Measurement of Umbilical Arterial Blood Flow to the Sheep Placenta and Fetus in Utero

DISTRIBUTION TO COTYLEDONS AND THE INTERCOTYLEDONARY CHORION

By Edgar L. Makowski, M.D., Giacomo Meschia, M.D., William Droegemueller, M.D., and Frederick C. Battaglia, M.D.

ABSTRACT
A method of estimating the magnitude and distribution of umbilical blood flow by means of radioactive microspheres in sheep fetuses in utero is described. Simultaneous measurements of total umbilical flow by this method and the steady-state diffusion technique showed agreement within ±11%. In 11 fetuses of 90 to 150 days gestational age, the distribution of umbilical flow to the intercotyledonary chorion was 6.2 ± 0.8% of the total. This information has been used to estimate the effect of venous admixture of cotyledonary and noncotyledonary blood on the umbilical vein-uterine vein concentration difference of inert molecules with flow-limited transplacental clearance.

ADDITIONAL KEY WORDS radioactive microspheres antipyrine regional blood flows shunted blood flow transplacental diffusion

Several methods of measuring umbilical blood flow of lamb fetuses have been described (1-6). Electromagnetic flowmeters placed in the common umbilical vein give a precise and continuous recording of umbilical blood flow, but the flows in such circumstances may be abnormal because the fetus is exteriorized and subjected to considerable surgical trauma. To avoid these disadvantages, methods of determining umbilical flow of the fetus within the uterine cavity have been developed. These are indirect methods, since they are based on the application of Fick's principle to the transplacental diffusion of a test substance (equilibrium-diffusion [6] and steady-state diffusion methods [5]). In the steady-state diffusion method, antipyrine is infused at a constant rate in a fetal vein until the transplacental diffusion rate of the test substance becomes constant. Umbilical flow can then be calculated as the ratio of the transplacental diffusion rate over the artery-to-vein difference of the test substance in the umbilical circulation. The validity of the calculated flow depends primarily on an accurate estimate of the transplacental diffusion rate and on the assumption that the umbilical vein sample is representative of total umbilical outflow. A comparison in the exteriorized fetus of the steady-state diffusion method with the cannulating electromagnetic flowmeter method has shown satisfactory agreement (7), but there has been no comparison of two independent intrauterine methods.

The umbilical circulation of the sheep fetus is distributed to two structures: (1) the placental cotyledons and (2) the intercotyledonary chorion. In the cotyledons, maternal and fetal capillaries are in close apposition over a relatively large surface area, while in the intercotyledonary chorion the two capillary beds are separated by a greater...
distance over a smaller surface. Thus, in studies of transplacental exchange, it is important to measure the relative distribution of umbilical flow to these different regions. This can be done by means of radioactive microspheres injected into the fetal circulation and carried by the umbilical arterial blood to the target structures in amounts proportional to their regional blood flows. In the past, the microsphere technique has been used to assess ratios of flows but not their absolute values (8, 9). However, the arterial concentration of microspheres, if properly measured, can be used in conjunction with the total number of microspheres found in the target regions to calculate both the magnitude and relative distribution of flows. Accordingly, the experiments presented in this paper had the following purposes:

1. To develop a microsphere method that measures both magnitude and distribution of regional flows.
2. To compare intrauterine measurements of umbilical blood flow by the microsphere and the steady-state diffusion methods.
3. To estimate the magnitude of umbilical blood flow to the cotyledons and the intercotyledonary chorion at different gestational ages.

**Method**

**PRINCIPLE AND PRACTICAL APPLICATION**

If microspheres large enough to be cleared completely by the target organ are carried by the arterial blood from time zero to time x, the blood flow through the target organ may be calculated by the following equation:

\[
\text{ml blood/min} = \frac{\text{number of microspheres in target organs}}{\int_{t=0}^{t=x} C_t \, dt} \quad (1)
\]

Since microspheres suspended in blood settle easily, we found it impractical to collect arterial blood in a syringe and transfer a representative fraction of it to a test tube for analysis. Therefore, the technique adopted for collecting the integrated arterial sample was as follows: Approximately 1 ml of isotonic saline was placed in the bottom of a test tube used for gamma radiation counting. The space above the saline was filled with mineral oil and closed at the top by a rubber stopper with two holes. A polyethylene catheter was passed through one hole, and one end of the catheter was placed in the saline layer of the test tube and the other end connected to the umbilical artery catheter. Thick-walled polyvinyl tubing (2.5 mm o.d., 1.0 mm i.d.) was passed through the second hole, and one end of the tubing was placed in the upper oil layer of the test tube and the other end connected to a 10-ml glass syringe. One-third of the syringe was filled with mineral oil and the syringe was placed in a Harvard pump. The blood sample was collected at a constant rate below the oil layer by pumping the oil from the test tube into the syringe. The microspheres were then concentrated in a pellet at the bottom of the test tube by centrifugation. The radioactivity of the pellet was determined with a gamma scintillation counter of the well type (Packard model 3001). In this way, the total number of radioactive microspheres withdrawn from the arterial catheter in a known period could be precisely determined. Thus, saline dilution of the blood sample and the duration of sampling did not affect the results, provided that the withdrawal was started before the injection of microspheres into the fetus and that the withdrawal was prolonged beyond the point at which the arterial microsphere concentration was zero. In these experiments, microspheres were infused for 2 minutes and the withdrawal time, which started a few seconds before the infusion, was 4 minutes. The rate of withdrawal by the Harvard pump was 1.125 ml/min. Blood flow was calculated according to the following equation:

\[
\text{blood flow (ml/min)} = \frac{\text{number of microspheres in the target organ}}{\text{number of microspheres in arterial sample}} \times 1.125. \quad (2)
\]

\(C_t\) represents the number of microspheres per milliliter of arterial blood at time \(t\). Experimentally, the integration can be done mechanically by withdrawing blood at a constant rate from the arterial system.

Plastic microspheres were labeled with \(^{51}\text{Cr}\) and \(^{105}\text{Yb}\) at a specific activity of 10 mc/g.\(^1\)

\(^1\)Supplied by Minnesota Mining and Manufacturing Co., St. Paul, Minnesota.
These two isotopes were chosen because they remain associated solely with the microspheres even when in contact with biological fluids, a property not shared by all isotopes used for labeling microspheres. This was confirmed in every experiment by finding no detectable radioactivity in the supernatant fluid after centrifugation. On occasion, the amount of free isotope in a given shipment was greater than 1%. Whenever this occurred, the microspheres were washed in isotonic saline and resuspended in a mixture of saline and glycerine (2:3 by volume) with a final concentration of 20 mg microspheres/ml solution. When injected, this concentration of glycerine and microspheres produced no change in the antipyrine clearance (Fig. 1). Thus, it has no effect on uterine or umbilical blood flows.

Microspheres of two mean diameters, 25 and 15 μ, were tested; 95% were within ±5 μ from the mean. The amount of radioactivity per microsphere was determined before each experiment in the following way: A drop of suspended microspheres was placed on a glass coverslip, and a photomicrograph of the drop was obtained. The glass coverslip was then placed in a test tube and the total radioactivity was determined by placing the test tube in a gamma scintillation counter of the well type. The amount of radioactivity per microsphere was calculated from the total counts per minute on the coverslip and the number of microspheres determined by visual counting from the photomicrograph. The techniques for tissue analysis of microsphere concentration are given in the next section.

**EXPERIMENTAL PROCEDURES**

Eleven pregnant ewes, either Dorsets or Westerns, with a single fetus of known gestational age were studied (Table 1). The fetal gestational age ranged from 90 to 150 days. The ewes were starved for 48 to 72 hours and given water ad libitum. Prior to the operation, they were sedated with pentobarbital (6 mg/kg iv) and given spinal anesthesia (6 mg tetracaine hydrochloride). Polyvinyl catheters (0.58 mm i.d.) were inserted in the main umbilical vessels from their peripheral branches in the two uterine horns according to techniques previously described (7, 10). Satisfactory catheterization of both umbilical arteries and veins was achieved in 8 of 11 experiments. In addition, a double lumen catheter made of two polyethylene catheters (0.58 and 0.28 mm i.d.) held together at the tip by a silicone sleeve was inserted into a fetal hindlimb vein. A 15% solution of antipyrine (5% in experiment 1) was continuously infused at the rate of 0.1 ml/min through the smaller catheter; the larger catheter was used for the injection of radioactive microspheres.

At the conclusion of the operation, both mother and fetus were given heparin intravenously, 10,000 units and 1,000 units, respectively. Fifty minutes or more after the start of antipyrine infusion into the fetal hindlimb vein, 0.6-ml samples were obtained from each umbilical artery and vein for antipyrine analyses. Four sets of samples obtained at 5-minute intervals for 20 minutes were analyzed for antipyrine concentration with a Technicon autoanalyzer (11). At the end of the 20 minutes, radioactive microspheres (150 μc) were injected manually into the fetal hindlimb vein over a period of 2 minutes. During the injection of microspheres, blood samples were withdrawn by the Harvard pump at a constant rate from one or both of the umbilical arteries for a total of 4 minutes according to the scheme described above. Integrated umbilical vein samples were obtained manually with plastic syringes. After completion of this procedure, sets of blood samples for antipyrine analyses were again obtained from maternal and fetal catheters at 5-minute intervals for 20 minutes. The experimental scheme is illustrated in Figure 2.

The pregnant uterus was then removed. All of the cotyledons and the entire intercotyledonary chorion were separated from both the uterine wall and the allantois, separately weighed and homogenized in a Waring blender (model CB-5) after the addition of approximately equal amounts of water by weight. The two masses of tissue were homogenized at 20,000 rpm for 20 minutes. The homogenate was cooled by immersing the container briefly in ice water and...
### Experimental Data on the Microsphere Method

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Days gestation</th>
<th>Fetus (grams)</th>
<th>Cotyledons (grams)</th>
<th>Intercot. (grams)</th>
<th>No. cotyledons</th>
<th>Counts/min/sphere</th>
<th>Umb. art. sample</th>
<th>No. microspheres</th>
<th>Umb. flow (ml/min)</th>
<th>% Flow to intercot.</th>
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*Microspheres were labeled with ^160^Yb in experiments 1, 2, and 6 and with ^51^Cr in the others. In experiments 1, 5, 9, and 11, the diameter of the microspheres was 15 μ and in the remainder 25 μ. Umb. = umbilical; Intercot. = intercotyledonary chorion.

*Sample from each umbilical artery obtained simultaneously.*
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homogenized again for 10 minutes. To obtain representative samples of homogenate, the bottom of the blender was modified to incorporate a spout which could be opened while the homogenizer blades were still in motion. Six aliquots from each homogenate were collected in test tubes placed directly under the spout. The test tubes were weighed on an analytical balance before and after filling. The average weight of an aliquot was 4 g. The microspheres were collected at the bottom of the test tube by centrifugation, and their radioactivity was determined, with a maximum variation among aliquots of 5%. The total counts per minute for each homogenate was calculated from the mean counts per minute per gram in the six aliquots and the total weight of the homogenate. The fraction of umbilical blood flow to each tissue group, intercotyledonary chorion and cotyledons, was calculated as the ratio of the total radioactivity in one homogenate to the total radioactivity of both. The total radioactivity of both the placental cotyledons and intercotyledonary chorion was used to calculate umbilical blood flow according to equation 2. Calculation of umbilical blood flow by the steady-state diffusion method was done according to the procedure previously described by Meschia et al. (5).

Results

Experimental Data on Microsphere Method.—The number of microspheres in the integrated umbilical artery sample, in the total tissue mass of cotyledons, and in that of the intercotyledonary chorion, as well as the percent of total flow distributed to the intercotyledonary chorion are presented in Table 1. In five experiments, integrated samples were obtained simultaneously from both umbilical arteries: the mean percent difference in total radioactivity was 1.7 ± 0.9 SEM. More than 99% of the radioactivity in the umbilical blood was cleared by the umbilical vascular bed. The percent of radioactivity shunted across the umbilical circulation was only 0.28 ± 0.13 and 0.15 ± 0.06 SEM for the 25- and 15-μ microspheres, respectively. The mean distribution of umbilical flow to the intercotyledonary chorion was 6.2 ± 0.8% of the total.

Experimental Data on the Steady-State Diffusion Method.—In the steady-state diffusion method, a possible source of error is that an umbilical venous catheter provides blood samples representative of only one of the umbilical veins. An estimate of this error was obtained by simultaneously sampling both veins. The concentration of antipyrine in the two umbilical veins was similar in seven of eight fetuses (Fig. 1). In these seven fetuses, the umbilical flows calculated
separately, according to the antipyrine concentration in either umbilical vein, had a mean percent difference from the average flow of $2.0 \pm 0.35 \text{ SEM (n = 45)}$. In experiment 7, there was too large a discrepancy between the two veins to allow a precise calculation of flow.

**Comparison of Flows Calculated by the Microsphere and the Steady-State Diffusion Methods.**—Total umbilical flows calculated according to the microsphere and steady-state diffusion methods were compared in 9 of the 11 fetuses (Fig. 3). In two fetuses, umbilical flows determined by the two techniques could not be compared because of a large inequality of antipyrine concentrations in the two umbilical veins (experiment 7) and loss of the integrated arterial sample due to breakage (experiment 10). During the injection of microspheres, the umbilical flow was not significantly different from the flow measured in the 20 minutes prior to the injection (mean percent difference $+0.3 \pm 1.8 \text{ SEM}$). The total umbilical blood flows measured by the microsphere technique are shown in Table 1.

**Changes in Cotyledonary and Intercotyledonal Flows Associated with Fetal Growth.—**In Figure 4, cotyledonary and intercotyledonary flows are plotted against fetal weight. As the fetus grows, cotyledonary flow increases.

**Discussion**

Both the steady-state diffusion and the microsphere methods for determining total umbilical blood flow have inherent although different sources of error. A possible source of error in the steady-state diffusion method is an inequality in the antipyrine concentrations in the two umbilical veins. We have confirmed the observation by Rudolph and Heymann (7) that this difference is usually small and have estimated that it introduces a 2% error in the calculation of flow. However, occasionally there is a large discrepancy between the two venous concentrations. This was demonstrated in experiment 7, in which severe hypotension in the mother when the flow was measured may have contributed to
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the discrepant antipyrine concentrations.

Since nearly all of the radioactive microspheres in the umbilical blood were cleared by the umbilical vascular bed, the microsphere method described in this paper circumvents the problem of obtaining a representative venous sample. However, a potential source of error in the microsphere method is represented by inaccuracy of umbilical arterial sampling due to either uneven distribution of microspheres in the arterial blood or faulty sampling techniques. The magnitude of this error was evaluated by obtaining blood samples simultaneously from both umbilical arteries (experiments 3, 5, 7, 8, and 11, Table 1). Our data suggest that this error is small (0.1 to 8%). A comparison of umbilical blood flows calculated by the antipyrine and microsphere methods shows that all but one of the nine observations fell within ±10% of an identity line (Fig. 3). Previous comparisons of umbilical blood flows in exteriorized fetuses measured by the antipyrine and electromagnetic flowmeter methods (7) had also shown agreement within the same limits. Thus these methods of intrauterine umbilical flow measurement, if properly performed, are free of large systematic and random errors.

The use of microspheres for measurement of regional blood flows requires that (1) the mixing of microspheres in the arterial blood be homogeneous; (2) small microspheres should be used to allow for a better resolution of regional blood flows; (3) only a negligible number should escape the target organ; (4) radioactive isotopes incorporated in the microspheres should not react with biologic fluids and become free; (5) a sufficiently large number of microspheres must be present in the structures to be analyzed; and (6) the tissue samples must be representative of the whole homogenate. All of these precautions were incorporated in our method. In addition, Phibbs et al. (12) have shown that there is no evidence of microspheres (50 μ in diameter) settling in medium-sized arteries during active flow, and Delaney and Crim (9) have demonstrated good agreement in estimating gastric flow distribution by radioactive potassium and microspheres. Thus microsphere distribution provides an accurate measurement of flow distribution to major anatomic structures, although the lower limit of resolution is still uncertain.

In this study, the average distribution of umbilical blood flow to the cotyledons was 94% of the total. In a preceding paper, we described the distribution of total uterine blood flow in pregnant sheep by a technique using microspheres 25 μ in diameter (13). During the last month of pregnancy, the average distribution of total uterine blood flow to the cotyledons was 84%, to the endometrium 13%, and to the myometrium 3%. Figure 5 illustrates the distribution of both umbilical and uterine blood flows in a sheep with a fetus weighing 3,000 g.

The information derived from these studies is relevant to analysis of data on transplacental diffusion. In experiments with the steady-state diffusion of inert molecules from fetus to mother, we have shown that the sheep placenta simulates a concurrent exchange system in which the concentrations of the
diffusing substance in the veins of the donor and recipient streams tend to equilibrate (5, 14). However, there is always inequality of concentrations in the main uterine and umbilical veins. As shown in Figure 6, the umbilical vein-to-uterine vein concentration difference for molecules with flow-limited transplacental clearance (e.g., antipyrine and tritiated water) averages 18% of the umbilical artery-to-uterine artery difference (14). The data obtained from three studies (present report, 13 and 14) permit a calculation of the umbilical and uterine venous concentration differences for antipyrine or tritiated water before admixture with noncotyledonal venous blood. These calculations have been made from the data summarized in Figure 5 on the assumption that there is a negligible exchange of a highly diffusible substance between the myoendometrial and intercotyledonal circulations. The two dotted lines in Figure 6 represent the two (fetal and maternal) cotyledonal venous concentrations thus calculated. The relative positions of these venous concentrations indicate that venous admixture of cotyledonal with non-cotyledonal blood in both uterine and umbilical circulations may explain approximately 50% of the measured concentration difference between main umbilical and uterine veins. Thus, there is a residual inefficiency of placental exchange within the cotyledons when compared to an ideal concurrent exchange system. This inefficiency could be the result of uneven perfusion ratios and intracotyledonal shunts (14). Experimental evidence (15, 16) favors this hypothesis.

The present data also contribute to a basic understanding of the respiratory function of the placenta. It is known that the umbilical vein $PO_2$ is significantly lower than the $PO_2$ in the uterine veins (17). Prior to completion of this study, one might have assumed that the low $PO_2$ in the umbilical vein was due to the presence of a large intercotyledonal shunt of umbilical blood flow, arterial-venous shunts, or both. It is now clear that this type of shunting plays a relatively minor role and that inefficiency of placental exchange represents the major factor. In addition, the combined information on diffusion of inert molecules and on flow distribution (Fig. 6) demonstrates that the pattern of flows within the placenta is an important aspect of this inefficiency.

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Circ Res. 1968;23:623-631
doi: 10.1161/01.RES.23.5.623

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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