Adaptive responses during anemia and its correction in lambs

JOHN A. WIDNESS, LANCE S. LOWE, EDWARD F. BELL, LEON F. BURMEISTER, DONALD M. MOCK, JAMES A. KISTARD, AND HARRY BARD

Departments of Pediatrics and Preventive Medicine, College of Medicine, The University of Iowa, Iowa City 52242; and Iowa Statewide Organ Procurement Organization, Iowa City, Iowa 52245; Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock 72205; and the Arkansas Children’s Hospital, Little Rock, Arkansas 72202; and Department of Pediatrics, University of Montreal Research Center, Hôpital Sainte-Justine, Montreal, Quebec, Canada H3T 1C5

Adaptive responses during anemia and its correction in lambs. J Appl Physiol 88: 1397–1406, 2000.—There is limited information available on which to base decisions regarding red blood cell (RBC) transfusion treatment in anemic newborn infants. Using a conscious newborn lamb model of progressive anemia, we sought to identify accessible metabolic and cardiovascular measures of hypoxia that might provide guidance in the management of anemic infants. We hypothesized that severe phlebotomy-induced isovolemic anemia and its reversal after RBC transfusion result in a defined pattern of adaptive responses. Anemia was produced over 2 days by serial phlebotomy (with plasma replacement) to Hb levels of 30–40 g/l. During the ensuing 2 days, Hb was restored to pretransfusion baseline levels by repeated RBC transfusion. Area-under-the-curve methodology was utilized for defining the Hb level at which individual study variables demonstrated significant change. Significant reciprocal changes (P < 0.05) of equivalent magnitude were observed during the phlebotomy and transfusion phases for cardiac output, plasma erythropoietin (Epo) concentration, oxygen extraction ratio, oxygen delivery, venous oxygen saturation, and blood lactate concentration. No significant change was observed in resting oxygen consumption. Cardiac output and plasma Epo concentration increased at Hb levels < 75 g/l, oxygen delivery and oxygen extraction ratio decreased at Hb levels < 60 g/l, and venous oxygen saturation decreased and blood lactate concentration increased at Hb levels < 55 g/l. We speculate that plasma Epo and blood lactate concentrations may be useful measures of clinically significant anemia in infants and may indicate when an infant might benefit from a RBC transfusion.

transfusion; lactate; hypoxemia; newborn

DURING THE EARLY WEEKS AFTER birth, primates and other mammals experience a gradual decline in blood Hb concentration as a result of rapid growth, shortened red blood cell (RBC) survival, increased oxygen availability accompanying the onset of respiration, and a lower tissue oxygen set point (35). In term infants, this fall in Hb typically reaches its nadir between the second and third months of life, with the greatest and earliest decline observed in the smallest, most premature infants. In the intensive care of critically ill newborn infants, blood sampling associated with laboratory testing hastens the onset of neonatal anemia and exacerbates its severity. Among premature infants weighing < 1,500 g at birth, daily phlebotomy blood loss of 4–5% of the blood volume is common in the first weeks of life when the severity of cardiopulmonary illness is greatest (4, 7, 28, 33, 36). Because the majority of RBC transfusions are administered during this same period (8, 26, 28, 36, 48), phlebotomy loss as a result of clinical monitoring is felt to be the primary cause of the large number of transfusions received by critically ill term and premature infants (4, 41, 50).

The fundamental basis for transfusion therapy is a decrease in oxygen transport capacity caused by a reduction in circulating RBC mass to the extent that cardiorespiratory status becomes impaired (45). Although the risks associated with RBC transfusion are well described, far less is known about the benefits of transfusing anemic infants at specific Hb levels (26, 41, 45, 50). Because of this uncertainty and because of developmental differences in oxygenation in neonates relative to adults (12), neonatal transfusion practices have varied widely over time and among institutions (28, 36, 48, 50).

Surprisingly, there have been no studies that have examined metabolic and cardiovascular adaptations resulting from slowly evolving anemia and its correction in unsedated neonatal animals in the absence of factors know to perturb oxygen delivery (DO2), e.g., hypoxia and decreased cardiac output (CO). Thus the objective of the present study was to identify clinically applicable indicators of the need for, and urgency of, RBC transfusions before the development of metabolic and cardiovascular decompensation accompanying critically low levels of DO2. We hypothesized that severe phlebotomy-induced isovolemic anemia, and its reversal after RBC transfusion, would result in a clearly defined pattern of adaptive cardiovascular and metabolic responses. Unanesthetized newborn lambs were...
selected for study because of their hematologic similarities to human infants.

METHODS

Animals. After we received approval from the local Animal Care and Use Review Committee, neonatal lambs of mixed Dorset and Suffolk stock were obtained from a local breeder. To avoid anemia during the baseline period (34), pregnant ewes were treated with 1 g of iron dextran by intramuscular injection every other week beginning at 100 days of gestation (term = 145 days). Beginning 24–48 h after birth, lambs were housed in an indoor, environmentally controlled facility in which the ambient temperature was maintained at 19°C. Between study sessions, lambs were nursed by their mothers. Except for experimentally induced anemia, all lambs were deemed in good health throughout the study period.

Surgical procedures. Surgery was performed when the lambs were 2–4 days of age. After induction of anesthesia with pentobarbital (13 mg/kg iv), lambs were intubated and mechanically ventilated. Thereafter, anesthesia was maintained with 0.05–2.0% halothane by inhalation. Benzathine penicillin (300,000 U/kg im) was administered before the first skin incision. Through a left inguinal incision, Tygon catheters (1D 0.05 in., OD 0.09 in.; Norton Performance Plastics, Akron, OH) were placed in the femoral artery and vein and advanced with their distal tips estimated to be immediately above the diaphragm. A left anterolateral thoracotomy incision was made parallel to the fourth intercostal space, and the ribs were separated by gentle traction. The pericardial sac was entered, and the great vessels were identified and isolated. After ligation of the ductus arteriosus, a 3–0 Ethicon purse-string suture was placed in the adventitia of the main pulmonary artery to monitor CO. Stroke volume, systemic D˙O2, oxygen consumption (V˙O2), oxygen extraction ratio (ERO2), and peripheral vascular resistance (PVR) were calculated from the directly measured variables as follows

\[
\text{ Stroke volume = CO ÷ heart rate (ml/kg) }
\]

\[
\text{ D˙O2 = CO } \times \text{ SaO2 } \times \text{ Hb } \times 1.39 \text{ ml O}_2/\text{g Hb (ml O}_2/\text{kg}^1 \cdot \text{min}^{-1}) \]

\[
\text{ V˙O2 = (CO } \times \text{ Hb } \times 1.39 \text{ ml O}_2/\text{g Hb } \times (\text{ SaO2 } - \text{ SvO2}) \text{ (ml O}_2/\text{kg}^1 \cdot \text{min}^{-1}) \]

\[
\text{ ERO2 = V˙O2 } ÷ \text{ D˙O2 (no units) } \]

\[
\text{ PVR = (arterial } - \text{ central venous blood pressure) ÷ CO } \]

chosen as the nadir for study because of the high mortality associated with levels below this (11, 18, 52, 53). During the transfusion phase, anemia was reversed in a stepwise fashion until Hb levels returned to baseline values. Transfusions were performed by using 4.8 ± 0.31 ml/kg of cross-matched adult sheep packed erythrocytes (hematocrit ≈90%) stored for <7 days in citrate phosphate dextrose adenine (CPDA). Transfusions were administered over 1.5–2 h, just before the lamb was returned to its mother but after data collection and blood sampling. Throughout the 4-day study period, benzathine penicillin (300,000 U/kg im) was administered every other day.

Lambs were weighed daily. Data collection periods during both the phlebotomy and transfusion phases consisted of 2-h sessions during which lambs were removed from their mothers and brought to the laboratory where they were maintained suspended in a cloth sling hung in a transport cart. Each of the 2-h study sessions was begun no sooner than 2 h after the previous phlebotomy or transfusion procedure (see below). Being placed in the sling had a calming effect on the lambs. After a 30- to 60-min period of acclimatization, during which lambs were left undisturbed, continuous cardiovascular data were recorded for ~60 min by using an eight-channel polygraph recorder (Gould, Cleveland, OH) interfaced with a data-acquisition software program (LabTech Notebook Data Acquisition Software, Laboratory Technologies, Wilmington, MA). Directly measured data included heart rate, mean arterial pressure, central venous pressure, and pulmonary artery blood flow. Because the ductus arteriosus had been ligated, pulmonary artery blood flow was used as a surrogate for CO. Stroke volume, systemic D˙O2, oxygen consumption (V˙O2), oxygen extraction ratio (ER02), and peripheral vascular resistance (PVR) were calculated from the directly measured variables as follows

\[
\text{ Stroke volume = CO ÷ heart rate (ml/kg) } \]

\[
\text{ D˙O2 = CO } \times \text{ SaO2 } \times \text{ Hb } \times 1.39 \text{ ml O}_2/\text{g Hb (ml O}_2/\text{kg}^1 \cdot \text{min}^{-1}) \]

\[
\text{ V˙O2 = (CO } \times \text{ Hb } \times 1.39 \text{ ml O}_2/\text{g Hb } \times (\text{ SaO2 } - \text{ SvO2}) \text{ (ml O}_2/\text{kg}^1 \cdot \text{min}^{-1}) \]

\[
\text{ ERO2 = V˙O2 } ÷ \text{ D˙O2 (no units) } \]

\[
\text{ PVR = (arterial } - \text{ central venous blood pressure) ÷ CO } \]
where \( \text{SaO}_2 \) is arterial oxygen saturation and \( \text{SvO}_2 \) is central venous oxygen saturation.

Respiratory rate as measured by chest impedance was recorded continuously over a 15- to 20-min portion of the same data collection period by using a Hewlett-Packard respiratory monitor (model 78212D) with a 50- to 60-Hz transducer (Hewlett-Packard, Palo Alto, CA). Rectal temperature was recorded by using a YSI 2600 O2/Temp Meter (Yellow Springs Instruments, Yellow Springs, OH).

All phlebotomies and transfusions took place at the end of each study after all measurements had been successfully completed. Afterwards, the lambs were returned to their mothers for a minimum of 2 h. The only exception to this was the second study day; after completion of the last phlebotomy and while experiencing the most profound level of anemia, lambs were not returned to their mothers after data collection but, instead, were first transfused and rested 2 h later. This was done as a precautionary measure to ensure survival.

At the completion of each 60-min recording period, simultaneous systemic and pulmonary arterial blood samples were collected anaerobically in heparinized syringes, placed on wet ice, and analyzed within 5 min of collection for \( \text{Hb}, \text{pH}, \text{PCO}_2, \text{PO}_2, \) oxygen saturation, and whole blood lactate. Additional blood was taken each morning with the first sampling for determination of plasma erythropoietin (Epo), whole blood 2,3-diphosphoglycerate (2,3-DPG), and \( \text{PO}_2 \), at which \( \text{Hb} \) is 50\% saturated with oxygen (\( P_{50} \)). After each blood transfusion, \( P_{50} \) was again measured. Because endogenous plasma Epo levels require a minimum of several hours to respond to changes in oxygenation before approaching steady-state levels (20), more frequent blood sampling for plasma Epo determinations was deemed inadvisable. Total body circulating RBC volume was measured three times: at the beginning of the study, just before the last phlebotomy, and after the last blood transfusion.

Laboratory determinations. \( \text{Hb}, \text{SV}_{\text{O}_2}, \) and \( \text{SaO}_2 \) were determined by using an IL-482 CO-oximeter (Instrumentation Laboratories, Lexington, MA). Blood samples were analyzed for \( \text{PO}_2, \text{PCO}_2, \) and \( \text{pH} \) by using an IL 1303 blood-gas analyzer and corrected to normal body temperature for lambs, i.e., to 39.5°C. \( P_{50} \) was determined by using the above-mentioned blood-gas analyzer and CO-oximeter and an IL-237 tonometer at \( \text{PCO}_2 = 40 \) Torr. \( P_{50} \) data were corrected to \( \text{pH} \) of 7.40 and normal lamb body temperature (23). The concentration of 2,3-DPG was determined spectrophotometrically on supernatant fractions of heparinized whole blood precipitated with 5% TCA (2:1) as described by Keitt (22). Lactate was measured on 50-µl whole blood samples by using a YSI model 27 analyzer (Yellow Springs Instruments).

Epo was measured on 100-µl plasma samples in triplicate by using a double-antibody RIA (47). Linear values for sheep Epo were obtained between 10 and 450 mU/ml by using sheep reference standards (EpoConn, Connaught Laboratories, Willowdale, Ontario). For each individual lamb study, samples were run in the same assay. Intra-assay coefficients of variation for these pools of plasma spanning the useful range of the RIA ranged from 4.7 to 11.1%.

Total RBC volume was measured by using autologous erythrocytes with \[^{14}C\]cyanate (New England Nuclear, Boston, MA) as described by Mook et al. (31). Circulating RBC volume determined with \[^{14}C\]cyanate agrees almost perfectly with RBC volume determined with \[^{51}Cr\], i.e., correlation coefficient = 0.99.

Data handling and statistical analysis. The area-under-the-curve methodology of Matthews et al. (29) was combined with the interpolation method of Cilley et al. (11) as the primary statistical method for estimating the \( \text{Hb} \) level at which study variables demonstrated change. In applying this methodology, the magnitude of response of each study variable was quantitated for individual lambs by measuring the area under the curve for the variable plotted against 5-g/l \( \text{Hb} \) intervals. This was done separately for both the phlebotomy and transfusion phases. As illustrated for a representative animal with the use of whole blood lactate measured during the phlebotomy phase as an example (Fig. 2), \( \text{Hb} \) intervals with missing lactate values were conservatively estimated by substituting lactate values from the next higher 5-g/l \( \text{Hb} \) interval in which a measured lactate value was available. In inspecting Fig. 2, it is evident that the application of this methodology results in underestimation of the \( \text{Hb} \) concentration at which a change occurs. The only parameters included in the final analyses defining the precise \( \text{Hb} \) level at which change occurred were those specifically perturbed by anemia, i.e., those demonstrating equivalent and reciprocal area-under-the-curve responses during the phlebotomy and transfusion at the same \( \text{Hb} \) interval. These parameters included \( \text{SV}_{\text{O}_2}, \text{CO}, \text{ER}_2, \) plasma Epo, whole blood lactate, and \( \text{VO}_2 \).

Because RBC volume measurements were taken during only 3 of the 10 study periods, the area-under-the-curve methodology was deemed inappropriate for examining the utility of RBC volume measurements in predicting change in \( \text{Hb} \) or in testing for associations with other oxygenation parameters. Instead, correlation coefficients of RBC volume and oxygenation parameters demonstrating change in the area-under-the-curve analysis were determined separately for each lamb. The mean group correlation coefficient for each oxygenation parameter was then tested for significance by a one-sample \( t \)-test. An identical correlation analysis with oxygenation study parameters was performed by substituting the simultaneously determined \( \text{Hb} \) concentration. To avoid bias, only \( \text{Hb} \) data obtained at the same three study periods as the RBC volume measurements were included, i.e., baseline, severe anemia, and complete recovery. The correlation coefficients of those variables demonstrating significant association with both RBC volume and \( \text{Hb} \) were compared by paired \( t \)-test to determine if RBC volume or \( \text{Hb} \) demonstrated a significantly better correlation.

Results are expressed as means ± SE. A \( P \) value < 0.05 (two-tailed) was considered statistically significant. Statisti-
cal computations were done by using commercially available software (Statview II, Abacus Concepts, Berkeley, CA). Variables demonstrating nonnormal distributions were transformed logarithmically before testing. Single-factor ANOVA with repeated measures was used to test for differences at specified Hb intervals indicated in Fig. 2 or the specified sampling periods indicated in Fig. 1. Dunnett’s post hoc testing procedure was performed by using the baseline period data for comparisons with all other periods for significant F tests.

RESULTS

The mean (± SE) age of the lambs at the time of the first phlebotomy study was 5.7 ± 0.2 days. There was no significant change in body weight during the study period; body weight before the first phlebotomy was 5.74 ± 0.25 kg, and the weight after the last transfusion was 5.75 ± 0.33 kg. Autopsies done at the completion of each study demonstrated proper positioning of all catheters and flow probes.

Study periods during which variables demonstrated significant change. Hb concentration decreased from baseline values of 96.9 ± 3.3 g/l to a nadir of 36.0 ± 1.3 g/l on the second day. After the last transfusion on day 4, Hb returned to near baseline levels, i.e., 93.9 ± 1.4 g/l (Fig. 3A). RBC volume measurements taken at baseline, during anemia, and after recovery were 27.1 ± 4.6, 11.1 ± 0.7, and 24.6 ± 1.6 ml/kg, respectively. These changes were of similar proportion to those for Hb at the same study periods.

Significant progressive (P < 0.05) changes were observed for SvO2, plasma Epo, and blood lactate as anemia intensified. Moreover, reciprocal changes were observed in all three as Hb levels increased during the study’s transfusion phase. During the phlebotomy phase, all lambs experienced a decrease in SvO2 from baseline values of 48.1 ± 10.5%. In six, the decrease was > 2 SD, and in three of the remaining four the decrease was > 1 SD (Fig. 3B). By the end of the phlebotomy phase, 9 of the 10 lambs manifested a progressive > 3 SD increase in plasma Epo from baseline, whereas Epo levels in the remaining animal increased by > 2 SD (Fig. 3C). The increase observed in blood lactate level was more variable than that in plasma Epo. Seven lambs increased blood lactate levels by > 3 SD of baseline, one increased by > 2 SD, whereas the remaining two manifested no measurable change in lactate (Fig. 3D).

Arterial pH, PaCO2, and PaO2 values all fell within normal limits during the baseline period (i.e., 7.39 ± 0.01, 44.6 ± 1.5 Torr, and 80.4 ± 16.3 Torr, respectively) and demonstrated little change thereafter (Fig. 4, A-C). Only a brief, small but significant, decrease in PaCO2 occurred coincident with the period of profound anemia during which blood lactate levels increased. Neither pH or PaO2 changed significantly during either the phlebotomy or transfusion phases.

Changes that occurred in the affinity of Hb for oxygen and in 2,3-DPG levels during the course of study did not
follow the pattern observed for Hb. The significant decline was first observed in 2,3-DPG just before the first RBC transfusion and continued throughout the remainder of the study (Fig. 5A). This was followed by a significant increase in P50 coincident with the administration of adult sheep blood during the study’s transfusion phase (Fig. 5B).

Several cardiovascular and respiratory study parameters demonstrated no significant change during the phlebotomy and transfusion phases, whereas others demonstrated marked changes. Although respiratory rate decreased during anemia (Fig. 6A), inspection of individual pneumograms revealed no episodes of apnea, tachypnea, or periodic breathing. Baseline pulmonary arterial pressure, central venous pressure, and body temperature did not change during the course of the study (not shown) and were within expected limits, i.e., 22.8 ± 6.9 mmHg, 3 ± 2 mmHg, and 39.6 ± 0.5°C, respectively. Although tidal volume was not measured, lambs may have breathed more slowly and deeply during the periods of most profound anemia, thereby leading to an increase in minute ventilation. Despite the decrease in respiratory rate, this speculation is consistent with the decrease observed in arterial Pco2. Arterial blood pressure followed a similar pattern to that of respiration (Fig. 6B). PVR decreased significantly as Hb levels fell (data not shown). The nonsignificant trend toward an increase in heart rate (Fig. 6C) combined with a significant rise in stroke volume (not shown) resulted in a gradual, but significant, rise in CO during the phlebotomy phase (Fig. 6D).

Of the calculated cardiovascular parameters indicative of oxygenation status, D˙O2 and ERO2 demonstrated significant progressive change as anemia worsened during the phlebotomy phase and returned back to baseline levels as Hb level normalized. The pattern of decrease of D˙O2 levels (Fig. 7A) as Hb concentration fell approximately mirrored the increase in ERO2 levels (Fig. 7B). Despite a pronounced decrease in D˙O2 and increase in ERO2 as Hb reached its nadir, VO2 did not change (Fig. 7C).

Area-under-the-curve analysis: the primary outcome methodology. The area-under-the-curve analysis used in estimating the Hb level at which statistically significant change occurred was reserved only for those oxygenation parameters demonstrating equivalent and reciprocal responses at the same Hb interval during the phlebotomy and transfusion phases. Because the Hb nadir was not identical in all study lambs, the spectrum of Hb intervals included in the area-under-the-curve analyses was indicative of less anemia than was the case with individual lambs. For those oxygenation parameters demonstrating progressive area-under-the-curve change from the baseline Hb interval, the Hb intervals at which statistical significance changes were identified were as follows: CO and plasma Epo increased at Hb < 75 g/l; D˙O2 decreased and ERO2 increased at Hb < 60 g/l; SvO2 decreased and blood lactate increased at Hb levels < 55 g/l (Fig. 8). VO2 did not demonstrate significant change at the range of Hb levels studied (data not shown). Although each of these parameters demonstrated significant change as anemia worsened and returned to baseline as anemia was corrected, the magnitude of and the progressive nature

![Fig. 5. Mean (± SE) data of 2,3-diphosphoglycerate (2,3-DPG; A) and P50 (B) in 10 newborn lambs during phlebotomy and transfusion study phases. Analysis by single-factor repeated measures ANOVA revealed significant differences from baseline levels for both measurements. Results of Dunnett’s post hoc significance are as follows: *P < 0.05 and †P < 0.01.](http://jap.physiology.org/)

![Fig. 6. Mean (± SE) cardiopulmonary status data in 10 newborn lambs during phlebotomy and transfusion study phases for respiratory rate (A), arterial blood pressure (B), heart rate (C), and cardiac output (D). bpm, Beats/min. Analysis by single-factor repeated measures ANOVA revealed significant differences from baseline levels for all variables except heart rate. Results of Dunnett's post hoc significance are as follows: *P < 0.05 and †P < 0.01.](http://jap.physiology.org/)
of change relative to baseline levels was most clearly evident for plasma Epo and blood lactate. Moreover, the variance of these two parameters was equivalent to or smaller than that of the other four oxygenation parameters.

Because the significant rise in P50 after transfusion with adult blood could have exerted an independent effect on the oxygenation parameters, a statistical power analysis was performed to estimate the magnitude of the error encountered in comparing the phlebotomy and transfusion phases of the experiment. To do this, the standard deviation of the mean difference between the two study phases was compared at each of the Hb intervals. The mean value for the mean standard deviations for each interval of the study variables shown in Fig. 8 was derived and multiplied by the appropriate statistical constants when it was assumed that power = 0.80 and α = 0.05 for 10 study subjects. When this value was expressed as a percentage of the mean area under the curve at each Hb interval, the mean variability observed ranged from 19.5 to 26.5% for all of the variables in Fig. 8, except lactate, which was slightly greater, i.e., 33%.

Comparison of total body RBC volume and Hb with oxygenation parameters. In the univariate correlation analysis, both RBC volume and Hb concentration demonstrated significant correlation with several of the oxygenation parameters that demonstrated change in the area-under-the-curve analysis (Table 1). When the degree of association of RBC volume and Hb level was compared, RBC volume was not associated to a more significant degree than Hb with $D_O_2$, CO, plasma Epo, $S_v O_2$, or $E R O_2$.

**DISCUSSION**

The newborn lamb model utilized here simulates, albeit on a shorter time scale, anemia gradually developing in infants as a result of cumulative blood loss for clinical monitoring. The inclusion of events during both phlebotomy and transfusion adds to the specificity, identification, and quantification of the adaptive responses that might serve as indicators of the need for blood transfusion. Few preclinical studies in nonanesthetized newborn animals have examined the relationship of oxygenation parameters in the presence of severe neonatal anemia. None has included the results of plasma Epo, a sensitive and specific indicator of tissue hypoxia, and none has evaluated adaptive responses during both phlebotomy and transfusion over an extended period. In the present study, our hypothesis that a progression of adaptive cardiovascular and metabolic responses would be observed in response to stepwise progressive phlebotomy-induced isovolemic anemia in newborn lambs and their subsequent reversal by transfusion was confirmed. The sequence of responses indicative of progressive tissue hypoxia accompanying a 60% decrease from baseline Hb level was initiated with a fall in CO and a marked rise in plasma Epo. This was followed by an increase in ERO2 and a decrease in DO2. A decrease in SvO2 and an increase in blood lactate were the last changes detected. The severity of anemia was never so marked that a decline in resting VO2 was detected.

Progression of events with worsening isovolemic anemia and its correction. Tissue hypoxia attributable to anemia is difficult to assess at the cellular level in in vivo studies. Nonetheless, the progression we observed in adaptive cardiovascular and metabolic changes as anemia worsened supports the anaerobic threshold hypothesis (11, 17, 18, 52). According to this hypothesis, homeostatic adaptations take place in response to progressive tissue hypoxia to maintain VO2 within fixed, narrowly defined normal limits, thus avoiding cardiovascular collapse and imminent death as tissue DO2 declines to critically low levels. Caution, however, is required to avoid overdrawing conclusions regarding the exact sequence of the changes in the cardiovascular and oxygenation study variables. This is necessary because the calculated parameters are based on those directly measured, e.g., DO2 is determined based on CO, ERO2 is determined based on SvO2, and so forth. Our discussion of the sequence of events needs to be viewed in this context with appropriate allowance for the degree of uncertainty identified in the error estimation performed with the area-under-the-curve analyses.

In the present study, the first of the homeostatic changes accompanying the decline in Hb was increases in CO and plasma Epo. These events occurred as the lambs’ Hb levels fell <75 g/l, with both returning to normal after the restoration of Hb levels to baseline during the transfusion phase. Consistent with the findings of Holzman et al. (18) in newborn lambs, the
increase we observed in CO was predominantly attributable to an increase in stroke volume, with a lesser, nonsignificant increase in heart rate. The decline in PVR as a result of declining Hb levels, and therefore in decreasing blood viscosity, likely contributed to the increase observed in stroke volume. In clinical studies of human neonates, small but significant changes (7–15 beats/min) in heart rate have been noted by some (5, 25), but not all (24), investigators as anemia worsens. Unfortunately, these changes in neonatal heart rate have all been within the normal range. Based on the present data, we speculate that this is attributable to less profound anemia than that observed in the present study or to a lesser role of heart rate in controlling CO in the human infant. If the latter is true, continuous heart rate monitoring in human neonates will be of little value in assessing their transfusion needs.

In contrast to the short-term adaptive responses in CO to anemia, the increase in plasma Epo concentration represents the body’s long-term Epo adaptation directed toward increasing Hb. Although the precise cellular and molecular hypoxia-mediated events leading to increases in Epo gene expression remain unknown, there is agreement that most Epo production in the adult takes place in the kidney (and to a lesser degree in the liver) (15). In contrast, in the fetus, and perhaps in the young infant (13), the liver is the primary organ of Epo production (15). The progressive increases in plasma Epo first detected in the lambs at Hb levels <75 g/l were in marked contrast to the more modest changes observed in CO and in the other cardiovascular parameters. Between Hb levels of 75 and 40 g/l, the average plasma Epo increased 43-fold relative to baseline (range: 5- to 233-fold), i.e., logarithmically transformed Epo values nearly doubled (Fig. 8B). These increases in plasma Epo concentration are of similar magnitude to those associated with both acute hypoxemia and anemia in fetal sheep (16, 38, 47).

### Table 1. Association of red blood cell volume and Hb with oxygenation parameters studied

<table>
<thead>
<tr>
<th>Oxygenation parameter</th>
<th>Mean r with RCV</th>
<th>Mean r with Hb</th>
<th>P Value for Hb vs. RCV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen delivery</td>
<td>0.48*</td>
<td>0.66†</td>
<td>0.14</td>
<td>7</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>−0.59*</td>
<td>−0.77†</td>
<td>0.11</td>
<td>7</td>
</tr>
<tr>
<td>Plasma In Epo</td>
<td>−0.90‡</td>
<td>−0.96‡</td>
<td>0.07</td>
<td>9</td>
</tr>
<tr>
<td>Venous oxygen saturation</td>
<td>0.55*</td>
<td>0.68†</td>
<td>0.22</td>
<td>9</td>
</tr>
<tr>
<td>Oxygen extraction ratio</td>
<td>−0.60*</td>
<td>−0.76†</td>
<td>0.19</td>
<td>7</td>
</tr>
<tr>
<td>Blood lactate</td>
<td>−0.32</td>
<td>−0.36</td>
<td>0.19</td>
<td>10</td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td>−0.25</td>
<td>−0.27</td>
<td>0.7</td>
<td>7</td>
</tr>
</tbody>
</table>

Epo, erythropoietin; RCV, red cell volume. n, no. of lambs. *P < 0.05, †P < 0.01 for 1-sample t-test.
and with hypoxemia and anemia of indeterminate duration in human fetuses (30, 43, 49). They differ markedly, however, from the much less marked increases in plasma Epo levels observed among chronically anemic human neonates (9, 24, 25, 30). This difference could be due to the lower oxygen environment of the fetus relative to the newborn.

Compensatory cardiovascular mechanisms in the lambs maintained $V_O^2$ at normal levels without a decrease in $D_O^2$, or an increase in $E_R O_2$, until Hb levels decreased <60 g/l. At the nadir Hb, $D_O^2$ had declined to $12 \pm 1.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, a value just above the critical level of $10-12 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at which lactate increases and $V_O^2$ decreases in large animals (10, 11, 52). At the lowest Hb levels, $E_R O_2$ values reached 0.70–0.80, a value much higher than that reported in critically ill adults and similar to those reported for severely anemic fetal and neonatal lambs (3, 14, 17, 42). In humans, Wilkerson et al. (51, 52) reported that $E_R O_2$ increases and $V_O^2$ decreases in large animals (10, 11, 52). Although $D_O^2$ in the present study approximates the 1–2 mM reported in human infants with isolated anemia immediately before blood transfusion (1, 19, 37, 40) but similar to levels observed in anemic large animals as $V_O^2$ decreases below critical levels of $D_O^2$ (11, 27, 52). The relatively low blood lactate levels reported in association with anemia in infants fall in the upper normal range and suggest that RBC transfusions are being administered well in advance of significantly impaired tissue oxygenation. In contrast, anemic infants with blood lactate levels similar to those of our lambs as the lambs' Hb levels approached their nadir merit careful evaluation and strong consideration of transfusion.

Despite the marked increase in blood lactate observed as the nadir of Hb was approached, resting $V_O^2$ remained unchanged. Previous acute normovolemic anemia studies have demonstrated that blood lactate levels increase as $V_O^2$ declines in close proximity to the point when critically low levels of $D_O^2$ are reached (11, 17, 18, 52). Although $D_O^2$ in the present study approximated critically low values reported by others, a significant decrease in $V_O^2$ in association with the rise in blood lactate was not demonstrated. This could have been due to our failure to directly measure $V_O^2$ and $D_O^2$ by independent means (11).

Correlation of RBC volume and Hb with indicators of tissue oxygenation. Total circulating RBC volume and Hb were compared for their respective associations with indicators of tissue oxygenation. Although not reaching statistical significance for any of the parameters tested, the correlation coefficients tended to be higher for Hb than for RBC volume. This finding differs from that of Jones et al. (21), who reported that RBC volume measurement provides a more accurate indication of the adequacy of tissue oxygenation than either Hb or hematocrit in human adults and infants. This discrepancy could be due to differences in the severity of illness of the study subjects, to species differences, or to methodological differences.

Changes in oxyhemoglobin affinity. The changes observed in $P_{50}$ and RBC 2,3-DPG were not equivalent during the phlebotomy and transfusion phases. Because the rise observed in $P_{50}$ during the transfusion phase will tend to increase $D_O^2$ and the release of oxygen to tissues, this disparity in $P_{50}$ during the two study phases also serves to complicate interpretation of several of the oxygenation parameters, e.g., $SvO_2$ and arterial and venous $P_O^2$. As suggested by the results of the area-under-the-curve error analysis, this could have contributed in making the relationship between Hb and the oxygenation parameters less precise.

In a previous report comparing two groups of newborn lambs made anemic by isovolemic transfusion with RBCs possessing low or high Hb-oxygen affinity ($P_{50} = 32.2 \pm 2.5$ and $19 \pm 1.06$ Torr, respectively), Hb levels as low as 40 g/l were tolerated in the low-oxygen affinity group without a decrease in $V_O^2$, whereas lambs in the high-oxygen affinity group experienced decreased $V_O^2$ (44). Thus the relatively high $P_{50}$ values (i.e., low-Hb-oxygen affinity) and our finding of no change in $V_O^2$ in the lambs in the present study are consistent with the previous report's low-Hb-oxygen affinity lambs.

The changes that took place in $P_{50}$ and RBC 2,3-DPG during the phlebotomy and transfusion phases likely reflect developmental events and/or were the result of transfusion with adult erythrocytes with their higher $P_{50}$ and lower RBC 2,3-DPG concentration (2, 27). During the first week after birth, newborn lambs rapidly switch from a high- to low-Hb-oxygen affinity with their $P_{50}$ increasing from ~18 to 30 Torr (2, 27). This change is mainly due to the rapid rise in RBC 2,3-DPG, which results in a commensurate decrease in oxygen affinity before the switch to adult Hb has been completed. Afterward, as the percentage of adult Hb further increases, levels of DPG decrease, and the postnatal increase in $P_{50}$ reaches a plateau by 10–14 days (2).

Comparison of anemia in lamb and infants. Although lambs and infants share developmental similarities of the cardiovascular and hematopoietic systems (27, 32), quantitative differences exist that need to be considered in extrapolating from one species to the other. With lambs' higher postnatal $P_{50}$ values (despite lower 2,3-DPG levels and differences in Hb structure), they may be better adapted for readily releasing oxygen to tissues and thus function more adequately at Hb levels that are normally 20–30% lower than their human infant counterparts (27, 34). Nonetheless, from a quali-
tative perspective, the sequence of adaptive changes to anemia observed for newborn lambs in the present study is similar to that reported in other newborn and adult animal and adult human studies. Furthermore, it must be reiterated that the present study addresses only the situation of isovolemic anemia in otherwise healthy neonatal lambs. Results of these studies must, therefore, be carefully extrapolated to animals or infants with superimposed acute blood loss, cardiac and/or pulmonary disease, sepsis, or surgery.

In summary, establishment of sound, experimentally based criteria for guiding decisions to administer RBC transfusions to anemic human infants is an important and pressing need. Literature in adults indicating that moderately lower levels of Hb are well tolerated in otherwise healthy individuals has not previously been extended to include newborns. Moreover, there have been no predilutional studies evaluating adaptive responses to anemia over extended periods during both phlebotomy and transfusion phases. Studies in newborn animals provide relevant comparative data yet avoid the ethical difficulties inherent in infant studies. In this study of isovolemic anemia in newborn lambs, we observed that Hb values interpreted in conjunction with adaptive cardiovascular and metabolic indicators of hypoxia, in particular within plasma Epo and blood lactate, provide an informative basis for assessing tissue oxygenation and thus the potential immediate benefit of erythrocyte transfusions. Measurement of blood lactate levels and plasma Epo have advantages over measurements of other responses, as both are easily performed, results of both are readily available in minutes to hours and, as shown by the present data, both demonstrate marked progressive changes in response to the development of anemia that are reversed after transfusion. Although quantitative differences in oxygenation parameters existing between the newborn lambs and human infants preclude precise extrapolation from one species to the other, qualitative similarities exist. Thus the present data in lambs support the assumptions on which infant erythrocyte transfusions are based and merit reexamination. Reasons for the marked elevations in plasma Epo and in blood lactate in anemic and hypoxic human fetuses, but not neonates, remain uncertain. One possibility is that our present reliance on Hb levels in isolation from other potentially informative laboratory indicators of the need for transfusion in infants, e.g., plasma Epo and blood lactate, is suboptimal. Before firm conclusions can be reached regarding this speculation, studies evaluating parameters of tissue oxygenation such as those included in this and other studies, carried out under a variety of clinical conditions, are needed.

We acknowledge the technical contributions of Delores Cordle, Robert Schmidt, Gary Lankford, David Viet, and Barbara Stewart; the secretarial contributions of Mark A. Hart; and helpful comments and critical review of the manuscript by Dr. Ronald G. Strauss.

This work was supported by National Heart, Lung, and Blood Institute Grant PO1-HL-46925 and Medical Research Council of Canada Grant MT-11552.

Address for reprint requests and other correspondence: J. A. Widness, Univ. of Iowa Hospitals & Clinics, 200 Hawkins Dr., W222-1 GH, Iowa City, IA 52242-1083 (E-mail: john-widness@uiowa.edu).

Received 30 November 1998; accepted in final form 28 December 1999.

REFERENCES


