Validation of the Antipyrine Method for Measuring Fetal Umbilical Blood Flow

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

ABSTRACT
Vinyl catheters were inserted into an umbilical vein, a hindlimb vein and either an umbilical or dorsalis pedis artery in 9 lamb fetuses with gestational ages of 110 to 147 days. The lambs were then delivered from the uterus, but placental flow was maintained. Cannulating electromagnetic flow transducers were inserted into both umbilical veins. Antipyrine solution was constantly infused into the hindlimb vein, and after equilibration, umbilical blood flow was calculated by the Fick method. The values for umbilical flow measured by the antipyrine technique were comparable with those recorded simultaneously with electromagnetic flowmeters. There was also no difference in antipyrine concentrations in the peripheral and main umbilical veins. The study thus validates the use of the Fick method with continuous antipyrine infusion for measuring fetal umbilical blood flow.

ADDITIONAL KEY WORDS electromagnetic flowmeters autoanalysis umbilical vessel catheterization sheep fetus

The first recorded attempts to measure umbilical blood flow are those of Cohnstein and Zuntz (1), who, in 1884, made direct measurements of flow in umbilical vessels of the exteriorized lamb, using a stromuhr. In 1934, Barcroft, Flexner and McClurkin (2) measured the cardiac output of 7 exteriorized lamb fetuses with open chests by means of a "cardiometer," a tambour that measured stroke output. They assumed placental flow to be one-third to two-thirds of cardiac output. More recently, Dawes and Mott (3) have taken advantage of more sophisticated instrumentation to measure blood flow in the umbilical veins with electromagnetic flowmeters. All these methods have the disadvantage that it has been necessary to remove the fetus from the uterus, and the effects of this maneuver on placental circulation are not known.

Meschia and his co-workers described a technique for measuring the umbilical blood flow of the fetus in utero (4). A double-lumen catheter was passed into a cotyledonary umbilical vein and a second catheter into an umbilical artery. Antipyrine was infused at constant rate into the distal limb of the umbilical venous catheter. After an equilibration period, it was assumed that antipyrine was being cleared from the fetal side of the placenta at approximately the same rate as it was being infused. Umbilical blood flow could then be measured by means of the Fick principle; that is by dividing the amount of antipyrine being infused per unit time by the umbilical arterial-venous antipyrine difference.

The validity of this method of determining umbilical flow has never been tested. We have compared the umbilical flow measured by the antipyrine method with the flow recorded simultaneously by electromagnetic flowmeters. We have also examined some of the potential problems of catheter placement and have modified the routes of infusion and sampling to overcome some of these difficulties in the technique.

Method
Nine pregnant ewes, with fetal gestational ages of 110 to 147 days, were given low spinal
analgesia with a mixture of 2 ml of 1% tetracaine hydrochloride (Pontocaine), 0.1 to 0.2 mg of epinephrine (0.1 to 0.2 ml of 1:1,000 solution) and 0.2 ml of 50% dextrose. The ewe was then blindfolded. Small amounts of sodium pentobarbital (60 to 120 mg) were given through an indwelling venous vinyl catheter if the ewe became restless. With the ewe lying on her right side, a vinyl catheter (0.03 inch i.d., 0.067 inch o.d.) was inserted through a superficial branch of the femoral artery and advanced into the iliac artery. A local anesthetic, lidocaine hydrochloride (Xylocaine) in 2% solution, was injected into the skin and subcutaneous tissues of the left flank, and 20 ml was injected into the peritoneal cavity. An incision 15 to 20 cm long was made and the left horn of the uterus exposed. After confirming the presence of placental cotyledons in the horn, a small incision (1 to 2 cm) was made near the junction of the horn with the uterine body toward the margin of peritoneal attachment, taking care to avoid the large uterine vessels. Careful blunt dissection through the myometrium exposed the chorion and amnion. The amnion was then gently pulled out until umbilical vessels of about 1 mm in diameter were encountered. Two 5-0 silk threads were placed under each vessel and a few drops of a solution that we have found useful in preventing constriction were applied to the vessels. This solution contains 50 ml of 1% hexylcaine hydrochloride (Cyaine) and 5.0 mg of phenoxybenzamine hydrochloride (Dibenzyline) in 100 ml of distilled water.

It was not possible to determine the direction of flow in the small umbilical veins with assurance, but the fetal side of the artery could readily be recognized from the pulsations. It was therefore particularly advantageous if an umbilical artery and vein coursing next to each other were isolated. Depending on the size of the vessels, vinyl catheters of 0.015 inch i.d., 0.035 inch o.d., or 0.030 inch i.d., 0.048 inch o.d., were inserted and directed toward the fetus. The arterial catheter was inserted for 7 to 10 cm and the venous catheter advanced 12 to 20 cm, depending on the estimated fetal size. This should place the tip of the catheter in a main umbilical vein. It was frequently necessary to lubricate the catheter with a drop of silicone oil (Dow Corning 360 Medical Fluid) to facilitate its insertion. To aid in blood sampling, the small catheters inserted into the vessels were connected to larger-bore vinyl tubing by means of cyclohexanone. The small uterine incision was then closed.

A fetal hindlimb was then recognized by palpation through the uterine wall. A purse-string suture was placed in the uterine wall and, with the limb pressed against the wall, an incision was made through the purse string. The limb was then pulled out through the purse-string suture, which could be tightened to avoid loss of amniotic fluid. A superficial vein was isolated under lidocaine anesthesia and a vinyl catheter 0.015 inch i.d., 0.035 inch o.d., was inserted. In some instances a catheter was also inserted into hind-limb and forelimb arteries. The limb was then replaced in the uterus and the purse string tied. The catheters were filled with heparinized saline. This completed the preparation for study of umbilical blood flow by the antipyrine method.

The lamb was then delivered through an incision in the body of the uterus. A saline-filled rubber glove was placed over its head before any respiratory movements could occur. The fetus was placed on a warming blanket at the level of the maternal abdomen, avoiding any tension on the umbilical cord. Rectal temperature was maintained at 38.5 to 39.5°C by covering the fetus with a second vinyl-covered warming pad. The umbilical cord was bathed with the hexylcaine-phenoxybenzamine solution and both main umbilical veins dissected free about 5 to 10 cm from the umbilicus. The lamb was given 1,000 units of sodium heparin intravenously. Thin-walled vinyl tubes, as large as could be accommodated by the umbilical vein, were connected to a 4- or 5-mm cannulating electromagnetic flow transducer and filled with heparinized saline. Great care was exercised to avoid entry of air into the system. These tubes were then inserted in each direction into a segment of umbilical vein isolated between bulldog clamps. The clamps were then removed. Flow was reestablished, and after it was clear that there was good flow in the cannulated vein, the procedure was repeated in the other vein. We used a Statham M4000 gated sine-wave electromagnetic flowmeter that presents a 400-cycle-per-second sinusoidal magnet excitation. The flow transducers were Statham Flo-probes, series QH with an internal diameter of 4 or 5 mm. The electrodes of these transducers are flush with the lumen, and the grounding electrodes from the transducer heads were inserted into the maternal abdomen. The system was calibrated by timed collections of 0.9% saline flowing through transducers. Calibrations were checked before and after each experiment and showed no variation over the period of the experiment. We have also shown that the calibration for the transducers was almost identical whether saline or blood was used, with a difference of less than 1% with hematocrits up to 60%. The electrical and mechanical zeros of the transducer flowmeter system were repeatedly checked during the experimental procedure by stopping flow, and over the experimental period there was a drift of mechanical zero of less than 2% of full-scale deflection when calibration was checked before and after each experiment and showed no variation over the period of the experiment. We have also shown that the calibration for the transducers was almost identical whether saline or blood was used, with a difference of less than 1% with hematocrits up to 60%. The electrical and mechanical zeros of the transducer flowmeter system were repeatedly checked during the experimental procedure by stopping flow, and over the experimental period there was a drift of mechanical zero of less than 2% of full-scale deflection when calibration was
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Comparison of Antipyrine Concentrations in Peripheral and Central Umbilical Veins

<table>
<thead>
<tr>
<th>Peripheral catheter position</th>
<th>Antipyrine concentration (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In major vessel</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>3.8</td>
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<tr>
<td>S6</td>
<td>4.8</td>
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<tr>
<td>S10</td>
<td>9.0</td>
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<tr>
<td>S11</td>
<td>8.6</td>
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<tr>
<td>S13</td>
<td>10.0</td>
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<tr>
<td>S14</td>
<td>5.4</td>
</tr>
<tr>
<td>In tributary</td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>4.8</td>
</tr>
<tr>
<td>S8</td>
<td>5.0</td>
</tr>
<tr>
<td>S15</td>
<td>18.2</td>
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<tr>
<td>Pointing away from fetus</td>
<td></td>
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<tr>
<td>S5</td>
<td>6.5</td>
</tr>
<tr>
<td>S6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

S = sheep.

adjusted for full-scale deflection to represent 240 ml/min of flow. The flows of the two veins were recorded simultaneously on a Grass or Offner direct-writing recorder.

Antipyrine solution (6 g/100 ml) was continuously infused by a Harvard variable-speed infusion pump, usually at a rate of 0.184 ml/min, to provide about 11 mg/min of antipyrine in a fetus with an estimated weight of 2.0 to 2.5 kg. In a full-term lamb, as much as 15 mg/min was infused. In some animals umbilical arterial and venous blood was sampled repeatedly to assess the time taken for equilibration, but all comparisons of antipyrine and flowmeter measurements were made after a minimum of 60 min of infusion. Blood samples were drawn into heparinized syringes from the umbilical arterial and venous catheters; antipyrine concentration was measured on a Technicon Autoanalyzer as described by Meschia (5). We have modified his method in the following manner: 0.6 ml of whole blood was placed in a small cup in the sampler set at 30 tests per hour with distilled water between each cup, giving a rate of analysis of 15 tests per hour. A double concentration of NaNO₂ stock solution of 4 g/1,000 ml in distilled water was used and a double dialysis performed; this gave a 20 to 25% increase in concentration peaks. A sample of fetal blood obtained before antipyrine infusion was used as a blank. Standard solutions of antipyrine containing 2.5, 5, 7.5, 10, 15 and 20 mg/100 ml were prepared. A complete calibration was run at the beginning and end of each experiment, and standards approximating the blood levels for antipyrine were interspersed between every 10 blood samples. The standard solutions of antipyrine were remarkably stable for at least 2 weeks and calibrations were almost identical from day to day.

In 9 animals, blood samples for antipyrine analysis were obtained simultaneously from the peripheral umbilical venous catheter and from each main umbilical vein; the latter samples were withdrawn by inserting a needle into the vinyl tubing connected to the flow probe.

At the end of the procedure the lamb and ewe were killed. The uterus was removed intact and the umbilical vessels were carefully dissected to locate the tip of the umbilical venous catheter.

Fetal umbilical blood flow was calculated using the Fick principle, from the equation

\[ Q_a (\text{ml/min}) = \frac{I_A (\text{mg/min})}{C_{A_n} - C_{A_v} (\text{mg/100 ml})} \times 100. \]

\[ Q_a = \text{fetal umbilical flow, } I_A = \text{amount of antipyrine being infused,} \]

\[ C_{A_n} = \text{concentration of antipyrine in umbilical (or hindlimb) artery,} \]

\[ C_{A_v} = \text{concentration of antipyrine in umbilical vein.} \]
Results

UNIFORMITY OF ANTIPYRINE CONCENTRATIONS

Since we planned to use the antipyrine method for measuring fetal umbilical blood flow of the lamb in utero, it was necessary to determine how representative of mean umbilical venous antipyrine concentration was the sample obtained from the catheter advanced from a peripheral umbilical vein.

Dissection of the placenta showed that the tip was in one of the main umbilical veins in 6 animals, in a secondary tributary in 2 and in 1 animal (in which the catheter was advanced with difficulty only 7 cm) in a peripheral vein in the horn, pointing away from the fetus and into a single cotyledon.

The antipyrine concentrations in blood from the two umbilical veins are shown in Table 1. Analysis of variance or t-test showed no statistical differences between them. There was also no statistical difference between the concentration of antipyrine obtained from blood in the peripheral umbilical vein and that in each major vein. Samples from the 1 animal in which the catheter pointed in the wrong direction showed very poor correlation with central venous concentration.

The concentrations of antipyrine were similar in umbilical arterial and hindlimb arterial blood, but lower by as much as 0.9 mg/100 ml in forelimb arterial blood in the two animals in which it was compared. This makes it impossible to use this sample for estimation of flows.

ELECTROMAGNETIC FLOWMETER RECORDINGS

The umbilical venous flow was monitored continuously with the electromagnetic flowmeter. Once the initial disturbance created by insertion of the cannulating transducers was overcome, the flows in each umbilical vein were stable for 2 hr or longer. At times a sudden movement of the ewe produced a change in the flows, usually related to a disturbance in the position of the vinyl cannulas in the veins. Repositioning reestablished the flows.

The lamb could tolerate complete occlusion of one umbilical vein for the several minutes required to insert the cannulating transducer without major disturbance. When we occluded one vein while monitoring flow in the other vein, we observed a consistent increase in flow in that vein, but the increase was not sufficient to raise flow to the control level. When the umbilical venous catheter was in the occluded vein, a marked rise in venous pressure occurred.

The actual levels of flow on the recordings could be accurately estimated to within 3 ml/min. The calibration was usually adjusted so that 6 ml/min of flow produced a deflection of 1 mm on the recording paper; it was stable and at the end of the procedure had not changed. Zero flow levels were checked repeatedly by clamping the vinyl cannula in each vein.

COMPARISON OF FLOWS MEASURED BY THE ANTIPYRINE METHOD WITH FLOWMETER RECORDING

Blood samples from the umbilical artery and vein were withdrawn for these comparative studies only after it was clear from the flowmeter recordings that umbilical venous flow had shown no major fluctuations for 5 to 10 min and had been stable for at least 2 to 3 min. The actual levels of umbilical venous flow recorded in this preparation were considerably lower than those in lambs we...
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The comparison of umbilical venous flow recorded by the two methods is shown in Figure 1. All but two of the 25 observations in the 8 animals fell within ±10% of the line of identity.

Discussion

There has been a good deal of controversy regarding the reliability of physiological measurements made in sheep and goat fetuses exteriorized from the uterus but with utero-placental circulation still continuing. There has been wide variation in the magnitude of reported fetal umbilical flows. The most recent report of values obtained by direct measurement with electromagnetic flowmeters showed umbilical flows of 217 ± 19 SEM ml/min per kg fetal weight for lamb fetuses 87 to 95 days old, and 170 ± 14 ml/min per kg for fetuses aged 137 to 141 days (3).

Meschia et al. (4), using the antipyrine method with the lamb in utero, reported umbilical flows of 233 ± 19 ml/min per kg in lambs aged 102 to 139 days. They suggested that the lower levels in Dawes' and Mott's studies were due to interference with placenta flow related to the experimental procedure; they further proposed that delivery of the fetus at 87 to 95 days produced less interference with fetal circulation than delivery at a later gestational age, thus explaining the variation in umbilical flows. This contention was based on the assumption that the antipyrine technique provided accurate measurements of umbilical blood flow, though it had not been checked by any other method.

We have demonstrated that if certain precautions are taken, umbilical blood flow can be measured with reasonable accuracy by the antipyrine method, not only when flow is normal but also when it has been markedly reduced, and there may be some degree of separation of placental attachment to the uterus.

A point of major concern to Meschia et al. (4) and to us was whether the blood obtained from the umbilical venous catheter was representative of mixed umbilical venous blood. Since the two umbilical veins in the lamb join only within the abdomen, and since each vein receives the drainage of a specific group of cotyledons, it is possible that differences in diffusion rates across the placenta in different cotyledons could have caused differences in antipyrine concentration in the two umbilical veins. It was therefore gratifying to find that the concentrations in the two main umbilical veins, as well as in the tributaries, were almost identical. It is important to be sure that the umbilical venous catheter is directed toward the fetus; the catheter, even if inserted in the correct direction in the vein, may accidentally pass peripherally. This occurred in one of our lambs; the catheter was almost lodged in a cotyledon, and the antipyrine concentration was considerably lower than in the central vein.

We modified and also, we believe, simplified Meschia's techniques in several ways. Instead of using a double-lumen catheter in the umbilical vein both for infusion and sampling, we infused into a fetal hindlimb vein. This provided a similar distribution of antipyrine, except for the fact that the initial high concentration bypasses the liver. The infusion of antipyrine into the umbilical vein could result in some difficulties. If the tip of the catheter coiled on itself, the infusion site would be very close to the sampling site (we have noted this complication). Since the tip of the infusion catheter is 10 cm upstream of the opening of the sampling catheter, the site of sampling is quite proximal and is therefore less likely to sample from one of the main veins. Also, the large outer diameter of the double-lumen catheter may obstruct the vessel lumen, particularly in a small fetus.

The finding of a different concentration of
antipyrine in the forelimb artery is not surprising, since there are several shunting sites in the fetus.

The use of 0.6 ml of whole blood, instead of 0.4 ml of plasma as suggested by Meschia, simplified the analysis. The blood sample without antipyrine produced a very small deflection on the autoanalyzer, but this was not greater than that produced by fetal plasma. A small amount of infused antipyrine is cleared in the fetal kidneys and a small amount enters the total fetal body water as the arterial concentration slowly rises; also there is some equilibration with amniotic fluid. Meschia et al. (4) estimated that up to 3% of the infused antipyrine is lost by these routes. We felt that since renal blood flow is very low in the fetus, and since the fetal arterial concentration changes very slowly, these corrections were not important. If we did adopt a correction of 3%, the calculated flows would be decreased by this percentage, but this would not significantly improve the correlation observed with the electromagnetic flowmeter measurements.

On the basis of our studies we conclude that the antipyrine method is a useful and reliable method for measuring fetal umbilical blood flow.

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