

Fetal Blood Volume and Fetal Placental Blood Flow in Lambs¹ (37018)

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The placenta combines the function of oxygen exchange with that of water exchange (1). Water is transferred between mother and fetus in the placental capillaries under the influence of hydrostatic (2) and osmotic pressures (3-5). Although alterations in fetal placental blood flow are known to bring about alterations in fetal oxygenation (6, 7), they also bring about shifts in fetal placental capillary blood pressure (2), and hence in fetal water content and blood volume. Blood volume is a major determinant of central venous pressure. In view of the great sensitivity of the cardiac output of the fetal (8) and neonatal (9) hearts to changes in central venous pressure, we investigated whether changes in fetal blood volume affect fetal placental blood flow. This flow is more than 30% of the combined outputs of the right and left fetal hearts (5). If in the fetus placental blood flow depends on blood volume, a primary disturbance of placental flow would, through its effect on blood volume, cause an opposite secondary change in placental flow that tends to negate the disturbance. In effect, there would be a homeostatic control of fetal placental blood flow.

Methods. Pregnant ewes and their fetuses were anesthetized with halothane. All operations were performed with sterile procedures. The fetuses were partially removed from the uterus and electromagnetic flow sensors were installed on the fetal aortas below the renal arteries. A vinyl catheter was advanced 1 cm into a fetal femoral artery and tied in place and the other femoral artery was tied off. A second catheter was advanced through

a femoral vein until its tip was in the proximal part of the inferior caval vein and a third catheter was left open in the amniotic cavity. Amnion and chorion were carefully closed and the uterus was closed separately with a purse string suture. The cables and catheters were conducted subcutaneously to the flank of the ewe where their ends were kept in a pouch. The ewes were immediately returned to the experimental laboratory where they were kept in the company of several ewes at all times. They appeared to be at ease during subsequent experiments, which were performed without sedatives.

The flow sensors were calibrated with blood *in vitro* at flow rates encompassing the values found later *in vivo*. Standard errors of the calibration factors were between 0.6 and 2%. The calibration factors varied less than 2% with variations in hematocrit within the ranges observed in the experiments and the effect of osmolarity (270-360 mOsm) was less than 1%. When a Carolina Medical Electronics flow meter was used, zero flows *in vivo* were obtained before each measurement by use of a mechanical occluder installed immediately distal from the sensor during surgery. The Omnicraft Mediflow ABC 1000 flow meter was equipped with an electronic zeroing device of which we found the accuracy to be better than 2% of full-scale deflection under all circumstances.

Since fetal distal aortic flow is not entirely fetal placental flow, the exact proportion was obtained by intravenous injection of at least 50,000 radioactive microspheres of 50 μ diameter (5) and post mortem counting of the amounts of radioactivity trapped in the placenta and in the fetus below the level of L4-L5. These spheres have been shown not to recirculate (10). A femoral-artery blood

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TABLE I. Mean Placental Blood Flow in Nine Fetal Lambs on the Third Day Following Implantation of Distal Aortic Flow Meters.

Fetus #	Fetal weight (kg)	Fetal heart rate	Placental fraction ^a of distal aortic flow (%)	Placental blood flow (ml min ⁻¹ kg ⁻¹)
1	2.5	185	77.4 ^b	143
2	2.6	180	74.5 ^c	156
3	2.2	180	86.7	187
4	2.3	167	78.7	196
5	0.9	190	83.8 ^d	175
6	3.6	170	80.7	201
7	4.5	187	77.9	160
8	2.7	210	92.0	224
9	3.2	165	82.5	147
Mean	2.7	181		177
S.D.	0.95	13		26
S.E.M.	0.33	4.6		9.2

^a In two additional fetuses whose femoral arteries were not both tied off these fractions were 88.3% and 69.4%.

^b Not directly determined in this fetus. Mean for fetuses without both femoral arteries tied off.

^c Femoral arteries not both tied off.

^d Not directly determined in this fetus. Mean for fetuses with both femoral arteries tied off.

sample was taken from the fetus during the period of injection to calculate placental blood flow by microsphere dilution (11) for comparison with the flows measured electromagnetically at the same time. At least 500 spheres were present in the blood sample, insuring a S.D. of the count of less than 4.5%. The counting techniques have been described previously (5).

Hematocrits were determined in quadruplicate and averaged. The difference between the four measures rarely exceeded 1% and in such cases the measurements were repeated. Fetal hemoglobin concentrations were determined by a commercial cyanmethemoglobin method (B. D. Unopette) in a Beckman-G spectrophotometer at a wave length of 540 nm.

Results and calculations. Nine fetuses survived the operation. Their weights, listed in Table I, varied from 0.9 to 4.5 kg and their estimated gestational ages from 100 to 140 days (term approx. 150 days). All experiments were performed three or more days after surgery, except in the one instance noted below.

The distribution of distal aortic blood flow was measured in nine fetuses (two of these

were used for other experiments later, unrelated to the subject of this paper). Since blood volumes were deliberately changed in the experiments that will be described, the distribution of distal aortic flow was measured in fetuses whose blood volumes varied from 80 to 100% of normal with the following results. In six fetuses in whom both femoral arteries were tied off the mean placental fraction was 84% (SEM 2%, N = 8), and there was no significant correlation between blood volume and the placental fraction of distal aortic flow ($r = -0.15$, $p \approx 0.75$, N = 8). In the remaining fetuses, this fraction was on the average 77% (SEM 5.7%, N = 3). Table I lists the fractions recorded in the individual fetuses.

Distal aortic flows were measured simultaneously with both techniques on six occasions in five fetuses. Treated as paired data, they showed that the electromagnetic flow meter measurements were an average of 5.3% (SEM 6.0%) higher than the microsphere dilution measurements. This difference was not statistically significant.

Fetal placental blood flow was recorded after surgery at approximately daily intervals. Figure 1 shows the individual results. Im-

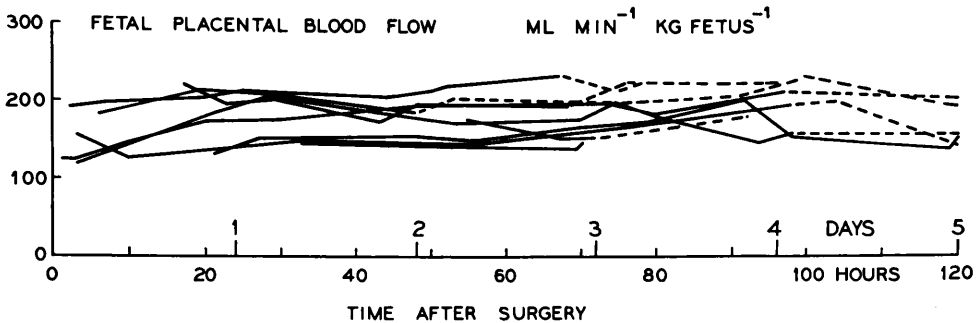


FIG. 1. Umbilical blood flow recorded in nine unanesthetized fetuses after recovery from surgery. The solid lines connect measurements taken before any further experiments were done on the fetuses. The broken lines connect measurements taken after the fetus had been subjected to blood letting or mannitol infusion into the ewe. Only measurements that were taken when blood volume was between 95% and 105% of the control value after blood letting experiments, or more than 8 hr after mannitol experiments are included.

mediately after surgery fetal placental blood flow was below average and it climbed to a stable level in about 24 hr. On the third day after surgery the nine fetuses had a mean placental blood flow per kg body weight of 177 (SEM 9.3) ml/(min kg). Table I lists the individual data.

The blood volumes of six of these fetuses were changed by blood letting. In one fetus this was done 49 hr after surgery, in the others 3–7 days after surgery. Some fetuses were bled on several successive days. Initial fetal blood volume was estimated from the values published by Creasy (12) (134.7 ± 3.1 SEM ml/kg) and the initial fetal red cell mass was calculated from fetal blood volume and hematocrit. The red cell mass of the blood collected during bleeding was measured, and subsequent fetal blood volumes were calculated from the remaining red cell mass and hematocrit taken at the moment fetal placental blood flow was measured. Fetal blood volume and fetal placental blood flow were normalized by setting the control values equal to 100%. The control values of flow and hematocrit for each fetus were obtained during a period of at least one hour before the first experiment on the first experimental day. In each fetus, placental blood flow diminished when blood volume was reduced and increased again during post-hemorrhagic hemodilution in the hours following blood letting. The relationships between blood volume and placental flow were

statistically significant in five of the six fetuses. In the sixth the relationship was also highly linear ($r = 0.94$) but not quite significant, probably because only four experimental points were obtained in this (first) experiment. Figure 2 shows the results of all of these experiments.

The regression equation predicting flow from volume from the data in Figure 2 is

$$\text{Flow (\%)} = -70.8 + 1.71 \cdot \text{Volume (\%)}$$

The coefficient of linear correlation is 0.85 and the 99% confidence limits of the slope of 1.71 are 1.36 and 2.05. On the average, therefore, a 1% reduction in blood volume caused a 1.7% change in fetal placental blood flow. Figure 2 shows that this relationship is valid for fetal blood volumes between 80 and 110% of normal.

The relationship between fetal blood volume and placental flow is independent of time. The points in Figure 2 indicate by their shape on which day of an experiment the results were obtained. Points representing results obtained immediately after blood letting and points representing results obtained later appear to belong to the same population. Although recovery of placental blood flow was always seen after hemorrhage, the recovery was associated with a decrease in fetal hematocrit and therefore with an increase in fetal blood volume.

To investigate whether the decrease in placental blood flow was caused in part by a

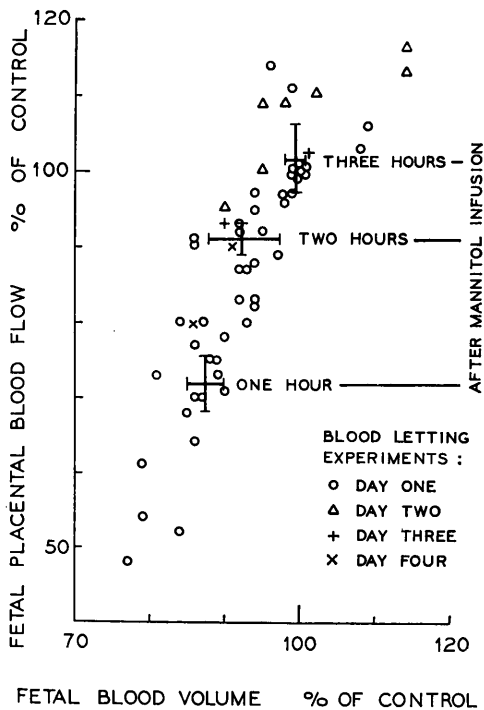


FIG. 2. Fetal placental blood flow as a function of fetal blood volume, both expressed as percentages of their control values obtained in the hour preceding the first experiment on the first experimental day. The individual points are from blood letting experiments. Some of the six fetuses in this group were experimented on a second, third, or fourth day (fifth, sixth, or seventh day after surgery). The crosses indicate the mean and standard error of fetal placental blood flow and fetal blood volume during the first, second, and third hours following the infusion of mannitol into the ewes.

decrease in fetal hemoglobin levels, fetal blood volume was reduced in the remaining three fetuses by the infusion of hypertonic mannitol into the jugular vein of the ewes. The consequent hyperosmolarity of the maternal blood caused the withdrawal of fetal water across the placenta (3-5). No blood samples were taken from these fetuses. Mean placental blood flow decreased to 72% (SEM 4%) of initial flow at one hour after infusion. Thereafter it returned to control levels being 91% (SEM 2%) of control value and 102% (SEM 5%) of control value at 2 and 3 hr after infusion, respectively.

The changes in fetal blood volume associated with the infusion of mannitol were

estimated from data obtained in a separate group of 5 fetuses, equipped with catheters only, who were part of a larger group published previously (5). Similar amounts of mannitol were infused into these ewes (900 ml of a 2 M mannitol solution with 30 g NaCl/liter). In these five fetuses hemoglobin levels at 1, 2, and 3 hr after mannitol infusion were 114.8% (SEM 3.6%), 108.1% (SEM 5.2%), and 100.3% (SEM 1.5%) of their initial values, respectively. Fetal blood volumes, again expressed as a percent of their initial value, were calculated from the changes in hemoglobin levels.

The fetal placental blood flows and the fetal blood volumes found in these two groups of animals at 1, 2, and 3 hr after the infusion of mannitol are plotted in Figure 2 with their respective standard errors. It is seen that the relationship between fetal blood volume and fetal placental blood flow as revealed by the infusion of mannitol into the ewe is superimposed on the relationship obtained after blood letting of the fetus, even though the infusion of mannitol is associated with an increase in fetal hemoglobin level whereas the blood letting is associated with a decrease in fetal hemoglobin level.

Discussion. In this group of nine fetuses, the mean value of fetal placental blood flow is in between the mean values found by other groups (11, 13, 14) using microsphere or antipyrine clearance techniques. It is slightly below the value of 199 ml/(min kg) (SEM 20) found by us with the microsphere technique in a previous series of 15 chronically prepared fetuses (5), but the difference is not statistically significant. We believe that the decrease in placental blood flow in Fig. 2 cannot be due to selective vasoconstriction of the vascular bed that accommodates the non-placental fraction of distal aortic blood flow. This fraction is only 16%, whereas the recorded reduction in distal aortic flow is almost 50% in some instances. Moreover, the placental fraction of distal aortic flow did not correlate significantly with fetal blood volume. The reduction in distal aortic blood flow is therefore accepted as representing an approximately proportional reduction in placental flow.

The strong dependence of fetal placental blood flow on fetal blood volume, illustrated by Fig. 2, is probably an expression of the Frank-Starling mechanism of the fetal heart.⁴ We have shown that the Frank-Starling mechanism is present even in the embryonic heart at the time of organogenesis (15) and Fournon and Héber (8) and Downing, Talner and Gardner (9) have shown its existence in the fetal and neonatal heart, respectively. The mechanism is known to be powerful; a doubling of cardiac output is brought about by an increase of only a few millimeters mercury in central venous pressure (9).

The place of the relationship between blood volume and placental blood flow in the regulation of fetal placental blood flow is determined by the fact that, in the long run, the fetus cannot tolerate net water transfer between fetal and maternal plasma except for the amount required for fetal growth. In the placenta, therefore, fetal capillary blood pressure must be equal to maternal capillary blood pressure, corrected for any small difference in osmotic pressures. If for any reason fetal capillary blood pressure in the placenta exceeds the equilibrium value, the fetus will lose water to the maternal circulation (2). The present experiments show that the consequent loss of fetal circulating blood volume causes a diminution in fetal placental blood flow which, in turn, is known to decrease fetal placental capillary blood pressure (2). This process continues until a steady state is reestablished. The relationship demonstrated in Fig. 2 is thus believed to be a crucial step in the stabilization of fetal

placental blood flow.

Summary. In the conscious, nonmedicated sheep fetus, blood volume strongly influences placental blood flow, a change of 1% in volume being associated with a change of $1.7 \pm 0.1\%$ in flow. This relationship is valid between blood volumes of 80 to 110% of normal, is independent of time over a period of several days and independent of concomitant changes in fetal hemoglobin concentration.

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⁴ Central venous pressure of the fetuses was not systematically recorded in the present experiments. In the 17 instances in which it was recorded, it correlated with fetal blood volume ($r = 0.70$, $p < 0.01$). This correlation points to the Frank-Starling mechanism as the cause of the observed relation between blood volume and placental flow although it cannot prove it without measurements of fetal cardiac output.