Measurement of human fetoplacental blood volume in erythroblastosis fetalis

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The fetoplacental blood volume of the human fetus was measured with the change in hematocrit at the time of intravascular transfusion for severe erythroblastosis. A total of 121 measurements were made between 18 and 31 weeks' gestation. The volume ranged from 117 ml/kg at 18 weeks to 93.1 ml/kg at 31 weeks. These values compare closely with those reported for the sheep fetus and, when they are extrapolated to term, are similar to the blood volume of human newborns plus the residual placental blood volume. (AM J OBSTET GYNECOL 1987;157:50-3.)

Key words: Blood volume, fetal circulation, human fetus

Estimation of the fetoplacental blood volume of the human fetus and the change of this volume with gestational age would be of great interest as a basic physiologic parameter and has potential practical importance in the management of a number of fetal conditions. In suspected fetal bleeding, either external or fetal-maternal, one could better assess the significance of blood loss if the magnitude of hemorrhage could be related to fetal blood volume. Likewise, with diagnostic fetal blood sampling, the volume removed is more significant when expressed as a fraction of the circulating volume than as an absolute sample volume. In treating erythroblastosis fetalis with fetal transfusion, an estimate of normal circulating volume would be useful in both selecting the volume of donor blood and predicting the posttransfusion hematocrit value.

Although there are several reports of blood volume measurements in experimental animals, human data are limited to one study in which radioiodinated albumin was used in 19 human fetuses at the time of midtrimester hysterotomy pregnancy terminations.[1] The blood volume of newborn infants has been measured in a number of studies. However, the circulatory changes that accompany delivery and adaptation to neonatal life limit the degree to which these volumes can be extrapolated and applied to the fetus.

The treatment of severe erythroblastosis fetalis with intrauterine intravascular transfusion[2, 3] provides an opportunity to measure the circulating blood volume of the fetus using the change in fetal hematocrit values in response to transfusion with a specific volume of known hematocrit donor blood. This approach has been validated in animal studies in which there was close agreement between the values obtained with the hematocrit change with transfusion and the value obtained with ^{51}Cr-labeled red blood cells.[4] Furthermore, estimates of the blood volume in neonates with the change in hematocrit after partial exchange transfusion are in satisfactory agreement with published values obtained by indicator dilution procedures.[5]

In this article we will present the estimates of fetoplacental blood volume determined at the time of intrauterine intravascular transfusion based on 121 procedures between 18 and 31 weeks' gestation. Because all of the data needed for this determination are obtained routinely at the time of fetal transfusion, there was no additional risk to the fetus or mother.

Methods

Fetal transfusion. Fetoscopically directed intrauterine intravascular transfusions were carried out for severe erythroblastosis as described previously.[2, 3] Gestational age was determined by calculation from menstrual dates and confirmed by ultrasonographic measurement of the fetal biparietal diameter.[6] At the time of fetoscopy a fetal umbilical artery or vein was punctured and a sample of pure fetal blood (1 to 4 ml) obtained for hematologic and biochemical studies. Transfusion was then performed with packed red blood cells of known hematocrit value that had been cross-matched against the mother. The fetus was transfused with from 5 to 50 ml donor blood, depending on the gestational age and donor and fetal hematocrit values. At the end of the transfusion the needle was flushed with saline solution (0.5 ml), and after 60 seconds a sample of pure blood was aspirated. The first 0.5 ml aliquot of this was discarded. The fetal pretransfusion and posttransfusion hematocrit values and the hematocrit value of the donor blood were determined

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with a Coulter "S Plus" electronic cell counter (Coulter Electronics Inc., Hialeah, Florida). In some cases the posttransfusion hematocrit value was found to be <40%. In these cases additional blood was often infused, but data from this additional transfusion were not used in blood volume estimation. If there was any evidence of contamination of the fetal blood samples with amniotic fluid or difficulty obtaining the posttransfusion sample, the data were not used in the computation of fetoplacental blood volume. Data were available for analysis from 121 transfusions in 57 fetuses between 18 and 31 weeks' gestation.

Computation of blood volume. Blood volume estimation was based on the following assumptions: (1) Infused blood was thoroughly mixed with the circulating blood volume before obtaining the final fetal hematocrit sample. (2) The fetal blood volume at the end of the transfusion (V_f) was the sum of the initial fetoplacental blood volume (V_i) and the volume of the transfusion (V_d) less the volume of the initial fetal blood sample (V_s): $V_f = V_i + V_d - V_s$ (equation 1). (3) The red blood cell (RBC) volume in the fetal circulation at the end of the transfusion is equal to the sum of the initial hematocrit value (H_i) multiplied by the initial blood volume (V_i) and the volume of the transfusion (V_d) multiplied by the hematocrit value of the donor blood (H_d) less the volume of the sample (V_s) multiplied by the initial hematocrit value (H_i): RBC volume = $V_iH_i + V_dH_d - V_sH_i$ (equation 2). (4) The hematocrit value at the end of the transfusion (H_f) equals the final red cell volume divided by the final blood volume:

$$H_f = \frac{V_iH_i + V_dH_d - V_sH_i}{V_i + V_d - V_s} \qquad \text{Equation 3}$$

Equation 3 can be rearranged to give:

$$V_i = \frac{V_d(H_d - H_f) + V_s(H_f - H_i)}{H_f - H_i} \qquad \text{Equation 4}$$

All of the quantities on the right of equation 4 are measured at the time of fetal transfusion, so V_i, the initial fetoplacental blood volume, can be calculated.

Statistical analysis. Gestational age was rounded off to the nearest completed week of amenorrhea. Polynomial regression by the least-squares method was performed on an IBM Pc personal computer using a program written by Giuricin Matteo and Riccardo Brachelente of Florence, Italy.

Results

Fetoplacental blood volume. Fig. 1 shows the relationship of fetoplacental blood volume to gestational age in fetuses with erythroblastosis fetalis. Twenty-seven fetuses showed ultrasonographic evidence of hydrops fetalis at the time of their transfusion. In Fig. 1 the hydropic and nonhydropic fetuses are plotted together. As can be seen, there is no difference between the blood volume estimates for these two groups. The relationship of fetoplacental blood volume to gestational age was described better by a quadratic than a linear function. At 18 weeks the best estimate of fetoplacental blood volume, from the regression line, was 25.87 ml and at 31 weeks it was 151.7 ml.

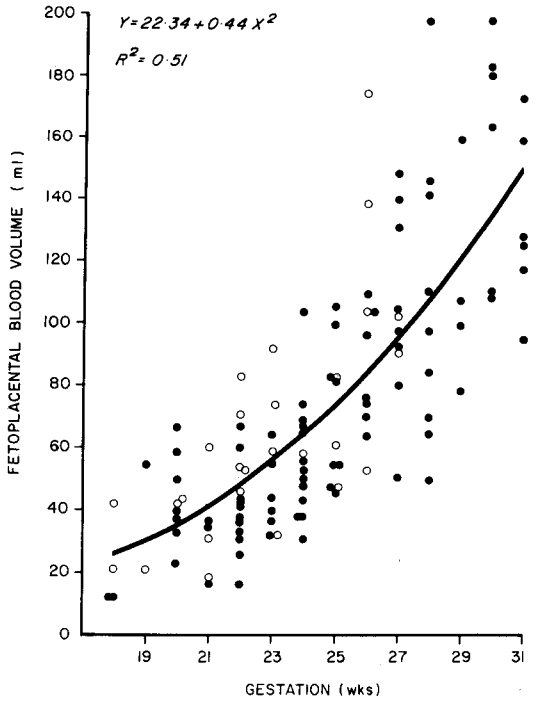

Fig. 1. Estimated fetoplacental blood volume. Open symbols represent hydropic fetuses.

In Fig. 2 the fetoplacental blood volume is expressed as milliliters per kilogram fetal weight. At 18 weeks' gestation the volume was 117 ml/kg and at 31 weeks it was 93.1 ml/kg. The overall mean was 101 ± 13.6 (2 × SD) ml/kg. The volume per kilogram fetal weight, computed in this way, was correlated with gestational age. Because fetal weight was not known for patients in this study at the time the blood volume was estimated, we used the fiftieth percentile for normal fetal weight as determined by Brenner et al.[7] and the blood volume estimate from the regression line in Fig. 1. Although these weights were derived for the patient population of Chapel Hill, North Carolina, and not the United Kingdom, it is unlikely that this would cause a significant error because the British population has a similar newborn weight distribution. The fetuses in this study had erythroblastosis fetalis, but ultrasonographic parameters of fetal growth, including head circumference, abdominal circumference (except in cases of hydrops fetalis), biparietal diameter, and femur length, were within the normal range for gestation.[8] Brenner et al. did find that there was significant variation in fetal

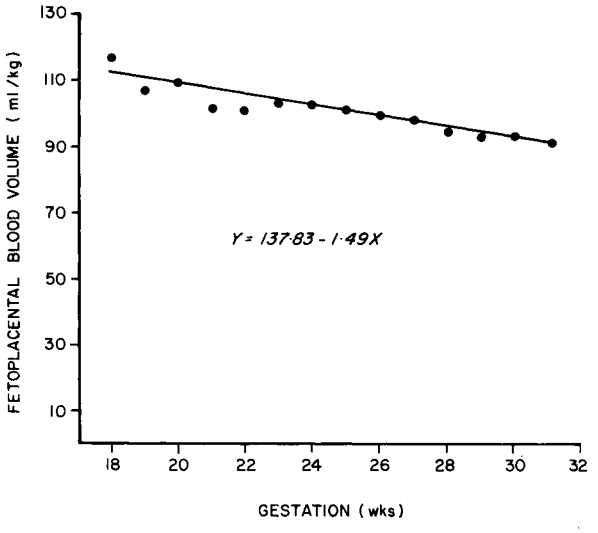

Fig. 2. Estimated fetoplacental blood volume expressed as milliliters per kilogram estimated fetal weight.

weight with fetal sex and maternal social class, but these effects were detected only after 36 weeks' gestation. Because our study involved fetuses <32 weeks' gestational age, it seems unlikely that there would be any significant difference between the weights of our fetuses and those reported by Brenner et al.

Comment

In this article we report the first estimates of human fetoplacental blood volume in continuing pregnancy. Using the change in fetal hematocrit values after intrauterine intravascular transfusion, we obtained values that are similar to those reported by Brace[4] for the sheep fetus and comparable with those for the human newborn and placenta after delivery.[9, 10] Although newborn infants with erythroblastosis fetalis have expanded plasma and contracted red cell volumes, their total blood volumes are usually normal. In a few severe cases their blood volume is contracted.[11] Although we made no blood volume measurements in normal fetuses, we found that hydropic and nonhydropic fetuses had similar estimated blood volume.

The usual methods of determining blood volume that can be applied in adults or children cannot be used for the human fetus. Dye-dilution measurements require access to the circulation for the injection of the dye and then sampling of the blood at various times.[12] Furthermore, the safety and validity of such dye-dilution determinations have not been established for the human fetus. Techniques that entail use of radioactive labels, such as ^{51}Cr-labeled red blood cells, ^{131}I-albumin, or ^{131}I-fibrinogen, cannot be used because of the fetal radiation exposure. Stable isotope labeling of red cells with ^{50}Cr and subsequent activation to ^{51}Cr by neutron irradiation have been used in the newborn, but the necessary equipment is available in only a very few centers.[13]

In measuring volume by our technique we made a number of assumptions that may have introduced systematic errors. The first of these assumptions is that the mixing of donor blood with the fetoplacental blood is complete by 1 minute after the end of the transfusion. However, using ^{51}Cr-labeled red cells, Brace[4] has shown that after a single injection of labeled cells into a fetal vein, it took >5 minutes for complete mixing to occur. Our transfusions ranged in volume from 5 to 50 ml. They were infused at a rate of 1 to 3 ml/min and therefore the duration was approximately 10 to 50 minutes. Blood infused at the start of the transfusion had adequate time for mixing and only blood at the end of the transfusion would be subject to incomplete mixing. A sample taken at 1 minute might give an erroneously high hematocrit value and therefore a low blood volume estimate. Our second key assumption is that the blood volume at the end of the transfusion is equal to the initial volume plus the volume of donor blood less the volume of the fetal blood sample (equation 1). This is clearly an approximation because the fetal blood volume has been expanded substantially by the transfusion and will immediately begin to return toward normal. Brace[14] has studied the effect of rapid blood volume expansion in the sheep fetus using saline solution and iso-oncotic dextran 70. He found that with a dextran infusion amounting to approximately 15% of the fetal blood volume, there was 80% intravascular retention after 10 minutes and that this decreased to 55% by 30 minutes after the start of the infusion. This rate of plasma loss is more rapid than that reported for the adult.[15] Because we did not subject patients to delayed fetal blood sampling, we were unable to assess the rate of the reequilibration of the plasma volume in the human fetus. As noted above, fetal transfusions are carried out over time periods ranging up to 50 minutes. In general, larger transfusions took more time and were used in later-gestation fetuses. Because our computation assumes complete intravascular retention, any error as a result of plasma loss would result in an underestimate of the initial fetoplacental blood volume and this error would tend to increase with larger donor volumes and therefore advancing gestational age.

An error introduced by either or both of these assumptions could explain the discrepancy between our mean value, 101.3 ml/kg, and the value obtained by Morris et al.,[1] 162 ± 4.12 ml/kg. However, Brace[4] has pointed out that in the sheep fetus, radiolabeled proteins, albumin, and fibrinogen give larger apparent blood volumes than do labeled blood cells. Using ^{125}I-albumin and ^{125}I-fibrinogen, he obtained a value of 126 ml and 124 ml, respectively. He observed a very rapid loss of labeled protein from the fetal circulation

immediately after injection. Since this resulted in a nonlinear semilogarithmic relationship of label concentration in the fetal blood to time, a true "time zero" value could not be obtained by extrapolation. Consequently, labeled proteins gave consistently larger blood volume estimates than did labeled red blood cells. He concluded that labeled proteins were inappropriate for estimating blood volume in the fetus. Morris et al. used ^{131}I-albumin and sampled fetal blood at two times and assumed a log-linear relationship with time in extrapolating to time zero. The blood volume Brace obtained with ^{51}Cr red cells was 110.3 ± 10.7 (SD) ml/kg fetal weight. Although the decline in blood volume per kilogram fetal weight we observed, from 117 ml/kg at 18 weeks to 93.5 ml/kg at 31 weeks, may be an artifact of the method of estimation, our mean value of 101.3 ± 6.8 (SD) ml/kg is not significantly different from Brace's value of 110.3 ± 10.7 (SD) ml/kg for the sheep fetus.

Reported blood volume for human newborns ranges from 66 to 110 ml/kg. This variability is dependent partly on the method of assessment and partly on the partition of blood between the infant and the placenta at delivery. Estimation of neonatal blood volume is fraught with most of the same problems encountered in estimation of fetal blood volume by indicator-dilution techniques. Delayed cord clamping and neonatal polycythemia have been associated with significantly higher neonatal blood volumes.[16, 17] Estimation of the residual placental blood volume has also been difficult. Some studies have simply measured the volume of blood that could be drained from the placenta after delivery.[16] Gruenwald[17] used the fetal hemoglobin concentration in placental homogenates and cord blood to estimate the placental blood volume. Both of these methods gave values ranging from 100 to 125 ml. If one assumes a term neonatal blood volume of 80 ml/kg and a term placental volume of 110 ml, a 3.3 kg fetus would be expected to have a fetoplacental blood volume of 374 ml or 113 ml/kg. This is remarkably similar to our value of 101 ml/kg and Brace's value[4] of 110 ml/kg.

In conclusion, in spite of a number of technical objections to our method of fetoplacental blood volume estimation, results have been obtained that are similar to those reported for chronic animal preparations and consistent with newborn plus placental blood volumes in humans assessed at delivery.

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