

with exogenous surfactant, the premature rabbit lung can be ventilated adequately with HFO at a comparatively low mean airway pressure, and that at these particular experimental conditions surfactant replacement effectively prevents the development of epithelial lesions.

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Rheology of Fetal and Maternal Blood

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ABSTRACT. Rheological parameters were measured in 10 pairs of mothers and newborns. Whole blood viscosity was similar despite a higher fetal hematocrit (47.0 ± 5.1 versus $35.5 \pm 12.0\%$, mean \pm SD, $p < 0.05$). When the hematocrit of the suspension of red cells in plasma was adjusted to 45%, the viscosity was significantly lower in the fetal blood over a wide range of shear rates (0.52 – 208 s^{-1}). The main reason for the lower viscosity in the fetal blood was the lower plasma viscosity as compared to the maternal blood (1.08 ± 0.05 versus 1.37 ± 0.08 centipoise, $p < 0.05$); this in turn was attributable to a lower total plasma protein concentration (4.74 ± 0.71 versus 6.47 ± 0.64 g/dl, $p < 0.05$). All protein fractions were lower in the fetal plasma. The assessment of red cell deformability by filtration through polycarbonate sieves revealed that the resistance of a fetal red cell was three times higher than that of a maternal red cell in a 2.6 - μ m pore, but there was no significant difference in resistance for these red cells in 6.9 - μ m pores. This higher filtration resistance of fetal red

cells through the small pores was mainly due to their large volume (115.4 ± 10.8 versus 93.5 ± 5.9 fl, $p < 0.001$). Measurements on membrane-free hemoglobin solutions indicated that the internal viscosity of these two types of red cells was not different. We conclude that in fetal blood the higher hematocrit and the presence of larger red cells, which cause impaired passage through pores < 5 μ m, are counterbalanced by a decreased plasma viscosity, resulting in a whole blood viscosity comparable to that of adults. (*Pediatr Res* 19:147-153, 1985)

Abbreviations

Hct, hematocrit
 RBC, red blood cell
 WBC, white blood cell
 MCV, mean corpuscular volume
 MCH, mean corpuscular hemoglobin
 MCHC, mean corpuscular hemoglobin concentration
 cP, centipoise

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Hyperviscosity syndrome in neonates is a serious condition, which may cause congestive heart failure, respiratory distress, and cyanosis (13, 24), cerebral infarction (2) and necrotizing

enterocolitis (15). It occurs in approximately 5% of all newborn infants (33). The etiology seems to be multifactorial (24), with polycythemia as a major factor (21, 24, 33).

It has been claimed that newborns have a higher whole blood viscosity than adults (3, 11, 21). Recently, however, Riopel *et al.* (31) found a lower blood viscosity in newborns. These findings prompted us to study the rheological properties of fetal and maternal blood in detail.

MATERIALS AND METHODS

Ten pairs of mothers and their newborn infants were studied. Informed consent was obtained from the mother. All pregnancies were at full gestational term, and elective cesarean section was performed. Prior to anesthesia (general or epidural), 30 ml of the maternal blood was drawn from an antecubital vein into Vacutainers containing heparin. Cord blood was obtained within 5 min after removal of the placenta from the uterus by drawing 30 ml from a clamped umbilical vein into Vacutainers containing heparin.

The Hct was measured in duplicate by the microhematocrit method. RBC and WBC counts were performed in a Coulter Counter (Coulter Electronics, Hialeah, FL). The Hb concentration was determined by the cyanmethemoglobin method, measuring the light absorption at a wavelength of 540 nm. The MCV, MCH, and MCHC were calculated from Hct, RBC count, and Hb concentration. The total plasma protein concentration was measured by using a refractometer (25). Plasma protein fractions were analyzed by means of electrophoresis. Plasma fibrinogen concentration was determined by the method of Ratnoff and Menzie (28).

The morphology of fetal and maternal red cells was studied in the last four pairs. Fresh samples were fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.4, 4° C) and postfixed in 2% OsO₄. Specimens for scanning electron microscopy (JSM-25, JEOL Corp., Peabody, MA) were dehydrated in ascending ethanol series, air-dried, and coated with gold-palladium. For transmission electron microscopy (model EM 9, Carl Zeiss, West Germany), the specimens were embedded in Epon. Thin sections were prepared and stained with 2% uranyl acetate and lead citrate. Some specimens were postfixed, prior to embedding, in OsO₄ containing 0.1% ruthenium red as an extracellular marker. Measurements on paper prints of electron micrographs were made with the aid of a magnifying precision scale (Scale Lupe, Peak, Brookstone Comp., Peterborough, NH).

Viscosity measurements were made with a Couette viscometer of high precision (7) at 37° C. It consists of two concentric cylinders separated by a uniform annular gap of 960 μm, which contains the sample. The inner cylinder can be rotated at constant speeds, with corresponding shear rates of 0.05–208 s⁻¹. The torque transmitted through the sample to the outer cylinder is balanced by a magnetic counter-torque through an electronic feedback system. The viscosity is calculated from the rotational speed, the torque value, and a geometrical conversion factor. For the viscosity measurement of suspensions of RBCs in plasma at a hematocrit of 45% (η_{P45}), whole blood was centrifuged for 5 min at 1500 × *g*, the buffy coat was removed, and the packed RBCs were mixed with the appropriate amount of plasma. For the determination of the viscosity of suspensions of RBCs in Ringer's solution at a hematocrit of 45% (η_{R45}), the red cells were washed 3 times in Ringer's solution containing 145 mM NaCl, 4 mM KCl, 2.2 mM CaCl₂, 5.5 mM dextrose, and 0.5 g/dl bovine albumin, adjusted to pH 7.4 with 0.1 N Tris buffer. The Ringer's solution had been prefiltered through 0.2-μm Millipore filters.

Red cell deformability was measured with a microsieving method. Red cells were washed three times in the Ringer's solution (see above). The washed RBCs were resuspended in the Ringer's solution to a hematocrit of 10.0%. Red cell filtration was performed at 37° C. The RBC suspension was pumped at a

constant flow rate of 0.82 ml/min through polycarbonate filters (Nuclepore Corp., Pleasanton, CA) with different pore sizes (nominal diameters of 3, 5, and 8 μm). The mean pore diameter and the pore density, as determined by scanning electron microscopy, were respectively 2.6 ± 0.2 μm and 23.6 × 10³/mm² for nominal 3-μm filters, 4.5 ± 0.6 μm and 4 × 10³/mm² for nominal 5-μm filters, and 6.9 ± 0.8 μm and 1 × 10³/mm² for nominal 8-μm filters. The same batch of filters was used throughout the study. The filtration pressure (*P*) was recorded as a function of time (*t*) using a pressure transducer (model MP 45-14, Validyne Engineering Corp., Northridge, CA). Before the measurement of a RBC suspension, Ringer's solution was pumped through the filter and the pressure recorded (*P*₀). Filtration of a RBC suspension gave an initial pressure reading (*P*_i), followed by a further rise in pressure with time. The ratio *P*_i/*P*₀ was calculated; it reflects the relative filtration resistance of the RBC suspension. The relative resistance of an individual RBC (*β*), *i.e.* the ratio of the resistance in a pore bearing a red cell to that in a pore filled with Ringer's solution alone, was computed (34):

$$\beta = \left[\frac{P_i}{P_0} - 1 \right] \frac{V}{h} + 1$$

where *V* is the ratio of RBC volume to the pore volume and *h* is the fractional volume of RBCs in suspension. The rising pressure after *P*_i reflects plugging of pores by cells (either RBCs or WBCs), which cannot pass the filter. It occurs regularly with 2.6-μm pores and only occasionally with 6.9-μm pores. The slope of the curve, corrected for *P*₀, was calculated as a measure of plugging (*K*_i, Ref. 10).

For the assessment of the internal viscosity (η_i) of RBCs, membrane-free hemoglobin solutions were prepared. The RBCs were washed three times with Ringer's solution (see above) and centrifuged at 1,500 × *g* for 10 min. The supernatant was removed and the packed red cells were lysed with toluene under vigorous stirring for 10 min. The suspension was transferred to cellulose nitrate centrifuge tubes and centrifuged at 30,000 rpm for 30 min. After the removal of the toluene, the solutions were frozen at -20° C. In the frozen state, the layer with membrane debris overlaying the hemoglobin solution was cut off. The hemoglobin solution was then thawed at room temperature. The hemoglobin concentration of the melted solution was measured (see above), adjusted to 31 g/dl by adding the appropriate amount of H₂O, and kept air-tight in a syringe. The viscosity of the hemoglobin solutions was measured in a Weissenberg rheogoniometer with a cone-plate geometry at 25° C (9). The rheogoniometer was preferred over the Couette viscometer because it allowed measurements with smaller samples (about 3 ml).

For statistical analysis, Student's *t* test for paired data was used. The results on plasma viscosity and proteins were analyzed with a one-way analysis of variance.

RESULTS

The hematological parameters of the mothers and their newborn infants are given in Table 1. Newborns had higher hematocrit, hemoglobin concentration, and white cell count, whereas the red cell count was not different from that of the mothers. The red cell indices revealed larger values for the MCV and MCH in newborns, but their MCHC was slightly lower.

The apparent viscosity of whole blood is shown in Figure 1. Although the newborns had a much higher hematocrit, the viscosity of their whole blood was very similar to that of the mothers at shear rates above 5 s⁻¹. At lower shear rates, the viscosity of the fetal blood tended to be lower, although the difference was not statistically significant. When the hematocrit was adjusted to 45% (Fig. 2), the fetal blood was significantly less viscous over a range of shear rate from 0.52 to 208 s⁻¹. The

Table 1. Hematological values (mean ± SD, n = 10)

	Mothers	Newborns
Hematocrit (%)	35.5 ± 12.0	47.0 ± 5.1*
Red cell count (10 ⁶ /μl)	4.06 ± 0.39	4.07 ± 0.40
Hemoglobin (g/dl)	12.8 ± 1.6	15.0 ± 1.8*
Mean corpuscular volume (fl)	93.2 ± 5.9	115.4 ± 10.8†
Mean corpuscular hemoglobin (pg)	31.7 ± 2.8	35.4 ± 1.0*
Mean corpuscular hemoglobin concentration (g/dl)	33.3 ± 1.0	31.9 ± 0.6†
White cell count (10 ³ /μl)	8.43 ± 2.91	14.94 ± 4.83†

* p < 0.05.
† p < 0.001.

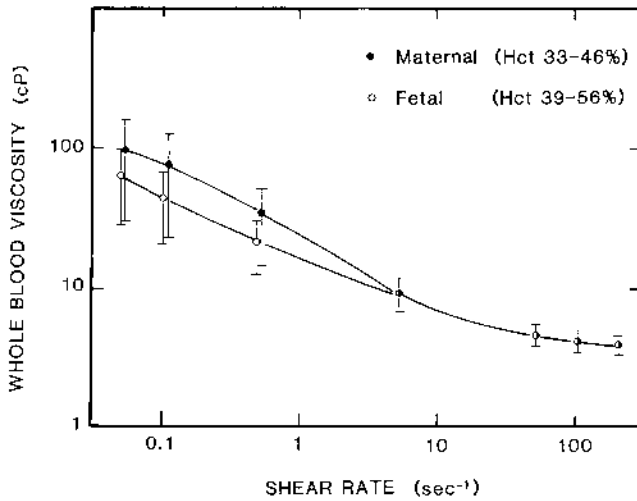


Fig. 1. Log-log plot of the viscosity of whole blood (ordinate, values in centipoise, mean ± SD) from 10 mothers and their newborns versus shear rate (abscissa). Measurements were made with a Couette viscometer at 37° C.

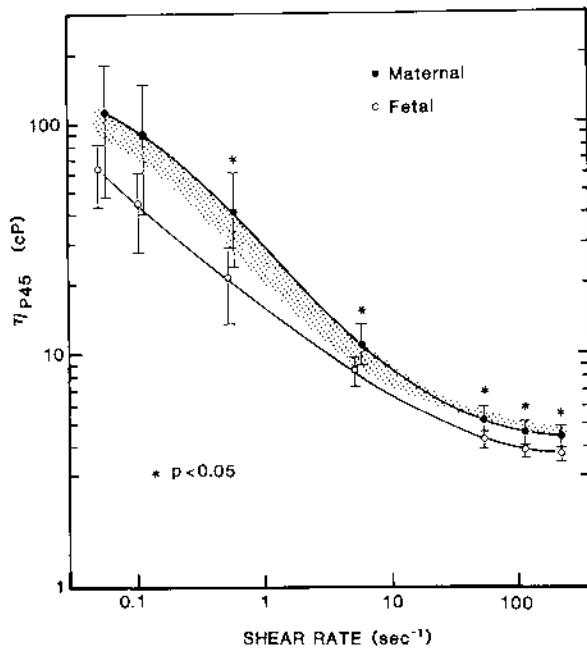


Fig. 2. Log-log plot of the viscosity of maternal and fetal red cells in plasma at 45% hematocrit (η_{R45} on ordinate, mean ± SD, n = 10) versus the shear rate (abscissa). Measurements were made with a Couette viscometer at 37° C. The shaded area represents the normal range of nonpregnant females (n = 10, mean ± SD).

viscosity of the maternal blood fell in the range obtained for normal, nonpregnant females.

When washed red cells were resuspended in Ringer's solution at a hematocrit of 45%, the viscosity was much lower than η_{R45} (Fig. 3). At shear rates above 100 s⁻¹, the viscosity of fetal RBCs in Ringer's solution remained lower than that of maternal RBCs (p < 0.05), whereas at shear rates below 10 s⁻¹ the suspension of fetal RBCs showed a tendency towards higher values than maternal RBCs.

The plasma viscosity is given in Table 2. It is significantly lower for the newborns when compared to their mothers or nonpregnant females. Plasma viscosity correlated best with the total plasma protein concentration (Fig. 4). The individual protein fractions also correlated with the plasma viscosity. The strongest correlation was found for α₂-globulins (r = 0.830, p < 0.001), α₁-globulins (r = 0.804, p < 0.001) and fibrinogen (r = 0.758, p < 0.001), with the weakest one for albumin (r = 0.622, p < 0.005) and γ-globulins (r = 0.594, p < 0.01). Compared to nonpregnant controls, the mothers had a slightly higher plasma viscosity, which could be due to the higher α-globulin concentrations.

The relative filtration pressures of RBC suspensions (P_i/P_o) were comparable for maternal and fetal RBCs through 6.9-μm pores (1.96 ± 0.42 and 1.88 ± 0.38, respectively) and 4.5-μm pores (2.03 ± 0.23 and 2.10 ± 0.38, respectively). Filtration through the 2.6-μm pores gave a much higher P_i/P_o for fetal RBCs (62.4 ± 13.8) compared to maternal RBCs (25.2 ± 15.9, p < 0.001). For 2.6-μm pore filters, a positive correlation between

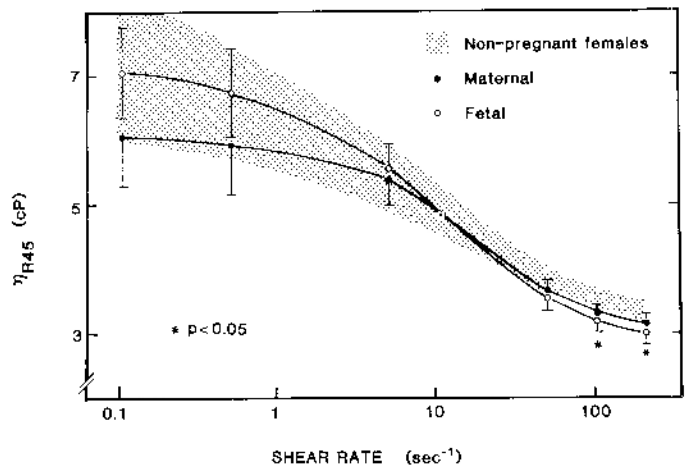


Fig. 3. Semilogarithmic plot of the viscosity of red cells in Ringer's solution at 45% hematocrit (η_{R45} on ordinate, mean ± SD, n = 10) versus the shear rate (abscissa). Measurements were made with a Couette viscometer at 37° C. The shaded area represents the normal range for nonpregnant females (n = 15, mean ± SD).

Table 2. Plasma viscosity and proteins (mean ± SD, n = 10)

	Nonpregnant females	Mothers	Newborns
Plasma viscosity (cP)	1.30 ± 0.04*	1.37 ± 0.08	1.08 ± 0.05*
Total plasma protein (g/dl)	7.21 ± 0.34*	6.47 ± 0.64	4.74 ± 0.71*
Fractions (g/dl)			
Albumin	4.28 ± 0.70*	3.49 ± 0.46	3.00 ± 0.54
α ₁ -Globulin	0.21 ± 0.04*	0.31 ± 0.09	0.18 ± 0.06*
α ₂ -Globulin	0.66 ± 0.13*	0.80 ± 0.18	0.34 ± 0.10*
β ₁ -Globulin	0.66 ± 0.18	0.69 ± 0.25	0.29 ± 0.10*
β ₂ -Globulin	0.39 ± 0.23*	0.24 ± 0.10	0.08 ± 0.04*
γ-Globulin	0.67 ± 0.25	0.72 ± 0.18	0.55 ± 0.16
Fibrinogen	0.33 ± 0.05	0.32 ± 0.07	0.21 ± 0.04*

* p < 0.05 compared to maternal values (one-way analysis of variance).

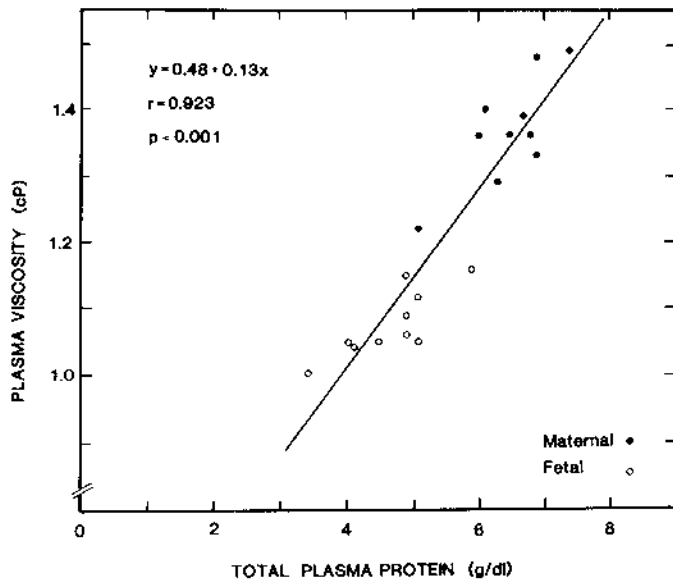


Fig. 4. Plasma viscosity (*ordinate*) plotted against total plasma protein concentration (*abscissa*) for maternal and fetal blood.

P_i/P_0 and MCV could be established (Fig. 5). The regression line yielded $y = 2.22x - 181.88$ ($r = 0.909$, $p < 0.001$).

The computed relative resistance in a pore bearing a red cell to that in a pore with Ringer's solution alone (β) was not different for large pores ($6.9 \mu\text{m}$), slightly elevated with the intermediate pore size ($4.5 \mu\text{m}$), and was over 3 times higher for the small $2.6 \mu\text{m}$ pores (Fig. 6). Although β was much higher, fetal RBCs did not plug filter pores, for all sizes studied, to a greater extent than maternal RBCs. This was documented by the lack of any statistically significant difference in the slope of the pressure-time curve (K_1). The K_1 values for maternal and fetal RBCs, respectively, were 34.1 ± 13.1 and $29.5 \pm 12.6 \text{ min}^{-1}$ for $2.6 \mu\text{m}$ pores, 1.5 ± 1.7 and $2.1 \pm 1.5 \text{ min}^{-1}$ for $4.5 \mu\text{m}$ pores, and 0.52 ± 0.60 and $0.55 \pm 0.44 \text{ min}^{-1}$ for $6.9 \mu\text{m}$ pores.

The viscosity of hemoglobin solutions at a concentration of 31 g/dl at 25°C and a shear rate of 540 s^{-1} was $8.5 \pm 3.8 \text{ cP}$ for the mothers and $8.8 \pm 1.4 \text{ cP}$ for the newborns. The amount of fetal hemoglobin was determined in three newborns; it was $74.8 \pm 5.8\%$ of the total hemoglobin.

The morphology of fetal red cells showed some characteristic features. On the cell surface, crater-like membrane invaginations (pits) could be found (Fig. 7). These cells have been previously described as "pocked" erythrocytes of the neonates (16). The interior of the cells also showed abnormalities. A large amount of intracytoplasmic vacuoles was seen (Fig. 8, *top*). In the four pairs of samples studied, such vacuoles were found in $37.3 \pm 1.8\%$ of the fetal RBCs and $19.6 \pm 5.4\%$ of the maternal RBCs ($n = 150$ cells for each subject, $p < 0.005$). Specimens postfixed with ruthenium red as an extracellular marker showed that only about 1% of these vacuoles have a communication with the cell surface (vacuoles filled with ruthenium red). There were also longitudinal, slit-like intracytoplasmic inclusions, which were generally parallel and close to the cell surface (Fig. 8, *middle*), but were ruthenium red negative. These membrane-coated structures were found more frequently in fetal than maternal red cells. Remnants of mitochondria were recognized by the presence of cristae (Fig. 8, *bottom*).

DISCUSSION

In this study, newborn cord blood viscosity and its major determinants, *i.e.* hematocrit, plasma viscosity, red cell deformation, and red cell aggregation (8), were investigated in a systematic manner. The results indicate that the cord blood of the newborn has a whole blood viscosity which is not higher than that of the simultaneously obtained maternal blood. This is

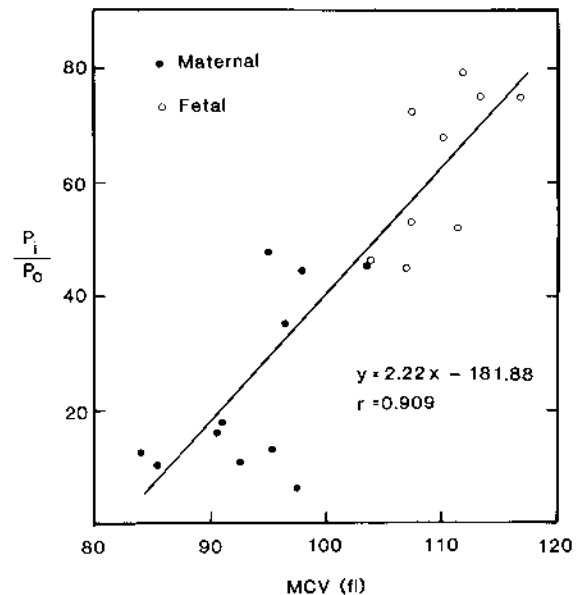


Fig. 5. The relationship between the ratio P_i/P_0 for Nuclepore filters with a mean pore diameter of $2.6 \mu\text{m}$ (*ordinate*) and MCV (*abscissa*). P_i is the initial filtration pressure generated by pumping a red cell suspension through a filter; P_0 is the corresponding value obtained for Ringer's solution alone. The ratio P_i/P_0 is an expression of the resistance to filtration of the red cells present in the suspension. A positive correlation between the two variables can be seen.

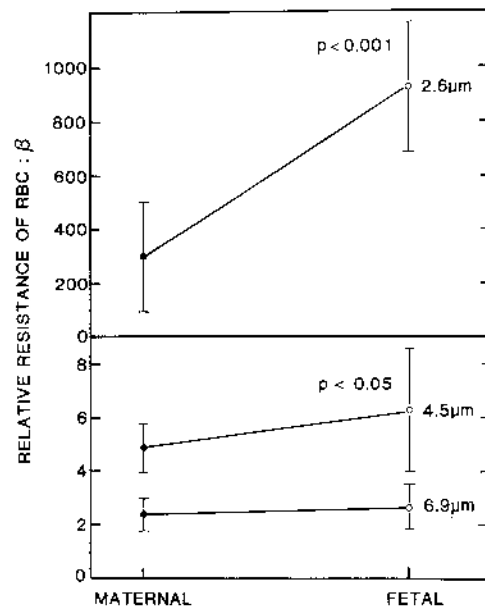


Fig. 6. Relative resistance in a pore containing a red cell to that in a pore with suspending medium alone, β , for maternal and fetal red cells (mean \pm SD, $n = 10$). Red cell filtration was performed with filter pores of 2.6 , 4.5 , and $6.9 \mu\text{m}$ at a constant flow rate of 0.82 ml/min .

surprising, since the hematocrit in the fetal blood (47.0%) was considerably higher than that in the maternal blood (35.5%). The fetal hematocrit found here is in agreement with other studies on cord blood (5, 23). When fetal and maternal blood samples were compared at the same hematocrit (η_{P45}), the viscosity of fetal blood became significantly lower, by about 50% at shear rates below 1 s^{-1} and 20% at shear rates above 50 s^{-1} .

One reason for the lower viscosity of fetal blood at the same hematocrit is the lower plasma viscosity. Plasma viscosity is mainly determined by plasma proteins. Fibrinogen is a major

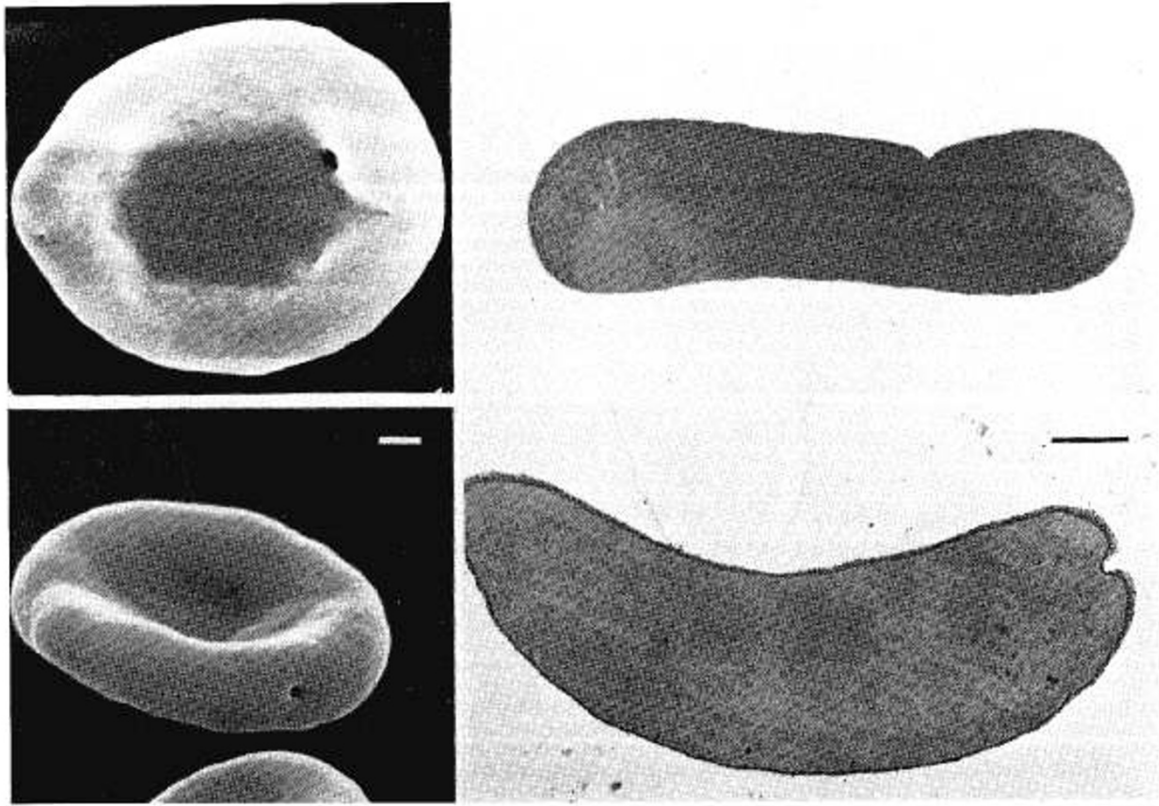


Fig. 7. Scanning electron microscopy (*left*, $\times 6,800$) and transmission electron microscopy pictures (*right*, $\times 12,900$) of so-called "pocked" RBCs of the newborn. The invaginations can be located in the dimple area (*top*) or on the edge (*bottom*) of the cell. The *bars* represent $1 \mu m$.

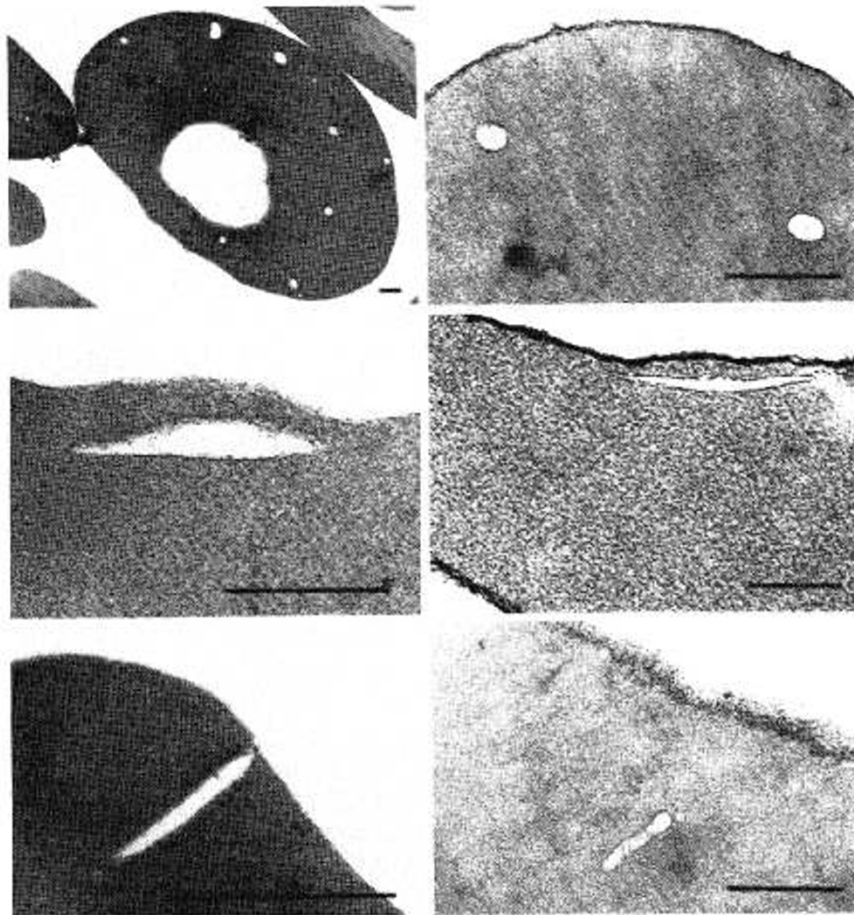


Fig. 8. Transmission electron microscopy pictures (between $\times 5,700$ and $60,000$) showing ultrastructural details of fetal red cells. Cytoplasmic vacuoles, often multiple (*top*), longitudinal structures parallel to the cell surface (*middle*), and elongated remnants of mitochondria containing cristae (*bottom*) can be seen. The samples on the *right* were postfixed with 0.1% ruthenium red as an extracellular marker, illustrating that these structures are not in connection with the cell surface. The *bars* represent $0.5 \mu m$.

determinant of plasma viscosity, and newborns clearly had lower fibrinogen levels. The fibrinogen concentration in mothers are lower than those reported in the literature (22, 27). Fibrinogen levels can fluctuate considerably during delivery within minutes (27), which may explain the difference between our study and those reports. Fibrinogen is involved in red cell aggregation, which occurs mainly at low shear rates (8). The low fibrinogen concentrations in newborns thus explain their lower blood viscosity at low shear rates. The facts that all plasma protein fractions correlate with plasma viscosity and that the total protein concentration gives the best correlation indicate that several fractions can contribute to the plasma viscosity.

When the plasma proteins were removed and washed red cells were suspended in Ringer's solution (η_{RAS}), the low viscosity of fetal blood at low shear rates was abolished or even partially reversed. At high shear rates, η_{RAS} was lower in the newborns. One reason for that could be the slightly lower MCHC of fetal RBCs, since the hemoglobin concentration is a major determinant of the intracellular viscosity, which may play an important role in determining blood viscosity at high shear rates (8). The viscosity of hemoglobin solutions at the same concentration, however, was not significantly different for mothers and newborns. In agreement with the literature (31), we found that 75% of hemoglobin in full term newborns is fetal hemoglobin, which has two γ -chains instead of two β -chains. This difference does not seem to influence the hemoglobin viscosity to a noticeable degree. Studies on the viscosity of fetal hemoglobin have not been reported to our knowledge.

Data on blood viscosity in newborns are controversial (3, 11, 21, 31). Our results support the very recent discovery of Riopel *et al.* (31) that, under normal conditions, blood viscosity is lower in the neonatal period because of a lower plasma viscosity. This has now been confirmed also for the newborn lamb and adult sheep (32). Earlier reports described an increased whole blood viscosity in normal newborns (3, 11, 21). Those studies were done with a Wells-Brookfield viscometer, which has a different geometry than the viscometer used in this study. The fetal hematocrit was higher in all those studies, which might be due to the fact that blood was drawn from the placenta after delivery (3, 11) or from the newborn after delivery (21), where hematocrit is regularly about 60% (3, 23). It is likely that the higher hematocrit accounts for the difference in whole blood viscosity between these earlier reports and the present data. In these earlier studies, other determinants of whole blood viscosity were either not measured (red cell deformability) or were similar to our data (plasma viscosity and red cell aggregation; Ref. 11), and there were no comparisons of the viscosity of red cell suspensions in plasma and Ringer's solution at the same hematocrit between mothers and newborns.

A decreased deformability of fetal erythrocytes has been reported (14) by using nominal 3- μ m pore filters and a very high constant filtration pressure (60 mm Hg). We extended such investigations to different sizes of filter pores. The resistance to passage through the narrow pores (2.6 μ m) is significantly higher for fetal RBCs, is nearly the same through 4.5- μ m pores, and is not different through 6.9- μ m pores. Narrow pores are more sensitive to changes in cell volume, while large pores are more sensitive to changes in internal viscosity (29). Our results therefore suggest that the decreased deformability of fetal red cells is mainly due to their increased volume. We found a 24% increase in volume, which is in agreement with Linderkamp *et al.* (20). The minimum cylindrical volume of fetal red cells, *i.e.* the thinnest cylinder through which a red cell can pass, was reported to be 8% higher than for maternal RBCs (20), others found it to be normal (6). Recently, Linderkamp *et al.* (19) measured the deformability of neonatal red cells with a rheoscope, where the cell volume or geometry is not important. They found no difference in the deformability of fetal and maternal RBCs, which agrees well with our data on 6.9- μ m pores, where the influence of the cell geometry is minimal (29). The fact that fetal RBCs do

not plug the filter pores to a greater extent than maternal cells further supports the hypothesis that fetal red cells differ from adult cells in volume rather than in membrane or cytoplasm properties. The larger cell volume creates a higher initial filtration pressure, but thereafter the same fractions of maternal and fetal cells are able to pass the filter per unit time. The data with 4.5- μ m filters suggest that the fetal cells have reached a volume which might contribute to the flow resistance through the capillary bed with a diameter of 4–5 μ m. Any additional decrease in red cell deformability such as severe acidosis (14) could therefore impair the microcirculation of the newborn.

The 3-fold increase in resistance for a fetal red cell to pass a 2.6- μ m pore suggests that passage through splenic sinuses would be difficult for these cells, and one would expect a decreased survival of fetal red cells. In fact, a mean survival of 60–80 days (normal, 120 days) has been reported (26). The presence of membrane invaginations and intracytoplasmic vacuoles in fetal red cells has been described (4, 16). Since the same type of red cells is found in adults after splenectomy (18) or in children with congenital agnecsis of the spleen (12), their presence in the newborn reflects a functional hyposplenism during the intrauterine life (1), which may be due to splenic immaturity, decreased blood flow through the spleen, or a combination of both. This explains why such large and less deformable red cells are circulating in the peripheral blood at birth instead of having been removed by the spleen during intrauterine life. After birth, the number of these "pocked" erythrocytes gradually decreased within 2 months (16), indicating the maturation of the splenic function and/or increased blood flow to the spleen. We would also expect a reduction of the MCV with a resulting improvement of the filterability through small pores during that time.

In conclusion, fetal blood has two major disadvantages from a rheological point of view: the high cell concentration and the large red cell volume. These disadvantages are counterbalanced *in vivo* by the low plasma viscosity and probably reduced red cell aggregation. However, this balance can be disturbed during the perinatal period. Extremely high hematocrits are present in infants with intrauterine growth retardation, infants of diabetic mothers, certain chromosomal abnormalities, chronic intrauterine hypoxia, twin-to-twin transfusions, or large placental transfusions with delayed cord clamping. High fetal fibrinogen concentration, which can be regarded as normal for adults, may contribute to such imbalance. Newborns with infections due to maternal contamination were found to have a 100% increase of fibrinogen level within the first 48 h of life (30). These changes may overcome the natural protection of the neonatal circulation and lead to the hyperviscosity syndrome in the newborn.

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New Tools for the Study of Niemann-Pick Disease: Analogues of Natural Substrate and Epstein-Barr Virus-transformed Lymphoid Cell Lines

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ABSTRACT. Acid sphingomyelinase activity was determined in Epstein-Barr virus-transformed lymphoid cell lines (LCL) established from patients affected with Niemann-Pick disease (NPD) using several substrates: sphingomyelin derivatives, radiolabeled [¹⁴C]sphingomyelin (SM), fluorescent *N*-(10-(1-pyrene)decanoyl)sphingomyelin (P₁₀-SM) or colored trinitrophenylaminolauryl-sphingomyelin, and the chromogenic non-natural substrate 2-*N*-(hexadecanoyl)amino-4-nitrophenylphosphoryl-choline. LCL from NPD Type A and Type B showed a severe deficiency of acid sphingomyelinase determined using either substrate, whereas LCL from normal subjects had

an activity close to that of blood leukocytes. Sphingomyelinase in normal LCL had the same pH optimum (5.0-5.2) and molecular form (pI 5.8) as the enzyme from other sources; identical profiles and activity levels were obtained using the various analogues of sphingomyelin. However, among these derivatives, the assay using P₁₀-SM appeared as the most useful and sensitive for enzymatic diagnosis of NPD. Electron microscopy of NPD LCL demonstrated the lysosomal storage. These results prove the validity of LCL as an experimental model system for NPD. (*Pediatr Res* 19:153-157, 1985)

Abbreviations

LCL, lymphoid cell lines
 NPD, Niemann-Pick disease
 SM, sphingomyelin
 P₁₀-SM, *N*-(10-(1-pyrene)decanoyl)sphingomyelin

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