to 3 h (27). In infants and children, stabilization of plasma concentrations after intravenous administration occurs no later than 2.5 h and persists for 3 to at least 24 h (28). After subcutaneous injection in neonates, a constant vol of distribution is observed for 3 to 12 h (29). These data led most investigators to select a single blood sample, obtained 3 h after administration of bromide, to calculate the bromide space (23, 29–34), although there was no evidence that stabilization of plasma bromide concentrations did not occur earlier after intravenous injection. In the past, we chose to use 1 h as a convenient time of sampling in both human and baboon neonates (24, 35–41). The resulting data for group averages were essentially identical to those obtained with the use of a 3-h sample. The data from the current study suggest that distribution of bromide after intravenous injection is not complete by 1 h, but is complete by 2 h and that the plasma concentrations remain stable for at least the next 3 h. For greater accuracy, blood sampling should occur no earlier than 2 h after injection.

The determination of plasma vol with T-1824, as originally described in adults, involved a single sampling of blood exactly 10 min after injection of the marker (42), and most investigators have used this simplified technique (6, 24, 35–38, 40, 43–49) rather than the most cumbersome method of employing a series of successive samples and extrapolation back to time 0. In neonates, although the rate of transcapillary escape of T-1824 is considerably greater than in adults—20 to 30%/h (5, 35, 44, 50, 51) *versus* 5%/h (42)—the error introduced by using a single sample is small (3%: at 20%/h average T-1824 rate of transcapillary escape, 3% are lost in 10 min) and well within the limits of accuracy of the laboratory assay (44). Even in circumstances when excessive loss of intravascular albumin (and T-1824 bound to it) may be suspected, as in erythroblastosis fetalis, the use of serial samples has little effect on the mean plasma vol determinations (48, 51). Barr *et al.* (49) compared the T-1824 concentrations at 10 min with that at time 0, obtained by linear retropolation of the disappearance curve, in both hypotensive and normotensive neonates and found no significant differences. Our data from baboons are in agreement with those from other investigators. They indicate that the disappearance curve of T-1824 from plasma is linear during the 1st h after injection, then becomes exponential. Calculations of plasma vol based on the 10-min concentration *versus* retropolation of the disappearance curve introduces an error of at most 5%.

This report does not address the issue of accuracy and validity of *in vivo* estimates of body water content and distribution, nor does it provide data favoring one marker *versus* another for any given fluid compartment except amniotic fluid. Validation studies with whole body chemical analyses are needed to resolve these important issues.

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