Circulatory Changes during Growth in the Fetal Lamb

By Abraham M. Rudolph, M.D., and Michael A. Heymann, M.B., B.Ch.

ABSTRACT

The changes in circulation with advancing gestation were investigated in 44 fetal lambs in utero; gestational ages ranged from about 60 days to 150 days. Spinal analgesia was administered to the ewe, polyvinyl catheters were inserted into fetal vessels, and umbilical blood flow was measured by the steady-state diffusion Fick method during antipyrine infusion. Cardiac output, distribution of cardiac output, and actual organ blood flows were calculated from injections of nuclide-labeled microspheres (50μm diam) into a forelimb and into an umbilical or hindlimb vein.

Umbilical Po2, Pco2 and pH did not change significantly during gestation. Umbilical blood flow and total cardiac output increased in proportion to fetal weight. The proportion of the combined ventricular output distributed to the placenta decreased from about 50% in the youngest fetuses to about 40% just before term. The proportion of the cardiac output distributed to the lungs, as well as the actual flow in relation to lung weight, increased throughout gestation, with a more rapid rise after about 120 days. There was also a late increase in intestinal flow. Cerebral blood flow increased gradually throughout gestation, both as a proportion of cardiac output and in relation to brain weight. There were no significant changes in percent of cardiac output, or flow related to weight in the kidney, heart, or skin and muscular tissues. The studies suggest that, since lung blood flow is a relatively small proportion of total cardiac output, it is not important in regulating distribution of blood flow, but that the peripheral circulation in skin and muscle, which receives a large percent of fetal cardiac output, is the site where vasomotor responses may effect major redistribution of the fetal circulation.

ADDITIONAL KEY WORDS radioactive microspheres antipyrine
Fick method cardiac output umbilical blood flow
ductus venosus flow cerebral flow myocardial flow renal flow
pulmonary flow gut flow

Growth of the fetus during gestation places an increasing burden on the placental circulation to provide the nutritional substances and oxygen requirements required for tissue metabolism. Since the rate of growth, and possibly the rate of development of functional maturity, varies greatly in different organs (1), it is possible that the blood flow to fetal organs may change markedly during fetal development. There are very few reports of levels of blood flow to specific fetal organs. The most frequent observations are those of umbilical blood flow. Most of these measurements have been made in exteriorized lambs when the ewe was anesthetized (2, 3). However, the Fick method of steady-state diffusion has been used for measuring umbilical blood flow in lambs in utero in relatively undisturbed states (4, 5). Blood flow to the lower limbs, in the left circumflex coronary artery and to the brain has been measured by Campbell et al. (8) with cannulating electromagnetic flow transducers in the appropriate...
vessels. These measurements, though valuable for assessing acute changes evoked by experimental interventions, could not provide reliable measurements of flow under reasonably normal intrauterine conditions. Similarly, measurements of pulmonary blood flow made with electromagnetic flow transducers (7) are subject to the same criticism. Thus, apart from the rather limited studies of umbilical blood flow at different gestational ages, there is no information available regarding changes in organ flow during fetal development.

We have examined lamb fetuses at various stages of gestational development and measured their total cardiac output and proportions of the cardiac output distributed to each organ and to the fetal body and placenta. In addition, we have calculated the actual blood flow to the various fetal tissues. These observations were made in lamb fetuses from about 60 days of gestation to just before term (±150 days); they were left in utero with as little disturbance as possible. Distribution of flow was measured after the injection of radioactively labeled microspheres, while umbilical blood flow was determined simultaneously during antipyrine infusion using the Fick steady-state diffusion technique (8).

The purpose of this study was to provide some indication of the patterns of flow and of actual tissue perfusion in the fetus at various gestational ages as a basis for examination of the effects of various physiological and pharmacological interventions.

Methods

The techniques used for studying the circulation of the fetal lamb in utero and for determining cardiac output and distribution of the cardiac output in the fetal lamb, have been described in detail previously (8). A brief account of the approach used and modifications of the previous techniques will be given here.

Forty-four fetal lambs were studied. The gestational periods were dated in 21 animals. We estimated the gestational ages of the other 23 lambs from the data of Barcroft (1). There were 14 twin pregnancies, and this was taken into account in calculation of gestational age. Since we did not have confirmed gestational periods in all 44 animals, we have correlated our observations with total fetal body weight.

The ewe was blindfolded, spinal analgesia accomplished with 2 ml tetracaine hydrochloride and 0.2 ml 50% dextrose solution, and the sheep placed on her right side. A polyvinyl catheter was placed in a maternal femoral artery for continuous monitoring of pressure and for repeated measurement of blood gases. Two percent lidocaine hydrochloride (Xylocaine) (15 to 20 ml) was injected into the peritoneal cavity and the uterus was exposed through an incision in the left flank. No measurements were made for at least 2 hours after the lidocaine was given, so that it is likely that any possible effect of the drug on the fetus would have disappeared (Morishima and Shnid-er, personal communication). Through a small uterine incision, usually in the pregnant horn, polyvinyl catheters were placed in a cotyledary umbilical vein and artery and passed proximally into major vessels. Catheters were also placed in a hindlimb artery and vein and into a forelimb vein, as previously described. In the three smallest fetuses the limbs were not isolated, but the fetal head was exposed and a small polyvinyl catheter was placed in the external jugular vein. The catheters were brought out through the uterine and maternal abdominal incisions which were then sutured closed. After allowing a period of stabilization of 30 to 60 minutes, an infusion of antipyrine solution (6 g/100 ml) was started at a rate of about 0.1 ml/min/kg of estimated fetal weight. After a further 45 to 60 minutes, blood samples for antipyrine were obtained repeatedly from the umbilical vein and the umbilical or femoral artery. (All further references to blood samples obtained from the lower part of the fetal body, whether from femoral or umbilical artery will be to umbilical artery.)

The distribution of blood flow to each organ from venous return of both superior and inferior venous cavae was determined by injection of radioactive microspheres 50 μ in diam into a forelimb vein (or external jugular vein in the three smallest fetuses), and into the umbilical vein (or in seven fetuses, into the hindlimb vein). These injections were made simultaneously over a 1- to 2-minute period. In the younger fetuses, a total of 1 to 1.5 ml, and in the older fetuses, up to 5 ml, of saline was injected. Blood samples were obtained from the umbilical vein and umbilical artery for antipyrine and blood gas measurements immediately before and within 2 to 3 minutes after the sphere injection.

In these studies two or more of the following radionuclide-labeled microspheres were used: 125I, 131I, 99mTc, 85Sr, 46Nb, and 85Sr.

The ewe was given a large dose of pentobar- bital and after the fetus had died, the uterus and its contents were removed.

The amount of each nuclide in each fetal organ and in the placenta was measured as previously described (8). A Nuclear Chicago automatic gamma changer and well-type detector were used, and a TMC 404C multiple channel pulse height analyzer recorded energy distribution pattern. The counts in each channel were recorded on paper punch tape (TMC 535). This information was then transferred to magnetic tape and the calculations of the amounts of each nuclide in each tissue performed on an IBM system 360 computer. Using the data for umbilical blood flow obtained from steady-state diffusion with antipyrine infusion, the actual flow to each organ from superior and inferior venae cavae and total organ flow was calculated (8).

The fetal and maternal arterial pressures were continuously monitored during the study using Statham P23Dc pressure transducers and a Beckman Dynograph 8 Channel direct-writing recorder. Blood gases and pH were measured in samples of maternal arterial and fetal arterial and umbilical venous blood with Radiometer pH, Pco₂, and Po₂ electrodes and blood gas meter.

Since in some of the studies it was necessary to manipulate the umbilical vessels to insert the catheters and we were concerned that we may have interfered with umbilical flow, we had to decide what we could consider a "normal" status. We assumed that the values for fetal pH, Po₂ and Pco₂ obtained by sampling from chronically implanted catheters maintained in fetal vessels in utero (9) represented normal fetal blood gas values. We included in our study only those fetuses with pH and Po₂ values of similar levels. There was no greater rejection of fetuses because of a lower pH and Po₂ at any specific gestational age.

Mean and standard errors were calculated for each of six weight groups for the various measurements reported. If there appeared to be any difference in the six groups, linear regressions were determined and a standard test of significance was performed. When one mean value appeared somewhat different from those in the other five groups, an analysis of variance was made.

**Results**

The fetuses were divided into six groups based on body weight. These weight groups and corresponding estimated gestational ages and the blood gas and pH measurements in each group are shown in Table 1.

The fetal arterial blood pH was slightly elevated but was essentially similar in all groups. Maternal arterial Po₂ was somewhat lower than that seen in sheep standing quietly, but was also similar in all groups. The maternal Po₂ was somewhat reduced from the levels in standing pregnant sheep, but all animals showed similar levels throughout the period of gestation studied (Fig. 1).

Umbilical venous blood pH was slightly higher in the 80- to 450-g fetuses than in the other groups, but this difference was not significant, and umbilical arterial blood pH, Pco₂ and Po₂ were also essentially the same in all the six weight groups. The umbilical arterial and umbilical venous Po₂ levels are presented in Figure 1.

**Umbilical Blood Flow**

Umbilical blood flow as measured by the Fick method was expressed in relation to fetal body weight (Table 1). The mean flow in the six weight groups varied between 192 and 244 ml/kg/min. The flows were similar in most groups, but in the fetuses weighing 451- to 900-g, they were slightly lower (192 ml/kg/min). The reason for this was not clear; analysis of variance showed that this difference was not statistically significant. By subtracting the proportion of nuclide which is held back in the hepatic-portal circulation from the total injected into the umbilical vein, it was possible to determine the percent of umbilical venous return which passed through the ductus venosus. There was a variation in this percent in the different weight groups, with a range of mean values from 41 to 63. However, in view of the individual variability, no obvious trend with gestational age was observed.

**Cardiac Output and Its Distribution**

The cardiac output was expressed as the combined output of both ventricles (Table 1). In Figure 2 the actual cardiac output was plotted against estimated fetal gestational age and quite clearly paralleled the increase in fetal weight with advancing gestation. Cardiac output per kilogram of fetal body weight was similar for all weight groups except for the 451- to 900-g group, in which mean cardiac output was 377 ml/min/kg, somewhat lower than in the other groups (Table 1).
## TABLE 1

<table>
<thead>
<tr>
<th>Measurements of Maternal and Fetal Blood Gases, Fetal Cardiac Output, Fetal Mean Arterial Pressure, Venous Flows, Distribution of Cardiac Output and Organ Blood Flows in Fetuses of Various Gestational Ages</th>
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<td>Fetal weight (grams)</td>
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difference of this group was tested by analysis of variance and found not to be statistically significant.

The proportion of the cardiac output which was distributed to the placenta definitely decreased with advancing gestational age (Fig. 3). It dropped from a mean level of 47 to 50% in the 60- to 85-day fetuses to a mean of about 40% near term. This decrease was examined by a linear regression, which was significant (P<.025).

The spleen received only about 1% of the total cardiac output through the period of gestation studied. Distribution of cardiac output to the kidneys was 1.8 to 2.9% but the suggestive decrease in the percent of cardiac output passing to the kidneys with advancing gestational age was of questionable significance (0.1>F>.05). The intestines received a relatively small proportion of the total cardiac output in the younger fetuses (mean of 2.7 to 3.6%), but after the 120th day there was a striking increase in the percent of cardiac output supplied to the gut (5.6 to 5.9%) (Fig. 3). The proportion of the cardiac output distributed to the myocardium was about 3.9% in the smallest fetuses; there appeared to be a steady decrease in this percent to a mean level of 2.3% in the 240- to 260-day group (Fig. 3). It dropped from a mean level of 47% in the 60- to 85-day fetuses to a mean of about 40% near term. This decrease was significant (P<.025) and was examined by a linear regression, which was significant (P<.002).
Increase in actual combined right and left ventricular (cardiac) output in relation to advancing gestational age. The curve closely follows that for increase of fetal weight.

Organ Blood Flows

The lungs showed a striking change in the proportion of the cardiac output they received during fetal growth. There was a rather large standard error for each age group, but there was a statistically significant rise in the percent of cardiac output passing to the lungs with increasing fetal weight with a mean value of 3.7% in the 80- to 450-g fetuses, rising to a mean of 7% in the group near term ($P < 0.001$).

The blood flow to the intestines was quite low in the small fetuses (mean of 29 to 42 ml/100 g/min in the youngest three groups). After this, there was a marked increase to 57 to 70 ml/100 g/min (Fig. 4). A linear regression of this relationship showed that this progressive increase of flow was highly
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Blood flow per 100 g of tissue to the brain, gut, and lung in relation to fetal body weight (mean ± SE).


The blood flow to the brain was quite low (30 ml/100 g/min) in the young group, but there was a progressive and considerable increase to a mean level of 122 ml/100 g/min in the term fetuses (Fig. 4). A linear regression was highly significant (P < 0.001). Blood flow to the carcass did not change significantly during gestation and averaged 17 to 30 ml/100 g/min in the various groups.

The pulmonary blood flow changes were quite striking (Fig. 4). In early gestation the flow was only 38 ml/100 g/min, but a steady increase in flow occurred with growth with a very striking increase in late gestation to a mean level of 125 ml/100 g/min (P < 0.001).

Discussion

The course of circulation of the mammalian fetus has been known for some time, but there has been very limited investigation of the changes in the circulation during intrauterine development. No studies of the magnitude of the blood returning in the inferior and superior venae cavae, or their patterns of distribution at different periods of gestation, have been carried out. Our present observations on lambs have been made on fetuses from about 60 days of gestation to term, viz., in the latter three-fifths of gestation. We are therefore not able to comment on earlier changes in the course or distribution of the circulation, and it is quite possible that more dramatic changes occur early in gestation. Unfortunately, we also did not have specific duration of gestation in all the animals studied and had to use fetal weights for comparison. Since it is possible that weights were not reliable because of interference with intrauterine development, some errors are possible. However, all the fetuses showed concurrence of their weights, crown-rump lengths, and general development with the findings expected for their gestational age, and we feel we can be reasonably sure about the accuracy of the gestational ages when the pregnancies were not time-dated.

A second problem was that, although the fetuses were in utero and no general anesthesia was used, these were acute studies performed with the ewe lying on her right
side after receiving spinal analgesia. It was necessary to decide what could be considered a "normal" state for the fetus. We decided to relate "normality" to the blood gas conditions of the fetus. On the assumption that the blood gases reported by Meschia et al. (9) in fetuses in which catheters were chronically implanted in the umbilical vessels represented normal values, we accepted those fetuses with similar blood gases as normal. We have also examined the cardiac output and its distribution and fetal blood gases under the experimental conditions of the present study, and also 1 or 2 days later in the standing ewe and found no significant differences in three animals (unpublished observations). It is, of course, possible that our experimental conditions could have produced variable effects at different periods of gestation, and these would not be taken into account. Maternal arterial $P_{O_2}$ was definitely lower in the ewe lying on her side than in the standing animal. The mean value in our animals was 73 mm Hg, whereas in the standing ewe it is 90 to 95 mm Hg. This is probably due to the development of either atelectasis or congestion and edema in the dependent lung. The pH and $P_{CO_2}$ were not, however, significantly different.

An additional factor that could have influenced our results is fetal movement. We did not monitor fetal movements, and although after returning the fetal lamb into the uterus it was our impression that the fetus was quiet and immobile, fetal movements, if present, could possibly have caused a rise in cardiac output and change in distribution of flow.

The fetal blood gas levels in both the umbilical artery and the umbilical vein were not significantly different throughout the period of gestation studied. This observation is in conflict with the reports of Barcroft (1), who suggested that there is a gradual decrease in umbilical arterial oxygen saturation toward term. We believe that the explanation for this difference is related to the fact that the latter observations were made in exteriorized fetuses in which there may have been some interference with umbilical placental circulation, and that this was more marked in the late gestation fetuses. We have previously reported on the decrease in umbilical blood flow which results from exteriorization of fetal lambs (10).

The patterns of venous return to the heart did not change during gestation. Similar proportions of umbilical venous blood passed through the ductus venosus in the various age groups. The superior and inferior vena caval returns each represented the same proportions of the total cardiac output throughout the gestational period, and also only a very small amount of superior vena caval blood crossed the foramen ovale in all fetuses. Umbilical blood flow increased in proportion to fetal weight, but there was a slightly lower level of flow per kilogram of fetal weight in the 86- to 100-day fetuses. Analysis of variance showed that this group was not significantly different from the others, but additional studies are required to confirm this. These findings are in agreement with those of Meschia et al. in lamb fetuses in utero (4), but are at variance with those of Dawes and Mott (2); the latter measurements were, however, made in exteriorized fetuses and suggested that the umbilical blood flow was higher in lamb fetuses of 86-95 days of gestation than it was in term fetuses.

It is of considerable interest that although umbilical blood flow was relatively constant in relation to fetal size during fetal growth, the umbilical blood flow represented a decreasing proportion of the total cardiac output with advancing gestation, dropping from about 50% of cardiac output early in gestation to about 40% at term. This would indicate that there had been a relative decrease in total fetal body circulatory resistance as compared with placental vascular resistance, and thus a redistribution of the cardiac output. It is apparent from our studies that most of this change can be explained by the relative increases in the blood flows to the lungs, gut, brain, and perhaps myocardium in the older fetuses. We cannot, however, exclude the possibility that some change in the placenta...
itself produces a relative increase in vascular resistance.

The proportion of cardiac output and the actual blood flows per 100 g of tissue distributed to the carcass, which includes skin and muscle, does not change significantly throughout gestation, so that these tissues do not participate in the redistribution of flows. If the drop in vascular resistances in the lungs, gut, and brain were alone responsible for the change in distribution, it might be expected that the additional blood flows to these organs would be diverted from the carcass rather than from the placenta. The peripheral circulation has been shown to be capable of considerable vasoreactive responses in the lamb fetus (6) whereas the umbilical-placental circulation is fairly passive (11). This would suggest, then, the possibility that an actual increase in placental vascular resistance relative to total body vascular resistance may well play a role in these changes toward term. The other possibility is that the relative decrease in peripheral vascular resistance during fetal growth is greater than the decrease in placental-vascular resistance.

The demand for increased circulation to various vital organs during growth is met by redistribution rather than by an increase of cardiac output. The combined ventricular output of the fetus does not change in relation to body weight throughout gestation. This response is different from that of the adult, in whom an increased demand for circulation in various organs or in the extremities is associated with some increase in total cardiac output. The reasons for this difference in response of the fetal and adult circulations are not apparent at this time.

The pulmonary circulation in the fetus has received considerable attention since it has been thought that the lungs may play an important role in redistributing the circulation during fetal asphyxia. It had been suggested that pulmonary vasoconstriction produced by hypoxemia and acidemia diverts blood ejected from the right ventricle away from the lungs and to the descending aorta through the ductus arteriosus, thus enhancing the passage of blood with low Po2 to the placenta. This cannot be an important mechanism in early gestation since the lung receives only a very small proportion of the cardiac output and even if lung flow were completely eliminated, there would not be any significant increase in placental flow. In the fetal lamb at term, when pulmonary flow is larger, pulmonary vasoconstriction by itself still could not exert any significant change in placental flow. It is probable that the peripheral circulation, which receives 35 to 40% of the cardiac output, is far more important in regulating the distribution of the total circulation. The peripheral circulation is quite reactive; the response to stimulation of sympathetic alpha-receptors is considerable during generalized fetal asphyxia (6).

The increased pulmonary blood flow at about the 100th day of gestation is in accord with the observations of Cassin et al. (12) that the actual vascular resistance in the lung is greater in lambs at 75-90 days of gestation than in those near term. However, they observed that vascular resistance was lower in the young lambs when related to wet lung weight. In our studies the flow per unit of lung weight increased with gestation age; this could be related to an increase in pulmonary arterial pressure with age. It is also of interest that the increased metabolic activity, which develops in the lung at this time, may be the stimulus for the development of an increased pulmonary blood flow.

It should be appreciated that the measurements of pulmonary blood flow by the methods used are subject to a greater degree of error than measurement of flow to other organs. Calculation of pulmonary blood flow depends on first determining the superior vena caval return and then estimating the proportion of superior vena caval flow that is distributed to the lungs. This is then added to the proportion of inferior vena caval blood passing to the lungs. The potential errors are
thus increased and this may explain the high standard error values for pulmonary blood flow measurements.

The selective increases in flow to various organs during gestation could be explained by at least two mechanisms. Increased growth of new vessels associated with maturation, or local vasodilation due to increased metabolic activity, or effects of nervous or hormonal stimulation could be responsible. In the gut, the rise in blood flow appeared to develop over a short time span, around the 110th day of gestation. It is not likely that development of new vessels could occur over a short period, but the initiation of increased enzyme activity and an increase in metabolism could result in vasodilation. In the brain, however, there was a gradual increase in blood flow throughout gestation and it is possible that, in this organ, the increase can be explained by enlargement of the total vascular bed. The increase in cerebral blood flow could not be accounted for by an increase in Pco2, which does cause cerebral vasodilation in the fetus (14, 15), since there was no significant change in Pco2 levels through the period of gestation observed.

The levels of cardiac output (expressed as the combined output of the left and right ventricles) were 377 to 549 ml/min/kg fetal body weight; these were similar for all gestational ages, and are higher than those reported previously. Dawes et al. (16), using the Fick method, and assuming a level of umbilical blood flow of 150 ml/min/kg fetal weight, estimated the cardiac output at 315 ml/min/kg. Mahon et al. (17), using indicator dilution techniques, found mean levels of combined ventricular output of about 362 ml/min/kg, and Assali et al. (18) recorded flows of about 335 ml/min/kg by means of electromagnetic flow transducers. In all these studies the sheep were anesthetized, the lambs were exteriorized, and in addition, in the series of Assali et al., rather extensive intrathoracic dissection had to be made. It is probable that the difference in experimental conditions account for the lower values for cardiac output previously reported.

The gestational changes in distribution of cardiac output and local organ blood flows we have observed may be valuable in judging the degree of maturation of various organs. The increase in pulmonary flow which coincides with the development of surface activity is of great interest. It would also be interesting to examine the gut in the sheep to determine whether an increase in enzyme activity is associated with the increase in flow at the 110th to 120th day of gestation. The measurements of blood flow and distribution of cardiac output in normal lamb fetuses should be of value in assessing the general circulatory adjustments to stress and to various pharmacological and physiological influences.

References
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