Fetal Blood Volume Responses to Acute Fetal Hemorrhage

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SUMMARY. The purpose of this study was to explore the changes in fetal blood volume that occur after fetal hemorrhage. Fifteen unanesthetized, chronically catheterized fetal sheep averaging 130 ± 3 (so) days gestation were studied 4 to 6 (average 5) days after catheter implantation. The fetuses were hemorrhaged by continuous withdrawal from an arterial catheter over 5 minutes. On the average (n = 15), 13.9 ± 5.3% of the initial volume was removed. Fluid gradually entered the fetal circulation during and after the hemorrhage. Thirty minutes after hemorrhage had been initiated, 53.0 ± 22.0% restitution of the lost volume occurred. Thus, short-term fetal blood volume restitution after fetal hemorrhage averaged about twice that of the adult. Hemorrhages averaging 7.3 ± 2.3% (n = 3), 12.9 ± 1.0% (n = 8), and 21.0 ± 3.3% (n = 4) were followed by 55.7 ± 13.3%, 54.7 ± 26.4%, and 47.7 ± 19.9% restitution, respectively. Thus, fractional volume replacement appears independent of the shed volume over the range of 5-25% volume loss. The protein concentration in the fluid which entered the fetal vasculature averaged 53% of the plasma protein concentration, suggesting that the fetal interstitium was the primary source of the fluid. In summary, the data suggest that the fetus is able to replace rapidly about half of the volume lost due to rapid hemorrhage, and appears considerably better at controlling its blood volume immediately after rapid hemorrhage than the adult. (Circ Res 52: 730-734, 1983)

THERE have been several studies of the effects of hemorrhage on the unanesthetized, chronically cannulated fetal lamb in utero. Past studies include the effects of hemorrhage on arterial pressure, heart rate, and the blood gases (Faber et al., 1974; Rurak, 1979; Toubas et al., 1981), placental blood flow (Faber et al., 1973; Toubas et al., 1981), plasma hormone concentrations (Rurak, 1979; Drummond et al., 1980; Iwamoto and Rudolph, 1981), cardiac output (Gilbert, 1980; Toubas et al., 1981), and cardiac output distribution (Toubas et al., 1981; Iwamoto and Rudolph, 1981). However, there has been no detailed study of the changes in fetal blood volume following fetal hemorrhage.

In comparison, there have been a variety of related studies in the adult. In general, following a blood volume reduction in the adult of 10-20%, about 20-30% of the lost volume is replaced by fluid from the interstitium in one-half hour (cf, Pirkle and Gann, 1975). We currently do not know the extent to which the fetus can compensate for blood volume losses.

The purposes of the present study were to explore the short-term changes in blood volume of the fetus after acute fetal hemorrhage, and to determine the fractional recovery in blood volume following blood loss.

Methods

Animal Preparation

I used the unanesthetized chronically catheterized fetal lamb preparation averaging 130 ± 3 (so) days gestation (range = 124-137 days) for these studies (term = 145-150 days). The preparation has been described in detail elsewhere (Gilbert, 1980). Briefly, catheters were inserted into femoral and brachial arteries and into femoral and cephalic veins so that the catheter tips were in the descending and ascending aorta and the inferior and superior vena cava, respectively. One additional catheter was sewn to the fetal chest at the level of the heart for recording amniotic fluid pressure. The animals were maintained on antibiotic therapy [500 mg ampicillin (Smith, Kline, and French) into the amniotic fluid and 2.5 ml combiotic (Pfizer) intramuscularly to the ewe daily], and catheters were flushed daily with heparin. Experiments were performed 4-6 (average 5) days after surgery to allow adequate time for recovery from surgical trauma.

Experimental Variables

The initial fetal blood volume was measured by standard indicator dilution techniques after injection of 2 ml of \textsuperscript{51}Cr-labeled autologous fetal blood. The labeled red cell space averages about 110 ml/kg in chronic fetal lambs, whereas the double indicator dilution blood volume averages 112-113 ml/kg after correction for the liver interstitium and extrapolation from samples taken at 1-minute intervals (Brace, 1983). The logic for using the red cell space as representing blood volume in this study is because of its ease of measurement, its closeness to the double indicator space, and the difficulty in determining the double indicator blood volume.

Changes in blood volume (BV) were calculated from the initial red cell volume, the volume of red cells withdrawn from the fetus, and from the changes in hematocrit (Hct) using the formula

\[ BV = BV_0 \times \text{FRCM} \times \frac{\text{Hct}_0}{\text{Hct}} \]  (1)
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where the subscript o represents control conditions and the fractional red cell mass (FRCM) is the volume of red cells circulating in the fetus divided by the initial red cell volume. The equation is based on the assumption that there is no significant release of red cells from storage sites such as the spleen, and this has been shown to be true for the chronic fetal lamb preparation (Brace, 1983).

The percent blood volume restitution (%R) after hemorrhage was calculated from the formula

\[ \%R = \left( \frac{V_f - \Delta V}{V_f} \right) \times 100 \]  

where \( V_f \) is the volume of blood removed from the fetus and \( \Delta V \) is the actual change in volume calculated from Equation 1.

Arterial blood samples (0.3 ml) were taken at 10-minute intervals for determinations in triplicate of microhematocrit and plasma protein concentration (American Optical, T5 meter). The hematocrits were read to 3 decimals on a calibrated microscope stage, and triplicates were averaged. The standard deviation of the triplicate hematocrits averaged 0.1 hematocrit unit. Thus, with an initial hematocrit of 30, a 1% change in blood volume can be detected with 99% confidence from a single sample. Additional arterial blood samples (0.25 ml) were taken at 30-minute intervals for measuring blood gases (Radiometer ABL2). The blood gas reference temperature was 39.5°C, the average fetal sheep temperature. No distinction was made for samples taken from the brachial artery vs. the femoral artery, although samples in an individual fetus were taken from only one artery.

Fetal arterial pressure, venous pressure, and heart rate, as well as amniotic fluid pressure, were continuously recorded on a Beckman R612 polygraph and simultaneously on disks with an on-line computer (Texas Instruments 990/10). The fetal vascular pressures were corrected for amniotic fluid pressure (i.e., amniotic fluid pressure was subtracted from the pressures in the fetal arterial and venous catheters) by the computer, and the corrected vascular pressures were recorded on the polygraph and disks. Fetal heart rate was calculated on a beat-to-beat basis using a Beckman cardiotachometer from the pressure pulses in an arterial catheter.

Protocol

Following a 30-minute control period, 25-87 ml of arterial blood were removed over 5 minutes by constant withdrawal into heparinized syringes. Thirty minutes after beginning the hemorrhage, the blood (warmed to 40°C just prior to reinfusion) was returned to the fetus over 5 minutes through a venous catheter. All variables were recorded for an additional 30 minutes after returning the blood.

Data Analysis

All data are expressed as the mean ± 1 so or 1 se. One-minute averages for heart rate, arterial pressure, and venous pressure were retrieved from the computer-stored data and plotted as means for each minute interval. To explore the relative changes in blood volume, we normalized blood volumes by dividing the values within each fetus by its average during the control period. The normalized data then were averaged to determine changes with time. To explore changes in the other variables, we subtracted average control values from each variable in each animal during the 30-minute control period, prior to performing interanimal averages. This procedure has the advantage that it reduces much of the variability due to animal-to-animal baseline differences. The data were analyzed for statistical significance using an analysis of variance with Duncan’s test. To determine whether the extent of hemorrhage affected the fetal responses, the animals were divided into three groups: (1) 4-10%, (2) 10.1-15%, and (3) 15.1-25% blood volume removal.

From the initial hematocrit during the control period (H0), the hematocrit after the hemorrhage (H), and the percent change in hematocrit (%ΔH), the percent change in the plasma protein concentration (%ΔP) can be calculated from the following equation, assuming that the fluid entering the vasculature contained no protein:

\[ \%\Delta P = \left( 100 \times \frac{\%\Delta H}{100 - H} \right) \]

This equation is based on the assumption that the whole body-to-large vessel hematocrit ratio (i.e., the f-cells ratio) has a value of one. The actual amount of protein in the entering fluid can then be determined by comparing the experimentally measured %ΔP with that calculated from the above equation, and using the volume changes calculated with Equation 1 above.

Results

Average values for each of the variables during the 30-minute control period are given in Table 1. At the end of the control period, removal of an average of 56 ± 5 (se) ml (13.9 ± 1.9% of the initial blood volume or 15.5 ± 1.4 ml/kg) of fetal arterial blood caused transient changes in blood volume, as shown in Figure 1. In this and subsequent figures, the vertical dotted lines at 30 and 35 minutes define the time of hemorrhage and, at 60 and 65 minutes, the time of blood reinfusion. The dashed line in Figure 1 represents the blood volume if no compensation had occurred. Blood volume was reduced by an average of 8.5%, 7.3%, and 6.5% at 10, 20, and 30 minutes after the hemorrhage began. These numbers are different from the volume of blood removed, i.e., 13.9%, because fluid was entering the vasculature from spaces such as the fetal interstitium. The percent replacement of the lost volume at these times averaged 36.2%, 45.3%, and 53.0%, respectively. The percent restitution was not a function of the volume of blood removed from the fetuses because hemorrhages of 7.3 ± 2.3% (so) (n = 3), 12.9 ± 1.0% (n = 8), and 21.0 ± 3.3% (n = 4) were

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Average (±SE) Control Conditions for All Fetuses (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>se</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>130.0 ± 1.0</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3590 ± 130.0</td>
</tr>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>43.2 ± 1.3</td>
</tr>
<tr>
<td>Venous pressure (mm Hg)</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>176 ± 6.0</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>31.9 ± 1.6</td>
</tr>
<tr>
<td>Plasma protein concentration (g/dl)</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Hct blood volume (ml/kg)</td>
<td>113.3 ± 2.5</td>
</tr>
<tr>
<td>Arterial oxygen tension (mm Hg)</td>
<td>24.1 ± 1.1</td>
</tr>
<tr>
<td>Arterial carbon dioxide tension (mm Hg)</td>
<td>55.5 ± 1.5</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.328 ± 0.008</td>
</tr>
</tbody>
</table>
followed by replacement of 55.7 ± 13.3%, 54.7 ± 26.4%, and 47.7 ± 19.9%, respectively, at the end of the hemorrhage period. Replacement of the shed blood caused blood volume to increase above normal, as shown in Figure 1, and then gradually return to normal, 20 and 30 minutes after blood infusion was begun. An average of approximately 1% of the initial blood volume had been removed as samples during the 90-minute period.

Arterial pressure gradually decreased during the 5-minute hemorrhage and only partially recovered during the subsequent 25 minutes. The arterial pressure pattern depended on the volume of blood removed from the fetuses. The upper half of Figure 2 shows a transient peak in arterial pressure about 10 minutes after the hemorrhage in the fetuses (n = 4) subjected to the larger volume losses (i.e., 21.0 ± 3.3% hemorrhage). The transient peak in arterial pressure did not occur in the groups subjected to the 7.3 ± 2.3% hemorrhage (n = 3) or the 12.9 ± 1.0% hemorrhage (n = 8), so the data from these two groups were lumped together. The lower half of Figure 2 shows the transient changes in arterial pressure in these fetuses (n = 11) subjected to 11.4 ± 2.9% hemorrhage. The changes in venous pressure, hematocrit, and plasma protein concentration were greater with the larger hemorrhages, but their patterns were not dependent on the extent of the hemorrhages and mean values are shown in Figure 3. The data in Figure 3 can be converted to absolute values by adding the initial values given in Table 1.

There was no statistically significant change in fetal heart rate, arterial pH, P{sub}O{sub}2, or P{sub}CO{sub}2 during or after the hemorrhage.

Discussion

Although the chronically prepared sheep fetus has been hemorrhaged in several previous studies, no one has examined in detail the blood volume changes that occur following the hemorrhage. Thus, the major purpose of this study was to examine the ability of the sheep fetus to regulate its blood volume after acute hemorrhage. In 15 unanesthetized fetal sheep, an average of 13.9% of the initial blood volume was removed in 5 minutes. Twenty-five minutes later, blood volume was reduced by an average of only 6.5%. Thus, the fetuses had replaced an average of 53% of the lost blood volume. This is remarkable, because such a large fraction of the lost volume was replaced in only 30 minutes. For comparison, under similar circumstances, the splenectomized adult dog is able to replace only about 20 to 30% of the hemorrhaged volume in one-half hour (cf, Pirkle and Gann, 1975). One additional surprise is the speed at which the blood volume shifts occur. Figure 1 shows that more than half of the restitution had occurred within 10 minutes after initiating the hemorrhage. Similarly, within 10 minutes after returning the blood to the fetus, the fluid which entered the vasculature following hemorrhage was lost from the blood stream.

Another interesting feature of the fetal response to hemorrhage is that the percent restitution of the lost volume was independent of the amount of blood removed from the fetus. Hemorrhages averaging approximately 7, 13, and 21% all were followed by restitutions averaging about 50%. This appears to suggest that the fetal responses which lead to blood volume replacement are linear functions of the amount of volume removed. Whether similar restitution of volume would also occur with larger volume losses is unknown.

The observation that the fetus is able to maintain blood volume close to normal in the face of mild to moderate hemorrhage raises the question: What is the source of the fluid which enters the fetal vascular during the hypovolemia? There are several possibilities: (1) lymphatic flow, (2) transcapillary reabsorption of interstitial fluid, and (3) transplacental absorption of maternal fluid.

It is possible to distinguish partially between the
interstitial and maternal sources by considering the composition of the fluid that entered the fetal vasculature. From the changes in hematocrit, one can calculate the resulting plasma protein concentration if the fluid that entered the vasculature had been protein free (see Methods). For example, if 50 ml of isotonic saline were added to 100 ml of blood with an initial hematocrit of 30, hematocrit would decrease by 33.3% while plasma protein concentration would decrease by 41.7%. Thus, adding protein-free fluid to blood causes a greater percentage decrease in protein concentration than in hematocrit. As seen in Figure 4, the measured percentage changes in protein concentration were significantly less than the expected changes if protein-free fluid had been added to the fetal blood. By comparing the calculated protein concentration with the measured, the data suggest that, during the 30-minute hypovolemic period, 8.2 ml/kg entered the fetal circulation with an average protein concentration of 1.9 g/dl (i.e., 52.7% of the initial plasma protein concentration). The data of Robillard et al. (1979) suggest essentially the same protein concentration of the fluid entering the fetal vasculature following hemorrhage because hematocrit fell by 22.2% and plasma protein concentration by 11.5%, whereas a 30.4% fall in plasma protein concentration should have occurred if protein-free fluid had entered the vasculature.

There are few possible sources of the protein. It could not cross the placenta because the sheep placenta is relatively impermeable, even to small molecular weight substances such as NaCl (Conrad and Faber, 1977), although placentas from guinea pigs (Schroder et al., 1982), rabbits, and humans (Longo, 1972) may allow a limited movement of larger molecules. Liver synthesis is not likely to be a major source over the short time of the study, although this has not been explored in the fetus. The only likely major source of the protein is the fetal interstitium. Our recent studies (Brace and Christian, 1981) suggest that the subcutaneous interstitial protein concentration in fetal lamb averages about 2 g/dl. Thus, because of the large amount of protein in the fluid entering the blood, it appears that the fetal interstitium is the major source of fluid. Even if the protein came from the liver interstitium where the protein concentration
may be close to the plasma protein concentration, the major source of fluid would still be the fetal interstitium. Therefore, the major shift of fluid into the fetal circulation during the first 30 minutes after acute hemorrhage does not appear to be from the mother, although some maternal-to-fetal fluid transfer undoubtedly occurred. Robillard et al. (1979) also concluded that the fetal interstitium is the major short-term source of fluid available to the fetus, based on the observation that the reabsorbed fluid following hemorrhage is isosmotic, whereas fluid rapidly transferred from the mother was expected to be hyposmotic because of the placenta's very low permeability to solutes.

The time course of the fluid and protein entering into the circulation may also provide insight as to their source. During the first, second, and third 10-minute periods of hypovolemia, an average of 5.7, 1.5, and 1.0 ml/kg of fluid entered the fetal circulation and contained protein concentrations averaging 1.3, 4.9, and 4.7 g/dl, respectively, as calculated from mass balances. With a plasma protein concentration averaging 3.6 g/dl, the high calculated protein concentrations over the latter two periods most likely result from a combination of lymphatic return plus a reduced protein loss rate from the fetal circulation, but this is only speculation which has yet to be explored.

One possible consequence of the large amount of fluid entering the fetal vasculature after hemorrhage is that arterial pressure should return close to normal. This has been previously reported by Faber et al. (1974) and Toubas et al. (1981) and others, as well as observed in this study; however, Toubas et al. suggested that arterial pressure may be maintained due to a greater ability of the fetal vasculature to constrict in response to blood loss or, possibly, due to a shift of blood from the placenta to the fetal body. In contrast, the present data suggest that fetal blood pressure is only moderately reduced after hemorrhage, partly because a large amount of the lost blood volume has been replaced by fluid absorbed mainly from the fetal interstitium. On the other hand, the fetal neuroendocrine system undoubtedly contributed to blood pressure maintenance after hemorrhage.

One other possible concern is that the carbon dioxide tension may appear higher and the pH lower in the fetuses of this study (Table 1) than some others report for their chronically catheterized fetal lambs for a period of days after surgery (cf, Drummond et al., 1980). First, the present values are consistent with other studies from this laboratory (cf, Lotgering et al., 1983). Second, although the carbon dioxide and pH differences among laboratories are unexplained, there are two factors which may partially explain some of the differences: (1) Our pregnant ewes have a resting carbon dioxide tension of 35 to 40 mm Hg (Lotgering study, 1983), which is higher than others report. This would elevate fetal CO₂ tension while reducing pH. (2) Our blood gases and pH were measured at 39°C and corrected to 39.5°C, the average fetal sheep temperature (Lotgering et al., 1983). The temperature correction increases CO₂ while reducing pH. Other factors are probably also involved, but these have yet to be identified.

In summary, the sheep fetus appears able to tolerate a blood loss of 5% to 25% of its initial blood volume over 5 minutes because the actual reduction in blood volume 25 minutes after the hemorrhage was less than half of the lost volume. The restitution of volume appears to occur primarily through absorption of fetal interstitial fluid because the reabsorbed fluid had an average protein concentration of approximately one-half of the plasma protein concentration.

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