Oxygen and Antipyrene Diffusion During In Vitro Human Placental Perfusion*

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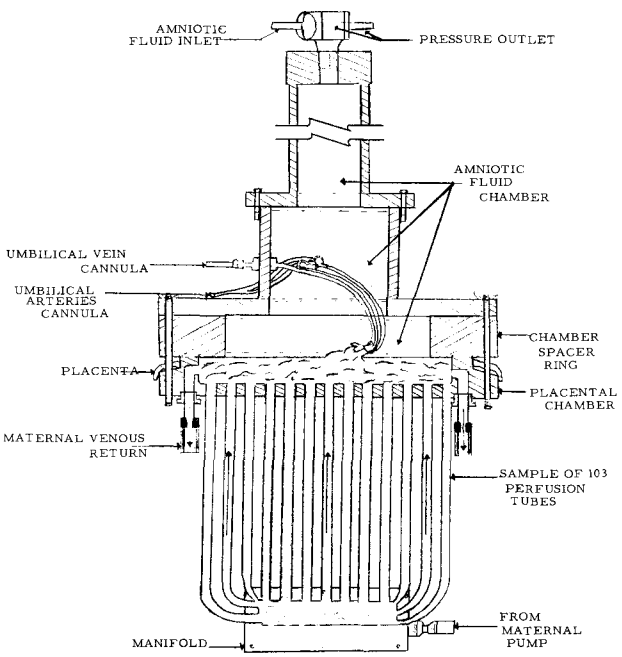
Abstract

Using an in vitro placental perfusion apparatus, the diffusion of oxygen and antipyrene across the human placenta was studied. At varying levels of maternal pO^2 there was no equilibration of fetal pO^2 across the placenta. Antipyrene diffusion was relatively slow. It is suggested that this apparatus is not physiologically accurate and that any further data from these in vitro methods be accompanied by measurements of other physiological parameters.

SCHEMATIC OF THE
ARTIFICIAL UTERUS AND AMNION CHAMBER



CROSS SECTION OF ARTIFICIAL UTERUS AND
AMNION CHAMBER

Introduction

The in vitro placental perfusion apparatus described by Krantz, et al (1) is designed to physiologically maintain the maternal and fetal placental circulations. While using this apparatus to study the placental transport of free fatty acids we noticed the apparent poor oxygenation of the fetal circulation blood.

Therefore, we studied the adequacy of this perfusion system by measuring oxygen in the maternal and fetal circulations, the diffusion of antipyrene, and the distribution of dye injected into the circulation.

Apparatus

The apparatus as described by Krantz, et al (1) includes a fetal circulation system, a maternal circulation system, and an artificial uterine chamber with a placental retaining ring.

In the maternal circulation system blood is propelled by a double roller pump into a manifold where the flow is divided among one hundred three (103) semirigid polyethylene tubes, (artificial spiral arteries), 0.840 cm I.D. which protrude o.4 cm into the uterine chamber and into the intervillous spaces of the placenta where maternal-fetal exchange takes place.

The placenta is held in place around its periphery by a retaining ring and spacer. Intimate contact between the artificial spiral arteries and the intervillous spaces is maintained by exerting a hydrostatic force of approximately 26 mm Hg on the fetal side of the placenta through the amniotic fluid chamber. (see Schematic)

The maternal venous blood is collected from the peripheral region of the placenta through four tubes communicating externally through the lateral wall of the artificial uterus. The venous return is then delivered to a disc oxygenator, (thirty 2.5 inch discs) and then by gravity flow into a reservoir with a water coil heat exchanger. From the reservoir the blood is delivered back to the pump for recirculation.

The fetal circulation system consists of a pulsatile flow pump delivering blood to both umbilical arteries through two polyethylene cannulas of 0.2 cm I.D. The venous return is collected by a single 0.3 cm. I.D. cannula inserted in the umbilical vein. the fetal blood then flows through a flowmeter into a reservoir with heat exchanger, (water bath) and then back to the pump for recirculation.

Materials

The maternal perfusion solution contains 6.74 g NaCl, .345 g KCl, .359 g $MgCl^2$, .238 g Na^2SO^4, 2.27 g $NaHCO^3$ .026 g KH^2PO^4 and .011 g Na^2HPO^4 per liter.

Fetal perfusion solution consists of 1 volume of heparin (10,000 USP units), 40 volumes of Rheomacrodex* and 260 volumes of maternal perfusion solution.

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In the maternal circulation citrated whole blood from the blood bank was titrated to pH 7.2-7.4 with o.3M THAM.** Fetal blood or sedimented adult blood from the blood bank, diluted 1:1 with fetal perfusion solution was used in the fetal circulation. Both fetal and adult blood used varied in length of storage. No difference in results was seen between blood collected on the day of perfusion from that which had been stored.

Oxygenation of the blood was achieved using 5 percent CO^2 in oxygen or 100 percent O^2.

Amniotic solution consisted of 6.01 g NaCl, .409 g $MgCl^2$, 1.95 g $NaHCO^3$, 0.052 g KH^2PO^4, and a .022 g NaH^2PO^4 per liter.

Procedures

A) Temperatures were determined by thermistors in the arterial side of each circulation near the point of entry to the placenta and near the site of blood sampling (as close to possible to the point of diffusion). Earlier observations showed that the temperature within the reservoir was not identical to that the the site of blood gas sampling.

B) Pressure was read in the arterial circulations from mercury monometers and is expressed as the maximum systolic pressure.

C) Flow was measured by diverting the venous circullation and collecting the perfusate in a graduated cylinder. Flow was measured before and after each blood sample was collected and was constant during that interval.

D) For gas studies blood was collected in glass syringes from sites closest to the placenta and near the site of temperature recording. The samples were immediately placed in ice and analyzed within 30 minutes. Samples were taken simultaneously from the maternal and fetal arterial and venous circulations.

Radiometer[2] equipment was used with the water bath reading 37.5C. Components consisted of a microelectrode pH meter 27, a platinum pO^2 electrode (E-50460), and a Severinghaus PCO^2 electrode (E-50360). The electrodes were calibrated immediately before each analysis. The oxygen electrode was calibrated using sodium bisulphite in borax solution for a zero point and room air for a high point.

For the calculations PO^2 was corrected to a temperature of 37°C. and a pH of 7.4(2). Oxygen saturation of hemoglobin was derived from a nomagram (2,3). Hemoglobin was calcualted as hematocrit x 0.32 grams percent for adult blood or hematocrit x 0.38 for fetal blood. The oxygen content was then calculated as follows: o^2 content (ml%) = Hemoglobin (gm%) x % saturation X 1.34 + pO^2 (mm Hg) X 0.003.

The oxygen consumption was calcualted as difference between arterial and venous o^2 content X flow (ml/min) x 10^{-2} giving O^2 consumption in ml/min per whole placenta.

E) Perfusion—The placenta was collected at the time of delivery and immediately the umbilical vein was injected with 50 ml of heparin (10,000 USP units) in maternal perfusion solution. The selection of placentas seriously limited the samples adequate for perfusion. The placenta had to be of the correct size with a fairly central insertion of the umbilical cord; adequately intact membranes for attachment to the apparatus and no obvious tears in the maternal surface.

The umbilical vessels were catheterized; the placenta was placed on the perfusion apparatus and the perfusion begun within 30 minutes of delivery with maternal and fetal perfusion solutions. "Amniotic" solution was added over the fetal surface of the placenta. 1000 ml of adult blood was added to the maternal reservoir while draining off 600 ml of perfusion fluid.

Diluted as previously described, 400 ml of sedimented adult or fetal blood cells were added to the fetal reservoir while draining off 200 ml of perfusion fluid. The longest period of perfusion was three hours.

F) One gram of antipyrene in 50 ml of basic perfusion solution was added to the maternal reservoir. Specimens for antipyrene determination were gathered from the maternal and fetal arteries and analyzed spectrophotometrically (4).

G) At the conclusion of several perfusions 50 ml of concentrated Toluidine blue dye was introduced into the maternal circulation. Perfusion was continued for twenty minutes to allow maximum penetration of the dye. After stopping the perfusion the placenta was immediately submerged in 30 percent formalin in water and "fixed" for six days. Following this histological sections were cut and mounted.

Results

Table 1 gives the range of values for temperature, arterial pressure, flow, and placental weight. Compared to probable IN VIVO values, the fetal arterial pressur is above normal and the fetal flow rate is below normal (5). When greater flows were attempted by raising the fetal pressure, a fetal to maternal circulation leak was a frequent complication. The data in this study was taken only from those perfusions where such leaks were not present. This was determined from the stable volume of blood in the respective reservoirs.

Table 2 shows the pO^2 values using different placentas. As would be expected the fetal artery and vein pO^2 are similar

Table 1. Physiological Parameters During Perfusion.

	Range of Values Maternal	Fetal
Temperature (Co)	30.6-37.8	33.0-38.2
Arterial Pressure (mm Hg)	40-60	70-130
Flow (ml/min)	300-776	40-114
Placental Weight (gm)	501-660	

since there is no tissue for oxygen uptake interveing. The difference in pO^2's between the maternal artery and vein represent oxygen consumption by the placenta. In Figure 1 it is seen that increases in the maternal pO^2 are not accompanied by increases in the fetal pO^2 as would be expected from previous publications using the apparatus (1) and from IN VIVO work with sheep (6).

When the maternal circulation was oxygenated, the oxygen consumption of the whole placenta ranged from 8.38 ml/min to 1.83 ml/min. In the literature recorded values have been recalculated and are 0.2-1 ml per 100 g net weight (6). If these are converted to whole placenta figures (x 600 g), the values become 1.2-6.0 ml per minute, which is similar to our range of values.

Similarly from IN VITRO placental tissue incubations the rate of 2.10 ml/min per placenta may be calculated in terms of whole placental oxygen utilization and again were found to be within the above range. As seen from Table 2 the amount of oxygen consumed did not vary directly with the maternal pO^2.

The lack of equilibration of the fetal circulation pO^2 to the maternal pO^2 led us to believe adequate diffusion was not occurring. We studied the equilibration of antipyrene between the two circulations. When 1 gram of antipyrene was added to the maternal circulation, fetal levels reached 85 percent and 98 percent of the maternal levels only after 60 minutes and 45 minutes respectively after injection (antipyrene diffuses at the same rate as water).

When toluidine blue was injecged into the maternal circulation, the maternal surface of the placenta was deeply stained by the dye. The cut surface of the placenta did not show any gross penegration of the dye.

Multiple slices were made to include the placental areas which were overlying the "spigots" of the maternal circulation. Discoloration was not found to be greater over these areas than the placental tissue between the "spigots".

Near the basal plate miscropsopic sections revelaed some dye staining occasional villi. A few of the red blood cells in the intervillous spaces near the basal plate were stained. No stain was found high in the placenta approaching the fetal amnio-chorionic membranes.

Discussion

The use of IN VITRO placental perfusion where the maternal spiral artery is simulated have been reported in this country by Krantz, et al (1) and Keller, et al (8). It is obvious that the chief anatomic difficulty in simulating the maternal spiral artery is penetrating the maternal decidua remaining on the placental surface and extending into the center of individual cotyledons. This shortcoming was well shown by our dye injections where no high penetration of the cotyledon by the dye occurred.

Table 2. Oxygenation of Blood in the Placental Circulation in Vitro.

	Perfusion Number	A) Maternal Artery pO_2 (mm Hg)	Maternal Vein pO_2	Fetal Vein pO_2	Fetal Artery pO_2	O_2 Consumption ml/min/placenta
a	4	35	30	16	17	7.77
b	10	50	43	32	28	3.76
b	11	62	42	22	24	8.38
c	6	69	55	28	27	1.83
	5	120	73	34	35	3.02
c	2	151	89	35	36	2.86
	9	151	87	17	19	3.15
	8	219	157	21	22	1.49
	7	362	115	40	42	6.17

	B) Fetal Artery	Fetal Vein	Maternal Vein	Maternal Artery	
3	280	46	23	23	2.78
c 6	528	59	50	53	1.51

Figures represent pO_2 in mm Hg corrected to 37° C. and pH = 7.40 (2).

A) Varying levels of pO_2 with the oxygenator in the maternal circulation.

B) Varying levels of pO_2 with the oxygenator in the fetal circulation.

a Oxygenator OFF
b Antipyrene Experiments
c Fetal Blood Used in the Fetal Circulation

The demonstration of oxygen diffusion across the placenta would be the best criterion of proving physiological adequacy of the apparatus. Keller, et al (9) argue that the adequacy of oxygen diffusion with their apparatus is shown by the maintenance of a fetal heart beat when a fetus and attached placenta were subjected to the IN VITRO maternal circulation. The prolonged maintenance of a heart beat in very premature infants who are not breathing after birth is a known clinical occurrence so this finding during IN VITRO perfusion is not proof of transplacental oxygenation. They mention visual evidence of the oxygenation of the fetal blood. No oxygen measurements were given.

Krantz, et al (1) state that when adult blood was used in the fetal circulation, the fetal pO^2 was 35 mm Hg at a maternal pO^2 of 250 mm Hg. When fetal blood was used in the fetal circulation, "the pO^2 of the blood completely equilibrated with maternal venous pO^2 each registering 135 mm Hg." We were unable to demonstrate this phenomena in our perfusions either with adult or fetal blood. Krants, et al do not give adequate data to evaluate this statement.

In no IN VIVO experiment has complete equilibration of pO^2 occurred between the maternal and fetal circulation. This may be explained by oxygen consumption by the placenta, shunting of fetal umbilical blood away from the placental membrane, uneven distribution of maternal and fetal blood perfusing the placenta, and resistance of the placental membranes to diffusion (10).

Table 3. Data from Perfusion #10

	Flow	Pressure	Temp	pH	pCO^2	pO^2	Hct
Maternal Artery	—	55	37.5	7.25	40	45	22
Maternal Vein	580	—	—	7.24	40	36	—
Fetal Artery	—	90	37.6	7.25	43	25	20
Fetal Vein	90	—	—	7.23	41	24	—

The figures are flow = ml/min; pressure = mm Hg; Temp = °C.; pCO^2 = mm Hg; pO^2 = mm Hg (uncorrected for temp and pH).

Figure 1. Legend. Changes in umbilical vein pO^2 with varying maternal arterial oxygenation. Each figure represents a different placental preparation.

However, we would expect increases in the maternal pO^2 to be reflected to some degree by increases in the fetal pO^2 as previously shown with sheep (6). In the absence of any such relationship we must presume that diffusion is not occurring to the extent seen in IN VIVO experiments. An alternate explanation is that the oxygen consumption of the placenta is increasing as the maternal pO^2 increases. However, this is not the case in our experiments.

Our oxygen consumption values fall in the range previously reported in studies where the fetal circulation only was perfused (6) and IN VITRO studies of placental homogenates (7). This can be explained in at least two ways. Oxygen diffusion may occur primarily higher in the intervillous space (where perfusion was inadequate), whereas the major site of placental oxygen consumption may occur lower in the intervillous space (where perfusion was adequate). A more likely explanation is that all the figures on oxygen consumption in human placental tissue are incorrectly low.

It was of interest that there was relatively rapid equilibration of anti-pyrene between the circulations. There is no IN VIVO data on humans with which to compare. In the pregnant monkey (11) equilibration of 4 amino antipyrene IN VIVO is much more rapid than our results. The portion of the blood volume exposed to the placenta per minute in those experiments were very small, whereas in our IN VITRO perfusion, the entire blood volume was exposed to the placenta every two to four minutes. We should have expected much more rapid equilibration than the IN VIVO experiment.

We would hope, in the future, that when IN VITRO placental perfusion studies are reported, a more critical evaluation of the adequacy of the perfusion method will accompany the data since our results seriously question the physiological adequacy of this system.

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In Vivo

Correspondence from Our Readers

Dear Sir:

We have read with interest your Winter Vol. III No. 1, particularly the article on page 18 concerning organs and tissues. While we are pleased that Pyrolite is becoming an accepted material in the area of the prosthetic heart valves, we consider the general statement made concerning the wear life premature. While we consider Pyrolite coatings have excellent wear characteristics, we consider the statement which implies a wear life of about 500 years may be misleading since it is taken out of context and needs additional qualification.

Yours sincerely,

Jack C. Bokros

Dear Ed:

It is always a genuine pleasure and a challenge for me to assist in the establishment of a new dialysis program. The Olive View Medical Center is one of the most remarkable hospitals I've seen. It has a total bed capacity of 850 beds.

This facility will be a twenty bed unit ultimately and will be set up for two (possibly three) six-hour shifts daily, The patients will share machines and we will be utilizing the coil reuse technique.

I would at this point like to define minimum supervision. Mrs. Irene Maples, Head Nurse, will be in charge of training a few R.M.'s and many L.V.N.'s and aides to handle the nursing aspect of dialysis. I will be in charge of training technicians to handle the technical aspect of dialysis and for maintaining the overall quality control of the dialysis unit. The line between the nurse and technician will intersect at many points. This will consist of hooking-up patients, declotting patients, teaching cannula care and other areas formerly taboo in certain parts of the country to the technician.

Yours truly

Leon C. Farbes
Chief Technician, D Dialysis Unit
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