Hematocrit Correlates Well with Circulating Red Blood Cell Volume in Very Low Birth Weight Infants

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ABSTRACT

Although circulating red blood cell (RBC) volume is a better measure of total body oxygen delivering capacity than hematocrit (HCT), circulating RBC volume is more difficult to measure. Thus, the HCT is often used in RBC transfusion decisions. However, several previous studies of low birth weight infants have reported that the correlation between HCT and circulating RBC volume is poor. Using a robust nonradioactive method based on *in vivo* dilution of biotinylated RBC enumerated by flow cytometry, the present study reexamined the correlation between HCT and circulating RBC volume in very low birth weight infants. Venous and capillary HCT levels were compared with circulating RBC volume measured using the biotin method. Twenty-six stable very low birth weight infants with birth weights less than 1300 g were studied on 43 occasions between 7 and 79 d of life. Venous HCT values correlated highly with circulating RBC volume ($r = 0.907$; $p < 0.0001$). However, the mean 95% confidence limits for prediction of circulating RBC volume from venous HCT (the average error of prediction) was ± 13.4 mL/kg. The correlation between HCT and circulating

RBC volume is strong in older stable very low birth weight infants. However, clinically important uncertainty exists in estimating circulating RBC volume and the associated RBC transfusion needs of an individual infant based on venous HCT. Because direct measurement of circulating RBC volume is not yet practical, the HCT (or the blood Hb concentration) remains the best available indirect indicator. (*Pediatr Res* 50: 525–531, 2001)

Abbreviations

HCT, hematocrit
MCV, mean corpuscular volume
RBC, red blood cell
RCC, red blood cell count
VLBW, very low birth weight
V, volume
W, weight
SG, specific gravity

Intensive care of critically ill newborn infants has increased blood sampling and exacerbated the severity of neonatal anemia. Daily phlebotomy blood losses of 4 to 5% of the total blood volume are common among VLBW infants (1–3) who are defined here as those infants with birth weight less than 1500 g. Not surprisingly, the majority of the multiple RBC transfusions such infants receive are administered during the first weeks of life when phlebotomy loss is greatest (1, 2, 4, 5). The present consensus is that phlebotomy loss from clinical monitoring is the primary cause of the relatively large transfusion needs of VLBW infants (1, 3, 6, 7).

The decision to administer a RBC transfusion is typically made on the basis of the infant's clinical condition and the whole blood HCT or blood Hb concentration (3, 6, 8). Although circulating RBC volume is a more accurate indicator of total body oxygen delivery capacity than whole blood HCT or Hb concentration (9, 10), HCT and Hb concentration are used clinically because they are quick, inexpensive, and analytically reliable. To understand the limitations of HCT as a guide to transfusion decisions, the relationship between HCT and circulating RBC volume in VLBW infants should be elucidated, and the sources of the previously reported poor correlation (9–14) should be segregated into methodologic artifacts and genuine characteristics of preterm physiology.

The aim of the present study was to examine the relationship between HCT and circulating RBC volume in stable VLBW infants. An accurate nonradioactive method based on biotinylated RBC was used to measure circulating RBC volume.

Received January 19, 2000; accepted November 29, 2000.

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Supported by P01 HL46925 National Institutes of Health and M01 RR00059 National Institutes of Health General Clinical Research Center Program.

METHODS

The present study was approved by the University of Iowa Committee on Human Research. Twenty-six infants with birth weight less than 1300 g were enrolled after informed consent was obtained from one or both parents. The mean ($\pm$ SD) birth weight was 974 ± 191 g; range = 551 to 1300 g. The mean gestational age at birth was 28 ± 2 wk; range = 24 to 31 wk. At the time of study, the infants' mean weight was 1426 ± 361 g, and the range = 781 to 2160 g; the mean postnatal age was 37 ± 16 d, and the range = 7 to 79 d. These infants were clinically stable at the time of measurement of HCT and circulating RBC volume.

Study Design

In infants scheduled to undergo homologous RBC transfusion, 0.2 mL of the infant's blood was obtained by venipuncture from a peripheral free-flowing vein; HCT and MCV were determined by an automated analyzer (Technicon H3RTX, Bayer, Inc., Tarrytown, NY, U.S.A.). This HCT value is referred to hereafter as the venous blood HCT. Within 3 min, biotin-labeled RBC from the transfusion donor were infused. After time for mixing of the biotinylated RBC (20 min), a sample of the infant's venous blood was obtained and submitted for determination of circulating RBC volume. Then, a RBC transfusion of 15 mL/kg was infused over 5 h as treatment for anemia.

Biotin Method for Circulating RBC Volume

An aliquot of the donor RBC was biotinylated, and a precisely determined volume (approximately 1 mL of the biotinylated RBC suspension) was infused. Circulating RBC volume was measured by the dilution of the biotinylated RBC in the endogenous unlabeled RBC.

Methodologic Detail

This method has been thoroughly validated against ^{14}C -cyanate in sheep (15) and against ^{51}Cr in adult volunteers (16). The method has been scaled down for VLBW infants. The method is described here in detail with emphasis on the modifications.

Wash solution. RBC wash solution was prepared by the addition of 4 mL of 50% dextrose (Baxter, Deerfield, IL, U.S.A.), 20 mL of 8.4% sodium bicarbonate (American Reagent Laboratories, Shirley, NY, U.S.A.), and 1 mL of sodium phosphates (American Reagent Laboratories) to 1 L of 0.9% sodium chloride (Baxter). Osmolarity for this solution is 356 mosmol/L.

Sulfo-N-hydroxysuccinimide biotin solution. A 0.04-mg/mL solution of sulfo-N-hydroxysuccinimide biotin (sulfo-NHS biotin; Pierce Chemical, Rockford, IL, U.S.A.) was prepared in wash solution and filter-sterilized immediately before use. One mL of sulfo-NHS biotin solution was added to 1 mL of washed RBC that had been resuspended in wash solution to the original HCT. The addition was made with mixing to ensure equal distribution and labeling density. The mixture was

incubated at room temperature for 30 min. The cells were washed four times to remove unreacted biotinylating reagent.

On the final resuspension, the HCT of the labeled donor blood was adjusted to approximately the same HCT as that of the infant recipient. Then, approximately 1 mL of the biotin-labeled donor blood was infused into the circulation of the recipient from a 1-mL syringe. The exact weight of blood infused was determined by weight difference of the syringe. After allowing 20 min for mixing, a 0.2-mL sample of venous blood was taken from the unwarmed heel of a limb not used for infusion of biotinylated RBC by free-flowing venipuncture or using a standardized capillary sampling device (Tenderfoot device, International Technidyne Corp., Edison, NJ, U.S.A.). Blood obtained using this device (whether the infant's heel is warmed or not) yields HCT values that are very similar to those obtained from arterial blood (17). Red cell count and HCT were determined by automated analysis (Technicon H3RTX) for the labeled RBC suspension and the donor blood.

The number of biotinylated RBC as a percentage of total (labeled plus unlabeled RBC) was measured by flow cytometry. During development of this method, we noted that for % biotinylated RBC less than approximately 5%, the percentage of biotinylated RBC measured by flow cytometry was greater than the actual % biotinylated RBC in samples constructed by *in vitro* mixing of labeled and unlabeled blood. The actual % biotinylated RBC was precisely determined using accurate gravimetric measurement of the volumes mixed. We also have demonstrated empirically that endogenous RBC contain less than 0.001% of cells that would register as biotinylated with flow cytometric windows set as shown in Figure 1. Likewise, the batches of biotinylated RBC are consistently >99% biotinylated. We believe this phenomenon is a result of one or both of the following: 1) denatured cells that are eliminated by

Figure 1. Histogram from flow cytometry of representative subject. Biotinylated RBC were labeled with fluoresceinated avidin, which increased the fluorescence intensity relative to unlabeled RBC. Gating windows (horizontal bars) were set to distinguish the two groups and to minimize the contribution from cell debris.

the choice of flow cytometric windows and 2) rare event counting. Because of this lack of one-to-one correspondence, values for % biotinylated RBC determined empirically by flow cytometry must be corrected to the actual % biotinylated RBC. This is accomplished by constructing a standard curve which recapitulates *in vitro* the dilution that occurs *in vivo* in the infant. Shown in Figure 2 is a standard curve constructed by diluting the biotinylated donor RBC in the unlabelled blood of an individual whose blood is of the same type as the infant. The equation for the regression line is given in Figure 2. For a representative infant whose flow cytometric % biotinylation was 1.97% (filled symbol), the actual % biotinylation was 1.55% = $[1.97 - (-0.012)]/1.292$.

Circulating RBC volume was calculated as previously described (16) on the basis of the dilution principle. The basic principle that underlies the dilution equation is conservation of mass; in this particular case, we assume that the number of biotinylated RBC is conserved. Stated mathematically, $N_1 = N_2$, where N_1 equals the number of biotinylated RBC in the infusate RBC, and N_2 equals the number of biotinylated RBC in the subject's circulation after infusion.

N_1 is calculated as the product of the % biotinylated RBC in the infusate times the total number of RBC infused. In turn, the total number of RBC infused is equal to the number of RBC per unit volume of infusate (RCC_i) multiplied by the volume of infusate (V_i). Finally, V_i is equal to the weight of the infusate (W_i) divided by the specific gravity of the infusate (SG_i). Thus,

$$N_1 = \% \text{ biotinylated in infusate} \times \text{total number of RBC infused} \quad (1)$$

Figure 2. The % biotinylated RBC determined by flow cytometry vs actual % biotinylated RBC. Flow cytometric % biotinylation of cells was calculated from the histogram shown in Figure 1. Actual % biotinylation of cells was determined from known mixtures of biotinylated and unbiotinylated cells as described in "Methods." The representative subject's data are displayed as a closed circle. All values are the mean of triplicate determinations. SD bars do not show if they are smaller than the diameter of the point.

$$N_1 = \% \text{ biotinylated in infusate} \times RCC_i \times V_i \quad (2)$$

$$N_1 = \% \text{ biotinylated in infusate} \times RCC_i \times (W_i/SG_i) \quad (3)$$

N_2 is equal to the number of biotinylated RBC per unit volume of the subject's mixed venous blood multiplied by the subject's blood volume. The subject's blood volume is equal to the subject's circulating RBC volume divided by the subject's HCT. The number of biotinylated RBC per unit volume is equal to the % biotinylated RBC in the subject's blood multiplied by the total number of RBC per unit volume of the subject's blood. In turn, the total number of RBC per unit volume is equal to the HCT of the subject's blood divided by the subject's MCV (MCV_s). Substituting the equivalent expressions for each term in the equation for N_2 , we obtain the following:

$$N_2 = \text{number of biotinylated RBC per unit volume of subject's blood} \times \text{blood volume} \quad (4)$$

$$N_2 = \text{number of biotinylated RBC per unit volume of subject's blood} \times (\text{circulating RBC volume}/\text{HCT}) \quad (5)$$

$$N_2 = \% \text{ biotinylated RBC per unit volume of subject's blood} \times \text{total number of RBC per unit volume of subject's blood} \times (\text{circulating RBC volume}/\text{HCT}) \quad (6)$$

$$N_2 = \% \text{ biotinylated RBC of subject's blood} \times (\text{HCT}/\text{MCV}_s) \times (\text{circulating RBC volume}/\text{HCT}) \quad (7)$$

HCT in the numerator and denominator of the equation for N_2 cancel to produce the following:

$$N_2 = \% \text{ biotinylated RBC of subject's blood} \times \text{circulating RBC volume}/\text{MCV}_s \quad (8)$$

$$\begin{aligned} & \% \text{ biotinylated in infusate} \times RCC_i \times (W_i/SG_i) \\ &= \% \text{ biotinylated in subject's blood} \times \text{circulating RBC volume}/\text{MCV}_s \quad (9) \end{aligned}$$

By conservation, N_1 must equal N_2 , yielding the equality:

Each of the terms in this equation is quantitated directly except for the subject's circulating RBC volume. Rearranging the equation for the circulating RBC volume yields the final equation:

circulating RBC volume

$$= \frac{\% \text{ biotinylated in infusate} \times RCC_i \times W_i \times \text{MCV}_s}{SG_i \times \% \text{ biotinylated RBC of subject's blood}} \quad (10)$$

Although an accurate relationship between HCT, circulating RBC volume, and blood volume must include the packing factor ($F \sim 0.97$), this pack factor appears in both numerator

and nominator of this derivation and cancels; therefore, equation 10 remains valid.

The units of the terms are as follows:

$$\text{mL} = \frac{\% \times \text{cells/fL} \times \text{g} \times \text{fL/cell}}{\text{g/mL} \times \%} \quad (11)$$

For the subject whose determination is depicted in Figure 2, weight of infusate = 1.3283 g, RCC = 0.00426 RBC/fL, % biotinylated of infusate = 99.88%, MCV = 117 fL, specific gravity of infusate = 1.050 g/mL, actual % biotinylated of subject sample = 1.55%. Using these values, we obtain the calculated circulating RBC volume = 40.63 mL.

Repeat Determinations of Circulating RBC Volume

To examine the relationship between HCT and circulating RBC volume over a greater range of HCT values, 17 of the 26 infants had a second determination of circulating RBC volume. Three were performed at approximately 24 h after the transfusion just before a second transfusion required for further treatment of anemia. Fourteen were performed after a mean interval of 21 d (range = 7–67). Together, these values provided a total of 43 pairs of circulating RBC volume and HCT measurements.

For a second measurement in the same individual, one must quantitate the incremental increase in % biotinylated RBC due to the second infusion of biotinylated RBC. Thus, the % biotinylated RBC persisting in circulation from the first infusion must be measured just before the second infusion. This baseline was subtracted from total % biotinylated RBC determined 20 min after the second infusion as previously described (15). The derivation of the formula for sequential measurements using labeled RBC has been previously given, and the application has been validated (15). Specific use of biotin-labeled RBC has been validated *in vitro* (data not shown) and *in vivo* in adult volunteers (Mock, DM, manuscript in preparation).

Statistical Methods

Significance of the correlation for linear regression between two variables (*e.g.* measured circulating RBC volume and automated venous HCT) was tested using commercially available software (Statview, Abacus Concepts, Berkeley, CA, U.S.A.). Correlation was considered significant if $p < 0.05$. Confidence limits for the linear regression line and for prediction of individual values were calculated according to Zar (18). Mean error of prediction was calculated as the average of the difference between the upper and lower 95% confidence limits for the HCT values at 1% intervals between 20 and 50% (18). Values of the intercepts of the two confidence lines for the linear regression were used to judge whether the intercept of the regression line was significantly different from the origin (18). Significance of the differences between venous and capillary of HCT values was tested using the paired *t* test.

RESULTS

Figure 3 depicts circulating RBC volume plotted against venous blood HCT for the 43 studies. Initial studies are denoted by open symbols, repeat studies by closed symbols. For the subgroup of 26 initial studies, correlation coefficient was 0.863; for the subgroup of 17 repeat studies, the correlation coefficient was 0.945. The regression lines for the two sets of data were not significantly different. Moreover, the linear regression lines for each of these subgroups were not significantly different from those obtained from the data of Bratteby (19) or from the data of Mollison *et al.* (20) as presented below. For clarity of presentation, all 43 pairs of data were pooled for subsequent analysis.

For the combined group, circulating RBC volume and venous blood HCT had a strong linear correlation, $r = 0.907$ and $p < 0.0001$. The 95% confidence limits for the slope of the regression line was 0.734 to 0.986. The mean error of prediction was 13.4 mL/kg for the HCT interval from 20 to 50%. The mean circulating RBC volume was 22.9 ± 7.6 mL/kg; the range was 11.9 to 43.9 mL/kg. The mean HCT was $33.5 \pm 8.0\%$; the range was 19 to 51.

As the total circulating RBC volume diminishes toward zero, the HCT must also approach zero. The intercept of the regression line was -4.9 mL/kg; contrary to logic, the origin did not fall within the 95% confidence interval (-9.05 to -0.67). Why did the regression line intersect the circulating RBC volume axis significantly below the origin? Examination of our data suggested that circulating RBC volume deviated positively from the regression line at HCT values greater than 45. Such a biphasic relationship between circulating RBC volume and HCT has been reported previously in infants (19, 20) and adults (21).

To examine the effect of higher HCT pairs, we excluded the three pairs of values with HCT greater than 45%. The regression line based on the remaining 40 pairs retained a good

Figure 3. Circulating RBC volume vs venous automated HCT. Symbols: ○ = initial; ● = repeat. $n = 43$. The 95% confidence limits for the regression line and prediction of individual values are depicted as (—) and (&OV0043;), respectively.

correlation (0.855) and had an intercept of -2.7 mL/kg; the 95% confidence limits for the intercept for this reduced data set (-7.7 to $+2.2$) included the origin. This result is consistent with the hypothesis of a biphasic relationship between circulating RBC volume and HCT.

As presented in "Discussion," techniques for measurement of HCT and the source of blood for determination of HCT are potential sources of artifact and, therefore, of the differences in correlation between circulating RBC volume and HCT reported in various studies. We examined the relative accuracy and the precision of the automated HCT method and the manual HCT method. In a group of 22 samples of VLBW infants, the average difference was $+0.6$ HCT points (automated-manual). For 10 measurements of the same sample using the Technicon H3RTX, the coefficient of variation was $+1.6\%$; for the manual capillary method, the coefficient of variation was $+1.5\%$. Thus, our comparison of HCT determined by automated Technicon H3RTX method *versus* manual capillary method revealed high relative accuracy and precision.

To produce accurate mixtures for the standard curve, HCT of both the biotinylated RBC infusate and the blood used as the source of unlabeled RBC must be accurately known. Thus, we sought to determine whether measurement of HCT on 100% biotinylated blood might have introduced an artifact specific to biotinylated blood. We determined HCT on the 100% biotinylated infusate blood by both automated and manual methods. Although absolute values were significantly different at $p < 0.0001$ by paired t test, the mean difference between the paired values was only 1.2%. Moreover, the coefficient of variation within each method was less than 2%. Thus, the analytical errors in HCT measurements do not appear to be a likely source of artifact.

To investigate source of blood HCT as a cause for artifacts, we took advantage of the fact that two additional sets of HCT measurements were available. Two capillary blood samples had been obtained on each infant using Becton Dickson Long Point Microlancet devices (Becton Dickson, Franklin Lakes, NJ, U.S.A.) as part of an ongoing study examining the clinical utility of HCT in transfusion decisions. The first capillary blood HCT was obtained at approximately 0800 the day of the transfusion. The second was obtained 2 to 4 h before transfusion (22, 23). These HCT were measured manually in duplicate as described previously (24) without correction for packing factor. When we compared capillary HCT 1 to capillary HCT 2 (which were determined only a few hours later in the same infant), we found a correlation coefficient of 0.92. However, the mean error of prediction was 5.8 HCT points, which corresponds to 19% of a HCT of 30. Thus, the technique used for obtaining the blood sample for HCT measurement and for the circulating RBC volume measurement appeared to be moderate sources of error.

Moreover, the circulating RBC volume still correlated well with capillary HCT. Figure 4 depicts the circulating RBC volume *versus* capillary blood HCT 1. The data correlated strongly (0.876) and significantly ($p < 0.0001$). Slope, y intercept, and correlation coefficient were quite similar to and not significantly different from those of Figure 3. The intercept of the regression line was -4.40 . For these data, the origin did

Figure 4. Circulating RBC volume vs HCT of capillary sample 1. Regression line and confidence limits as in Figure 3.

Figure 5. HCT of capillary blood sample 1 and 2 vs venous automated HCT of the same subject. Line of identity rather than regression line is depicted. Ninety-five percent confidence limits for prediction of individual values are depicted as (—).

fall within the 95% confidence interval for the y axis intercept (-9.28 to 0.47).

This large variability in HCT of capillary blood samples could have been caused by capillary sludging (25), by expression of lower HCT in blood by squeezing (26), or both. If the venous HCT is assumed to represent the true peripheral venous HCT, then sludging and squeezing artifacts should produce deviations above and below a line of identity, respectively, for a plot of all capillary HCT values (1 and 2) against the gold standard venous HCT (Fig. 5). Many data pairs fell more than the expected analytical error above or below the line of identity ($= \pm$ twice the SD; $= \pm 3\%$). Indeed, the mean error of prediction was 10.4%. For example, one infant had a capillary HCT = 40 and venous automated HCT = 33, suggesting a

sludging artifact. Another infant had a capillary blood HCT = 22 and a venous automated HCT = 29, suggesting squeeze artifact. The total number of capillary blood HCT values above the line of identity was 54 of 77; the total number below the line of identity was 15 of 77. Eight values were equal. The greater number above the line suggests a preponderance of sludging artifact. By χ^2 testing, this distribution was significantly different from random at $p < 0.0001$.

DISCUSSION

The data from this study provide evidence that there is a strong direct correlation between circulating RBC volume as measured by a biotinylated RBC method and venous HCT in stable VLBW infants. The strength of the correlation observed in this study is similar to that reported in adult studies (21) and in some previous studies in infants (19, 20).

The strength of the correlation between circulating RBC volume and HCT has been addressed in some previous studies of preterm infants. Our data confirm the conclusions previously reached by Mollison *et al.* (20) and Bratteby (19) who used venous blood for both RBC volume and HCT determinations. Mollison *et al.* studied 43 full term infants less than 1 d of age; using a ^{32}P RBC label or an immunoagglutination method for determining circulating RBC volume, they found a correlation of 0.901. Using the ^{51}Cr RBC label, Bratteby studied 40 full-term infants and 26 preterm infants between birth and 138 d of age. In addition to finding a high correlation (0.961) between circulating RBC volume and HCT in both term and preterm infants, Bratteby found the same slope for the regression lines for term and preterm infants (19, 27–30).

To compare those studies to the one presented here, data from the studies of Mollison *et al.* (20), Bratteby (19), and the current study are plotted in Figure 6. Only five data points from the two other studies fell beyond the confidence limits based on the current study (lines not depicted). Moreover, analysis of

covariance revealed that there was not a statistically significant difference in the slopes of the three lines. Overall, these three studies appear to describe the same strong reproducible correlation despite substantial differences in HCT and gestational age of the infants studied.

Using a variety of methods for measuring RBC volume and for obtaining the blood samples, other investigators (9, 10, 12–14) have detected a poor correlation between circulating RBC volume and HCT in VLBW infants. Reported correlation coefficients varied between 0.3 and 0.7. For example, using a stable isotope of chromium, Faxelius *et al.* (11) measured circulating RBC volume in 259 infants with birth weights between 878 and 5386 g before age 72 h; their series included 93 infants with birth weight less than 1500 g. These investigators detected a poor correlation ($r = 0.5$) between circulating RBC volume and venous HCT.

To what can we attribute the differences in the findings among the various studies? We have considered several sources of artifact and differences:

1 Error in the biotin method: Our studies measuring circulating RBC volume in adult subjects provide evidence that the biotin method is accurate (16). Circulating RBC volume by biotin correlated strongly with ^{51}Cr (correlation coefficient = 0.97). Precision was consistently high; coefficients of variation among replicates were less than $\pm 5\%$. It is important to note that this agreement between the two methods was observed when the same mixed venous sample containing both the ^{51}Cr -labeled RBC and biotin-labeled RBC was used for quantitation of the concentration of ^{51}Cr and % biotinylated RBC. In addition, the samples for determination of HCT and circulating RBC volume were obtained from venipuncture from a free-flowing vein. Given that free-flowing venous samples (or the equivalent) were used for determination of venous HCT and circulating RBC volume in this study, we conclude that analytical error in our study is not likely to have produced significant artifact and is even less likely to have produced the strong correlation observed.

2 Error in measurement of HCT: Although we found a small but statistically significant difference between the automated and manual methods for determining HCT, the difference was far too small to explain the poor correlation reported by others. On the basis of the analytical characteristics of the HCT data, we conclude that analytical error in measuring the HCT is not a likely source of substantial error whether a manual or automated method was used in the various studies.

3 Errors due to blood collection artifacts: Blood collection artifact is a potentially important source of poor correlation between HCT and circulating RBC volume as follows. The ^{51}Cr method, the nonradioactive chromium method (11), and the agglutination method all rely on measurement of the concentration of a label (^{51}Cr , ^{52}Cr , RBC antigen) in blood. In theory, squeezing or sludging artifacts that change the HCT of the sample should produce a compensatory change in the label concentration; thus, an accurate determination of circulating RBC volume would still occur. However, the artifact in HCT has an independent effect on HCT *per se* and thus would weaken the correlation between circulating RBC volume and HCT. In the biotin method, biotin-labeled RBC are enumerated

Figure 6. Comparison of data from three studies that detected correlation between circulating RBC volume and HCT. ● Present study; □ Bratteby; △ Mollison. Confidence lines are based on combined data for the three studies.

as a percentage of the total RBC; as a result, the method is more resistant to squeezing and sludging artifacts. As predicted, we have observed that even when the venous blood sample has a 50% HCT underestimate introduced by plasma addition or when a similar overestimate is produced by plasma removal, the % biotinylated RBC and the calculated circulating RBC volume are unchanged. Of course, the calculated blood volume would still be directly affected by any HCT artifact. We speculate that the robustness of the biotin method with respect to HCT artifacts resulted in a more accurate measurement of circulating RBC volume in this study. This permitted the detection of a tight correlation between circulating RBC volume and HCT.

4 Clinical status of subjects: If differences in the clinical status (*e.g.* pulmonary, hepatic, or renal disease, hypotension, or hypoxia) lead to disturbances in the relationship between circulating RBC volume and HCT, it would be logical to infer that differences in the clinical status of the populations of infants examined in the various studies could be a source for the differences in the correlations reported. Available descriptions of the populations do not lend themselves to meaningful comparisons of clinical status.

In summary, the strong correlation between circulating RBC volume and HCT observed in this study provides some justification for the continued use of HCT in transfusion decisions in stable VLBW infants despite recent studies describing weaker correlation in some low birth weight infants. The correlation coefficient of 0.907 observed here is similar to values of 0.88 and 0.92 reported in normal and anemic adults by Huber *et al.* (21) and by Bentley and Lewis (31). However, the ability to predict circulating RBC volume from HCT for an individual infant is limited. For example, in infants whose HCT was 30, the circulating RBC volume varied from 15 to 28 mL/kg, a range of considerable clinical significance. We speculate that direct measurement of circulating RBC volume may lead to superior decisions concerning transfusion therapy, but this measurement is not yet practical for routine clinical use. HCT (or blood Hb concentration) remains the most practical, albeit indirect, laboratory assessment of circulating RBC volume.

Acknowledgments. The authors thank Leon Burmeister, Ph.D., for statistical advice and Karen J. Johnson, R.N., Gretchen A. Cress, R.N., and Natalie W. Connolly, R.N., for assistance in collecting the blood samples and infusing the biotin-labeled erythrocytes. We also thank Gudrun Clausen and Robert L. Schmidt, who assisted with the biotin assays and the biotin labeling of the RBC.

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