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The Effect of Hematocrit Alterations on Cerebral Vascular CO_2 Reactivity in Newborn Baboons[1]

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ABSTRACT. To evaluate whether baseline hematocrit affects cerebral vascular reactivity to CO_2, we studied the cerebral blood flow velocity in the internal carotid artery of newborn baboons, using a pulsed Doppler technique with direct imaging. Velocity responses to varying arterial CO_2 tension ($Paco_2$) levels were first tested under baseline hematocrit (mean ± SD, 35.9 ± 4.7%), after hemodilution (hematocrit 20.3 ± 2.7%), and after hemoconcentration (hematocrit 52.7 ± 5.2%). The data were analyzed using multiple regression models, in which blood flow velocity values were the dependent variables, and $PaCO_2$, hematocrit, and their interaction terms (product) were the independent variables. Models for the maximum systolic velocity, time-averaged mean velocity, and the end-diastolic velocity revealed a highly significant $PaCO_2$ effect; with each mm Hg $PaCO_2$ increase, the velocities increased between 2.9 and 3.6% (21.8–27% per 1 kPa). $PaCO_2$ and hematocrit interaction terms were also highly significant and inversely related to velocity (negative slopes) in the maximum systolic velocity and time-averaged mean velocity models, suggesting that when the hematocrit is high, the $PaCO_2$-induced increase in flow velocity would be attenuated, and when the hematocrit is low, such a response would be accentuated. The hematocrit effect on $PaCO_2$ reactivity was maximal on maximum systolic velocity and least on end-diastolic velocity. Systolic velocity acceleration slope was significantly reduced when the hematocrit was high, and increased when the hematocrit was low. Based on these findings, we conclude that hematocrit is an important variable affecting the rate of kinetic energy change in the large vessels, thereby influencing cerebral vascular $PaCO_2$ reactivity as assessed in Doppler studies. Therefore, to avoid errors in the calculated resistance indices and $PaCO_2$ reactivity, hematocrit values should be included in the statistical analysis. (*Pediatr Res* 29: 385–390, 1991)

Abbreviations

CBF, cerebral blood flow
Ca_{O_2}, arterial oxygen content
V_{max}, maximum Doppler spectral velocity
V_{mn}, mean Doppler spectral velocity
V_{cd}, end-diastolic velocity
RI, resistance index
PI, pulsatility index
S/D, systolic-diastolic velocity ratio
$PaCO_2$, arterial CO_2 tension
PaO_2, arterial O_2 tension
$\hat{\beta}$, regression coefficient

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Doppler techniques have been used to measure CBF velocity and vascular resistance in animal and human vasculature, and in the uteroplacental circulation (1–10). RI, PI, and S/D are the measures of peripheral vascular resistance obtained frequently in Doppler studies (9). Because CBF changes in response to $PaCO_2$ (11–13), Doppler techniques have also been used to assess the reactivity of cerebral vasculature to $PaCO_2$ (1, 3, 10). $PaCO_2$ reactivity is known to vary with postnatal age, with maturity, and in disease states (10, 14, 15), but the factors affecting such variations are unknown.

Several lines of evidence suggest that alteration in oxygen availability by any combination of factors (hematocrit, PaO_2, CaO_2, or PO_2 at 50% Hb saturation) affects cerebral blood flow (16–19). In anemic hypoxia CBF increases and in polycythemia CBF falls. Hudak *et al.* (17) have speculated that the reduction in CBF in polycythemia is an independent effect of red cell mass, but others contend that this is due to an increased oxygen availability, rather than changes in viscosity (19).

Whatever the mechanism, it is clear that an increase in hematocrit not only affects CBF, but also influences local vascular rheologic properties (20, 21). We therefore questioned whether a change in baseline red cell mass affects the extent of CBF response to $PaCO_2$. Because there were no studies addressing this issue, we designed experiments to investigate the effect of changes in hematocrit on cerebral vascular CO_2 reactivity in the newborn baboon.

MATERIALS AND METHODS

Animal preparation. Term newborn baboons (*Papio anubis*) were used in these studies, which were approved by the institutional Animal Care Committee. After intramuscular ketamine (10 mg/kg), the animal was intubated and artificial ventilation was provided using a time-cycled, pressure-limited infant ventilator (Baby-Bird Inc., Palm Springs, CA). The baseline PaO_2 was maintained at 100–150 mm Hg, and $PaCO_2$ at 30–35 mm Hg, respectively (1 mm Hg = 0.133 kPa). An end-tidal CO_2 monitor (Datex System, Puritan-Bennett Inc., Los Angeles, CA) was connected to the endotracheal tube adapter to sample and display the partial pressure and concentration of CO_2 continuously.

Sedation was provided with intermittent i.v. phenobarbitone (20 mg/kg). Muscle paralysis was maintained using 75–100 μg/kg pancuronium bromide. Both femoral arteries and veins were catheterized, and 90 mL/kg/d of 10% dextrose in 0.5 normal saline was used as maintenance fluid. The rectal temperature was maintained at 37.5°C using a heated mattress and overhead radiant lamps. Heart rate, respiration, and the mean blood pressure were recorded continuously.

Hematocrit alterations. Two-hundred mL heparinized maternal baboon blood was centrifuged to separate the plasma and packed red blood cells (70% hematocrit) and stored at 4°C. After baseline experiments, hemodilution was achieved by an isovolumic exchange transfusion using maternal plasma warmed to room temperature. The exchange volume was calculated using the formula: blood volume in mL/kg × (observed − desired hematocrit/observed hematocrit), to drop the hematocrit by 40–45% of the baseline. Hemoconcentration was achieved by a similar procedure using maternal packed red blood cells to raise the hematocrit by 40–45% of the baseline. Simultaneous withdrawal of blood and infusion of plasma (or blood) were done via the femoral artery and venous catheters, respectively, maintaining identical rates such that the heart rate changes were <10 bpm, the mean blood pressure changes were <5 mm Hg, and the rectal temperature was maintained at 37.5°C. The procedure took about 45 min. Whole blood viscosity, hematocrit, and blood gas and acid base status were evaluated before and after each procedure. Thirty min of stabilization were allowed after animal preparation between exchange transfusions and $Paco_2$ changes.

$Paco_2$ reactivity. $Paco_2$ reactivity experiments were done at baseline hematocrit, and after hemodilution and hemoconcentration. Using a Doppler method (see below), we first measured the internal carotid artery flow velocity under control $Paco_2$ levels. Hypocapnia (15–20 mm Hg $Paco_2$) was then induced by increasing ventilator rate to 60/min, and hypercapnia (50–60 mm Hg $Paco_2$), by connecting 2% CO_2 flow (0.5 L/min) to the ventilator inspiratory tubing. End-tidal CO_2 readings were used to assess a steady state $Paco_2$ in hypocapnia experiments. Steady state $Paco_2$ was maintained by adjusting the ventilator rate or the CO_2 flow rate based on the results of blood gases measurements (triplicate) during each CO_2 experiment. Because even minimal $Paco_2$ change could affect velocity, it was critical that Doppler signals analyzed were those that coincided with the sample of blood drawn. We used the voice recorder and event markers to time these steps. To prevent hypoxia with the addition of CO_2, we increased the inhaled O_2 concentration by 35%.

Doppler methods. The blood flow velocity was measured using a phased array sector scanner (no. 77030A, Hewlett-Packard Co., Palo Alto, CA) with a 5.0-MHz short-focus transducer (4, 5, 22). Using a black-and-white imaging for the first four studies and a color-flow imaging for the final five, we scanned the vertex in the mid-sagittal plane to obtain a real time image of the internal carotid artery distal to its entry via the cavernous sinus. While maintaining the arterial image, the Doppler sample volume (0.5^3 mm) was positioned within the vessel such that the blood flow/Doppler incident angle was always between 0 and 10°; thus, the velocities measured were accurate to within 3% (23), requiring no angle correction.

The built-in electronic caliper cross-hair was placed at specified points on the wave forms, identifying the leading edge of spectral display and the steepest parts of the ascending and descending portions. The measurements were precise to ±0.167 cm for velocity, and ±0.005 s for time. Our results were videotaped, and five consecutive wave forms were measured to determine V_{max}, V_{ed}, and, by integrating the wave form over a cardiac cycle, the time-averaged V_{mn}. The systolic acceleration slope ($\Delta V/\Delta t$, where V is velocity, and t is the time to V_{max}) was also measured (22–23). Using velocity values, we computed Pourcelot's RI (RI = $V_{max} - V_{ed}/V_{max}$), Gosling's PI (PI = $V_{max} - V_{ed}/V_{mn}$), and S/D (S/D = V_{max}/V_{ed}) (9).

Whole blood viscosity. Whole blood viscosity was measured at 37°C using a constant temperature water bath and a Wells-Brookfield Cone/Plate viscometer (models Ex-100 and LVTDVCP-II, Brookfield Engineering Inc., Stoughton, MA). With a 1.565° cone/spindle (CP no. 42) and 0.5 mL blood, we measured viscosities at eight increments of shear rates, from 1.15 to 230 s^{-1}. The digitally displayed viscosity values were recorded. The system is accurate to within 1% in the operating range. Because of viscosity hysteresis, values obtained during decreasing shear rates were not analyzed.

Data were analyzed using a mainframe-based Statistical Analysis System package (24). Analysis of variance with post hoc comparison was used to compare the mean viscosity and Doppler result. The effect of hematocrit and $Paco_2$ on velocities were assessed using multiple regression models having the general formula $\hat{Y} = \hat{\alpha} + \hat{\beta}_1 \times X_1 + \hat{\beta}_2 \times X_2 \ldots$, where Doppler variables were dependent variables and $Paco_2$, hematocrit, and their interaction terms (product) were the independent variables. To control for the differences between baboons, we included indicator variables for each baboon ($Z_i = 1$ in the ith baboon, 0 otherwise) in the model. Because the same baboon is studied at different levels of hematocrit, there is a risk of correlated errors; therefore, correlation matrices of the residuals were checked. To avoid inflating n, we included only one set of velocity/blood gas values per animal at each of the hematocrit (low, normal, or high) and $Paco_2$ (low, normal, or high) combinations. The results for the major variables are reported as mean ± SD and $\hat{\beta}$ with their standard errors. A p value of <0.05 was considered to be statistically significant.

RESULTS

We studied nine baboons having a mean age of 13 d (range 7–20 d), and a mean (±SD) weight of 997 ± 207 g. After experiments under baseline hematocrit conditions in all nine animals, both plasma and packed red cell exchange transfusions were done in three, only plasma exchange in three others, and only packed red cell exchange transfusions in the remaining three. Hypo- and hypercapnia experiments at various hematocrit levels were done in all nine animals.

Exchange transfusion with maternal plasma dropped the hematocrit by 44%, and with maternal packed red blood cells increased hematocrit by 48% ($p < 0.0001$, Table 1). The vital signs did not change significantly, either during or after the procedures. Hyperoxia occurred in eight of nine animals, but the Pao_2 values were comparable among the three groups during the $Paco_2$ experiments.

Viscosity changes. After the packed red cell exchange transfusion, the whole blood viscosity values were 48 to 115% higher than the corresponding baseline values between the shear rates of 5.75 and 115 s^{-1} (analysis of variance with post hoc comparison; p values were between <0.05 and <0.001). After plasma exchange, the mean viscosity values were consistently lower than the corresponding baseline values at all shear rates, but the differences were not statistically significant.

Velocity results. The mean velocity values during control $Paco_2$ levels were higher at low hematocrit and lower at high hematocrit levels (Table 1). Because of large within-group variations, and perhaps due to the effect of subtle differences in hematocrit and Pco_2 between each animal (see below), the differences were not statistically significant. In low hematocrit experiments, the systolic acceleration slope was 50.2% higher as compared with normal hematocrit and 94.5% higher as compared with high hematocrit experiments ($p < 0.001$) (Table 1).

Multiple regression analysis (Table 2, Fig. 1). Fifty-eight of the possible 63 sets of values with different hematocrit-$Paco_2$ combinations were considered reliable for analysis. The $Paco_2$ regression slopes ($\hat{\beta}$ values, Table 2) represent $Paco_2$ reactivity; by relating these values to the baseline control condition velocities in Table 1, the $Paco_2$ reactivity can be expressed as % change in velocity/mm Hg $Paco_2$ (or per kPa).

$Paco_2$ had a highly significant effect on all components of the velocity wave form (Table 2). For each mm Hg $Paco_2$ increase, the V_{max} increased by 1.5 cm/s, or 2.9% of 52.5 cm/s baseline V_{max}; the V_{mn} increased by 1.1 cm/s, or 3.6% of 30.9 cm/s baseline V_{mn}, and V_{ed} increased by 0.57 cm/s, or 3.2% of 17.9 cm/s baseline V_{ed}. (Expressed in SI units, these $Paco_2$ reactivity

Table 1. *Baseline physiologic data**

Variables	Normal hematocrit (30–44%) ($n = 9$)	Low hematocrit (<30%) ($n = 6$)	High hematocrit (>44%) ($n = 6$)
Hematocrit (%)	35.9 ± 4.7	20.3 ± 2.7†	52.7 ± 5.2†
% change		−44%	+48%
Mean blood pressure (mm Hg)	82.5 ± 5.9	80.9 ± 7.7	93.2 ± 16.4
% change		+2%	+13%
Heart rate (bpm)	160 ± 29	181 ± 24	183 ± 40
% change		+13%	+14%
Blood gases‡			
P_{CO_2} (mm Hg)	31.8 ± 6.9	32.4 ± 6.0	33.7 ± 6.4
P_{O_2} (mm Hg)	211 ± 32	187 ± 23	196 ± 24
pH	7.33 ± 0.1	7.35 ± 0.1	7.32 ± 0.05
Velocity variables‡			
V_{max} (cm/s)	52.5 ± 10.1	70.9 ± 19.0	40.1 ± 14.1
V_{mn} (cm/s)	30.9 ± 8.8	41.6 ± 14.2	24.5 ± 8.4
V_{ed} (cm/s)	17.9 ± 6.0	21.5 ± 8.9	13.5 ± 5.2
RI	0.67 ± 0.07	0.71 ± 0.09	0.67 ± 0.05
PI	1.19 ± 0.32	1.31 ± 0.42	1.1 ± 0.17
S/D	3.19 ± 0.95	3.94 ± 1.65	3.07 ± 0.53
Systolic acceleration slope (cm/s^2)	548 ± 202	823 ± 195§	432 ± 137

* Values are mean ± SD.
† $p < 0.0001$, as compared with normal hematocrit group.
‡ The blood gas and velocity results were during baseline control ventilation. 1 mm Hg = 0.133 kPa.
§ $p < 0.001$ as compared with other two groups, analysis of variance with post hoc comparison.

Table 2. *Regression analyses: velocity data**

Dependent variables	Independent Variables: Pa_{CO_2} $\hat{\beta}$	Hematocrit $\hat{\beta}$	Interaction $\hat{\beta}$	Full model R^2
V_{max} (cm/s)	1.5†	−0.05	−0.02‡	0.74†
$SE_{\hat{\beta}}$	(0.35)	(0.32)	(0.008)	
V_{mn} (cm/s)	1.1†	0.15	−0.014§	0.75†
$SE_{\hat{\beta}}$	(0.25)	(0.22)	(0.006)	
V_{ed} (cm/s)	0.57†	0.06	− 0.005	0.78†
$SE_{\hat{\beta}}$	(0.16)	(0.14)	(0.004)	
Systolic acceleration slope (cm/s^2)	0.56	−12.7†	0.02	0.62†
$SE_{\hat{\beta}}$	(4.5)	(4.2)	(0.1)	
PI	−0.024‡	−0.022	0.0004	0.21‡
$SE_{\hat{\beta}}$	(0.14)	(0.012)	(0.0003)	
RI	−0.031†	−0.0036	0.00003	0.18‖
$SE_{\hat{\beta}}$	(0.0035)	(0.003)	(0.00008)	
S/D	−0.131‖	−0.13‡	−0.0024‖	0.25†
$SE_{\hat{\beta}}$	(0.034)	(0.05)	(0.0012)	

* $\hat{\beta}$ and their standard errors ($SE_{\hat{\beta}}$) are from the full regression models that included dummy variables.
† $p < 0.0001$.
‡ $p < 0.01$.
§ $p < 0.02$.
‖ $p < 0.05$.

values are equivalent to 21.8–27% velocity increase per kPa Pa_{CO_2} change.)

The effect of hematocrit per se was not statistically significant on velocity variables. However, Pa_{CO_2} and hematocrit interaction $\hat{\beta}$ were highly significant in the V_{max} and V_{mn} models, but not in the V_{ed} model. All interaction $\hat{\beta}$ values were negative, implying that the combined function of increasing Pa_{CO_2} and hematocrit had a negative influence on velocity changes. The interaction terms explained 26% variance in the V_{max}, 14% in the V_{mn}, and 4% (NS) in the V_{ed} models.

Hematocrit inversely affected systolic acceleration slope. The wave forms were steep followed by sudden downstroke in low

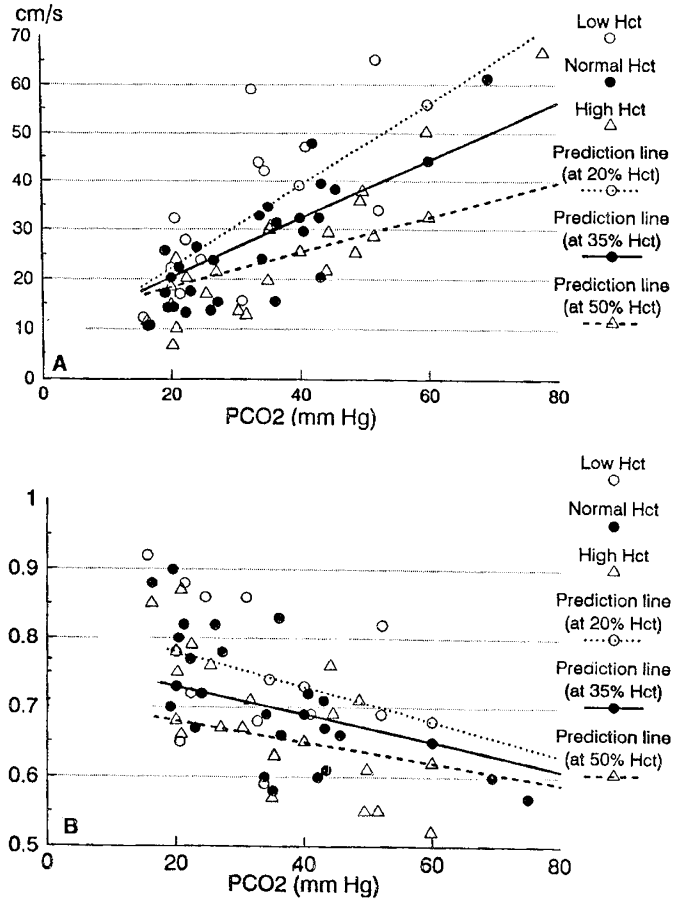

Fig. 1. Hematocrit effect on $Paco_2$ reactivity. *A*, temporal mean velocity: Velocity *vs* $Paco_2$ in three experimental groups is shown with symbols for low, normal, and high hematocrits (*Hct*) described in the key. Prediction lines were drawn using the multiple regression equations, including the interaction terms shown in Table 2. Calculations were done for three hematocrit and $Paco_2$ combinations: hematocrit values of 20, 35, and 50% and $Paco_2$ values of 20, 40, and 60 mm Hg. The prediction line representing $Paco_2$ response at 20% hematocrit is steeper as compared with prediction at 50% hematocrit, implying a strong interaction between changing hematocrit and $Paco_2$. *B*, RI: The values for the RI were computed from velocity values described in Materials and Methods. The three prediction lines were drawn from calculations similar to the temporal mean velocity (*A*). The change in RI is much greater for a given change in $Paco_2$ when the hematocrit is 20% than when it is 50%, as shown by nonparallel prediction lines at these hematocrits (1 mm Hg = 0.133 kPa).

hematocrit experiments, and smooth with rounded peaks in high hematocrit experiments. $Paco_2$ had no effect on this variable (Table 2).

The scatter plot in Figure 1*A* reveals the intricate relationships among the temporal mean velocity, $Paco_2$, and hematocrit-$Paco_2$ interaction. We plotted three prediction lines calculated using the V_{max} regression equation at 20, 35, and 50% hematocrits and at 20, 40, and 60 mm Hg $Paco_2$. V_{max} increased as a function of $Paco_2$ at all hematocrit levels; however, with increasing $Paco_2$ the prediction lines for 20 and 50% hematocrit diverge from each other, suggesting a strong inverse influence of hematocrit and Pco_2 combination on cerebral velocity. This implied that, as compared with baseline, the $Paco_2$-induced velocity increase would be much less at high hematocrit and much greater at lower hematocrit. A similar trend was noted in the V_{mn} and V_{ed} models (not shown).

Resistance measures. All models were statistically significant (Table 2). In Figure 1*B*, the prediction lines for RI drawn at various hematocrit values were calculated as in Figure 1*A*; these lines also reveal the inverse effect of high hematocrit on $Paco_2$-induced changes in RI. Diagrams for the PI and S/D were also similar (not shown).

Hematocrit effect on various segments of Doppler velocity wave forms. To evaluate the effect of hematocrit on various segments of Doppler-derived velocity wave form, we utilized the regression equations and computed velocities at various hematocrit and 40 mm Hg $Paco_2$. An increase in hematocrit to 50% (from 35%) would decrease V_{max} by 21.9%, V_{mn} by 17.8%, and V_{ed} only by 10%. Because the same regression equation was used, not surprisingly, a converse relationship of similar magnitude was seen when calculations were done for 20% hematocrit.

Other variables. Multiple regression models including heart rate, systolic and diastolic times, mean blood pressure, acceleration and deceleration times, and Pao_2 revealed no statistically significant effect of any of these variables on either the velocity or the computed resistance measures.

Model validity. None of the dummy variables was statistically significant. The correlation matrices of the residuals for each baboon showed no intuitively obvious pattern, and the plots of the residuals failed to show heteroscedasticity.

DISCUSSION

The main conclusion from our study was that baseline hematocrit should be included in the list of variables affecting cerebral vascular reactivity to Pco_2. Increasing hematocrit attenuates $Paco_2$-induced velocity increase, whereas decreasing hematocrit accentuates the same. This is indicated by a statistically significant inverse influence (negative $\hat{\beta}$) of hematocrit-$Paco_2$ interaction term (product) in the V_{max} and V_{mn} models, which means that the combined effect of these two variables was larger than the sum of their individual effect. This is also illustrated in Figure 1*A*, which was drawn by incorporating three hematocrit and $Paco_2$ values into the V_{max} regression model equation. The CBF velocity prediction lines for 20 and 50% hematocrit diverge from each other, such that for a given change in $Paco_2$, the velocity changes at 20% hematocrit would be much greater than at 50% hematocrit. If hematocrit affected velocity alone without influencing $Paco_2$ reactivity (nonsignificant interaction term), the prediction lines plotted in Figure 1*A* would remain parallel.

Three explanations can be provided to interpret our findings: *1*) the effect of hematocrit on local vessel caliber; *2*) Poiseuille's flow model and changes in kinetic energy due to hematocrit alterations; and *3*) changes in CO_2 buffering capacity due to altered red cell mass.

Variation in local viscosity and shear stresses on the vascular endothelium are believed to control the vessel caliber (25–28). Melkumyants *et al.* (25) showed that increasing viscosity of the perfused blood by hemoconcentration (69% hematocrit) caused a substantial increase in cat femoral artery diameter, whereas decreasing the viscosity by hemodilution (21% hematocrit) had an opposite effect of lesser magnitude. In both conditions, a new steady state vessel caliber was reached within minutes of viscosity changes. The diameter changes could be compensated by flow rate variations, such that the shear stress on the endothelium remained constant. This property, perhaps mediated by endothelium-derived relaxing factors, was unique to only an intact endothelium (25, 27). These and other reports (26, 28) suggest that the arterial wall responds to a physical value proportional to the product of bulk viscosity and flow rate and reacts to changes in local shear stresses by altering smooth muscle tone. Dintenfass (20) postulates that under constant physiologic conditions the bulk viscosity is a principal factor governing the caliber of large vessels. These studies suggest that the altered hematocrit in our experiments affected principally the large vessel caliber, influencing $Paco_2$ reactivity. From hemodynamic calculations, it can be shown that when the baseline vessel caliber is increased in the presence of a relatively constant pump force (heart rate and blood pressure) $Paco_2$-induced peripheral vaso-

dilation produces attenuated velocity increases, as compared with the baseline. This could have occurred in our high hematocrit experiments, whereas a converse phenomenon occurred in low hematocrit experiments.

Our findings can be explained also by adopting Poiseuille's steady state flow model (29). By simplifying the model, it can be shown that the volume flow rate is proportional to the ratio of the 4th power of the tube radius and viscosity (Appendix). When both viscosity and diameter increase, the increase in volume flow rate will be much less than when only the diameter increases. An example of this phenomenon is shown in the Appendix.

We found the systolic velocity acceleration to be inversely influenced by hematocrit (Tables 1 and 2). Marked increase in velocity acceleration has been reported in the umbilical circulation of human fetuses affected by severe anemia (30). To explain this phenomenon, we could use fundamental physical quantities such as force, length, mass, and time and apply them in simple mathematical analysis (dimensional analysis). It can then be shown that velocity acceleration is a function of inverse viscosity; the lower the viscosity, the higher the systolic velocity acceleration slope would be (Appendix). This observation, coupled with a higher V_{mn} in anemia as compared with polycythemia at any $Paco_2$ level, suggests that the rate of kinetic energy change was greater when the hematocrit was low (and lesser when the hematocrit was high), providing a hemodynamic explanation for the differences in $Paco_2$ reactivity noted by us. Although the whole blood viscosity dropped after plasma exchange, the drop was not large, perhaps because of a higher fibrinogen content of the maternal plasma (31).

After plasma exchange, because of reduced red cell mass, the CO_2 buffering capacity is also reduced. Therefore, a greater fraction of CO_2 is dissolved in the plasma at any given $Paco_2$ (32), which could also explain an increased $Paco_2$ reactivity in anemia (and a converse phenomenon in polycythemia). In contrast to the experiments of Melkumyants *et al.* (25, 28), Hudak *et al.* (33) reported that the cerebral vessels dilate in anemia. It is difficult to see how vessel diameter could be measured accurately using color-flow Doppler signals, inasmuch as the signals are influenced by velocity, flow/Doppler incident angle, and other factors (34). However, Hudak *et al.* did not provide the details of their technique in their abstract.

Sola *et al.* (35) noted that 4.5% CO_2 inhalation preserved cerebral and coronary flow in animals previously subjected to acute hemorrhage without replacement of shed blood; this occurred in part due to an increase in systemic blood pressure. However, the animals in our study were not hypovolemic and did not have hypotension or bradycardia during hypercarbia experiments. Therefore, we believe that systemic effects due to CO_2 inhalation in our study, if any, were minimal.

Despite our attempts, hyperoxia occurred during CO_2 experiments. Because Hb is 98% saturated at 100 mm Hg Po_2 and the dissolved fraction of oxygen increases by 0.3 mL/100 mm Hg Po_2 (32), we could estimate that at the levels of hyperoxia noted in our study (about 200 mm Hg Po_2, Table 1) the Cao_2 increase would be approximately 0.5 mL/100 mL. Such an increase in oxygen content is unlikely to have affected the CBF. However, there are no studies comparing the effects of Cao_2 values of over 20 mL/100 mL on CBF. In our study, the Po_2 values at various levels of hematocrit were comparable, and in the regression models we failed to demonstrate a significant Pao_2 effect. Similarly, by exchanging neonatal red cells with those of the mother, the Hb-O_2 affinity would be reduced due to an increase in adult Hb. However, we believe that the resultant increase in P_{50} (Po_2 at 50% Hb saturation) would have little effect on our findings because the Pao_2 values in our studies were always above 90 mm Hg and the differences in Hb-O_2 affinity would be minimal at the flat portion of the oxygen saturation curve (16, 32).

Although previous experiments have validated Doppler methods (3, 36–38), we hasten to add that at their best Doppler studies reflect qualitative circulatory changes, providing approximate estimates of the direction of change (37). Because even large arteries could be controlling changes in CBF (3, 39), direct extrapolation of the numeric findings from Doppler studies could be risky (37). Three main Doppler-derived ratios have been used to assess peripheral vascular resistance in fetal and neonatal circulation (9, 30, 40, 41) and to measure neonatal cerebral $Paco_2$ reactivity (1). All three ratios—RI, PI, and S/D—are calculated by obtaining values for different segments of the Doppler velocity wave forms. We found that the variations in baseline hematocrit have dissimilar effects on various segments of Doppler wave forms. Hematocrit most affected V_{max} and least affected V_{ed}. Therefore, to avoid error, intrasubject variations in hematocrit must be evaluated (or statistically controlled for) while interpreting changes in resistance ratios.

The extent to which the $Paco_2$ reactivity results in our baboon model are relevant to humans needs to be shown. Pryds *et al.* (14) found a mean 32.6% change in CBF per kPa CO_2 in premature babies, and Archer *et al.* (1) reported a 5.9 to 7% change in velocity for each mm Hg $Paco_2$ change (or 44–52% per kPa) in preterm infants. In newborn lambs, Sonesson and Herin (3) noted a 27% change in flow per kPa change in $Paco_2$. The $Paco_2$ reactivity values of 21.8–27% per kPa $Paco_2$ in our study compare well with the two above reports (3, 14).

Hematocrit-dependent attenuation and accentuation of $Paco_2$ reactivity, if present in human infants, could be clinically important. Anemic or polycythemic infants with respiratory distress, intentional hyperventilation for persistent pulmonary hypertension, and low hematocrit and $Paco_2$ levels maintained during neonatal open-heart surgery are some, albeit rare, clinical situations where extremes of hematocrit and $Paco_2$ combinations could occur.

In summary, we suggest that in the newborn primate the baseline hematocrit (and therefore viscosity) strongly influences cerebral vascular $Paco_2$ reactivity; the mechanisms involved in such a process are subject to speculation. The findings could be of importance in clinical situations where hematocrit and $Paco_2$ are likely to change simultaneously. To avoid errors in the Doppler-derived values for $Paco_2$ reactivity, velocity, and resistances indices, hematocrit changes should be included in the analysis.

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APPENDIX

I. According to the Poiseuille's steady state flow model (29), the volume flow rate ($\dot{Q}$) can be expressed as:

$$\dot{Q} = K \times \frac{\Delta P \times R^4}{L \times \eta} \quad (1)$$

where ΔP = pressure drop, L = tube length, R = tube radius, η = viscosity, and K = the numerical constant $\frac{\pi}{8}$ (0.393).

At a constant pressure gradient (ΔP) and tube length (L), the variables governing $\dot{Q}$ are, therefore, tube radius (R) and viscosity (η); thus, equation 1 can be simplified as:

$$\dot{Q} = C \times \frac{R^4}{\eta} \quad (2)$$

where C is the new constant ($K \times \frac{\Delta P}{L}$). Using the above equation, we calculated volume flow rates when three different sets of changes occur in viscosity and diameter. The results are shown in the table below.

% Change in volume flow rate

	Diameter change				
	0	+10%	+20%	+30%	+40%
Normal viscosity	0	46	107	186	284
20% higher viscosity as compared with normal	−17	22	73	138	220
20% lower viscosity as compared with normal	25	83	159	257	380

For a given diameter change, the % change in $\dot{Q}$ would be lower when the viscosity is high (*e.g.* polycythemia) than when the viscosity is low (anemia).

II. Dimensional analysis: Acceleration (Acc) can be expressed as:

$$Acc = f(\eta, \Delta P, D) \quad (1)$$

where η = viscosity, ΔP = pressure gradient, and D = tube diameter. With the assumption that acceleration is a single function of these variables, the equation can be written as:

$$Acc = K \times \eta^a \times \Delta P^b \times D^c \quad (2)$$

where a, b, and c are the exponents to which these variables must be raised and K is the dimensionless constant. By the known relationship of acceleration to mass, length, and time (physical principles), we can demonstrate that the exponent $a = -2$, $b = 2$, and $c = 3$. Equation 2 can then be written as:

$$Acc = K \times (\eta^{-2} \times \Delta P^2 \times D^3) \quad (3)$$

rearranging:

$$Acc = K \times \frac{\Delta P^2 \times D^3}{\eta^2} \quad (4)$$

Accordingly, if the pressure gradient ΔP and tube diameters D are constant, acceleration would be inversely related to the squared value of viscosity; if viscosity decreases (as in anemia), one would expect acceleration to increase, and a converse phenomenon to occur if viscosity increases (as in polycythemia).