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A Morphometrical Analysis of the Guinea Pig Placenta after Chronic Exposure to Carbon Monoxide

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Abstract

A MORPHOMETRICAL ANALYSIS OF THE GUINEA PIG PLACENTA AFTER CHRONIC EXPOSURE TO CARBON MONOXIDE

By Marcella J. Woolsey

We used morphometric analysis to determine if placental structure is modified by carbon monoxide. Camm-Hartley guinea pigs were exposed throughout gestation to chronic conditions of carbon monoxide (averaging about 180 parts per million). Maternal weight, placental weight, fetal weight, and fetal length were recorded at the time of placental fixation. Using a computerized image analyzer on photomicrographs obtained through light microscopy, we measured maternal and fetal percent vessel volumes, surface areas, and vessel numbers. Maternal vessel numbers and surface area remained basically unchanged but decreased slightly. Maternal percent volume decreased 15%. However, fetal capillary number increased 35% ($p<0.01$) from a control value of 2325 ± 184 per mm^2 (SE of mean) of tissue surface to 3140 ± 101 (SE of mean) per mm^2 of tissue surface and total fetal surface area increased 30% ($p<0.05$) from 0.430 ± 0.038 (SE of mean) m^2 to 0.558 ± 0.041 (SE of mean) m^2. Fetal percent volume remained unchanged. The ratio of placental to fetal weight increased 25% ($p<0.01$) from 67 ± 3 (SE of mean) g/kg to 84 ± 4 (SE of mean) g/kg. Fetal weight was correlated with both placental weight ($p<0.01$) and with total maternal surface area ($p<0.02$). The slight decrease in maternal surface area suggests that a slight decrease in placental function as determined by diffusing capacity should occur, while the increase in fetal capillaries suggests a compensatory response to hypoxic conditions.

LOMA LINDA UNIVERSITY

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Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Arts.

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INTRODUCTION

Maternal hypoxia, including both acute and chronic exposure to low oxygen concentrations, high altitude, and carbon monoxide is associated with several effects on the fetus; decreased body weight (Asmussen, '80; Delaquerriere-Richardson et al., '65; Fechter and Annau, '77; Gilbert et al., '79; Penney et al., '80), decreased litter size (Penney et al., 80; Williams and Smith, '35), changes in organ to body weight ratios (Garvey and Longo, '78; Gilbert et al., '79; Penney et al., '80), increased hematocrit (Delaquerriere-Richardson and Valdivia, '67; Gilbert et al, '79), and limb deformities (Astrup et al., '72).

Low arterial oxygen content from carbon monoxide exposure, due to the displacement of oxygen from hemoglobin, decreases the oxygen carrying capacity of arterial blood. Availability of oxygen to the tissues is further lowered by the increased affinity of hemoglobin for oxygen which is demonstrated by the left shift of the oxyhemoglobin curve (Roughton and Darling, '44). Longo ('76) and Longo and Hill ('77) have shown in fetal sheep that arterial oxygen tensions are particularly sensitive to changes in carboxyhemoglobin levels, especially after maternal exposure to carbon monoxide. The partial pressure of oxygen in fetal blood decreases in proportion to increased carboxyhemoglobin concentrations.

Although the adult may react to carbon monoxide hypoxia by increasing cardiac output (Ayres et al., '70), Longo et al., ('78) found in their comparative study that fetal lambs did not significantly increase cardiac output in response to hypoxic hypoxia or carbon monoxide hypoxia. Rather, fetal circulatory response is a redistribution of peripheral flow. Gilbert ('80) showed in sheep that fetal cardiac output (already four to

five times that of the adult per unit weight) cannot be increased by a significant amount because the fetal heart normally operates near the plateau of its Starling function curve.

However, an attempt at compensation seems to have occurred in guinea pigs which showed a 14% increase in absolute placental carbon monoxide diffusing capacity after chronic gestational exposure to 12% oxygen (Gilbert et al., '79). Placental diffusing capacity is defined as the rate of gas crossing the placenta for a given difference in the partial pressures between maternal and fetal blood expressed as ml/min. Torr. Carbon monoxide is used in place of oxygen to measure diffusing capacity because, unlike oxygen, it is not consumed in any significant amount by either the uterus or the placenta, and uneven flow distribution does not affect the carbon monoxide exchange or the mean partial pressure gradient in any appreciable degree (Longo et al., '67). Placental diffusing capacity affects fetal and maternal end-capillary oxygen partial pressures, umbilical oxygen partial pressures, and the placental oxygen exchange rate (Longo et al., '72). Possible limiting factors for diffusing capacity are capillary blood volume, placental surface exchange area, placental tissue thickness, and blood diffusing capacity.

Oxygen capacity of blood, one of the factors affecting blood diffusing capacity, is reduced by increasing blood carboxyhemoglobin concentrations. Would any of the placental parameters also be modified by carbon monoxide exposure? And would these modifications reflect changes in placental diffusing capacity?

In an attempt to answer these questions we used stereological

techniques to measure maternal and fetal surface areas, maternal and fetal percent vessel volumes, and mean diffusion distance (placental tissue thickness) in near term guinea pigs that had been exposed to carbon monoxide throughout pregnancy.

MATERIALS AND METHODS

We obtained 64 date-bred female Camm-Hartley guinea pigs 12 to 18 days pregnant. On their arrival we numbered, weighed, and assigned them to one of two groups: 1) the control group or 2) the carbon monoxide (CO) hypoxia group, each group containing 32 animals. The guinea pigs were kept in individual cages, fed Purina guinea pig chow, given water ad libitum, and weighed weekly.

The cages containing the CO group were placed in a plastic tent in a room separate from the controls. A mixture of compressed air and carbon monoxide entered the tent at a flow rate of 25 to 30 ml/min. We selected this rate to prevent carbon monoxide, water vapor, and ammonia from building up inside the tent. We monitored carbon monoxide concentrations two to three times daily (Ecolyzer, Energetics Science Inc.). They averaged about 180 parts per million (ppm) (range of 90 to 210 ppm). The tent was opened two to three times a week for cage cleaning and/or animal weighing.

At gestational age 63 $\pm$ 2 days we removed the animals from their cages, weighed each mother, and gave her a systemic anesthetic (Pentobarbital Sodium Solution, 60 mg/kg body wt) intraperitoneally. The abdominal area was shaved and injected with a local anesthetic (Lidocaine HCl 10%, 3-5 ml). The uterus was exposed with a mid-line incision and the uterine horns with their fetal contents gently expressed from the abdominal cavity. We used only one placenta from each mother to avoid any possible tissue changes due to delay in perfusion. The uterine wall and the amniotic sac were cut and retracted

to expose the umbilical vessels. Fetal position was adjusted to allow unobstructed access to one of the umbilical arteries, which we cannulated with a 22 gauge needle connected by tubing to an infusion pump (Model 922, Harvard Appl. Co. Inc.). Both the needle and the tubing were flushed with heparinized saline prior to each perfusion. A pressure transducer (Mark 200, Brush Instr.) recorded perfusion flow pressure.

After arterial cannulation, we activated the infusion pump, simultaneously cut the umbilical vein, and clamped the umbilical cord between the fetus and the cannula. We followed a modification of the perfusion method described by Kaufmann and Davidoff ('77) using 2.2% glutaraldehyde (pH = 7.28 to 7.33, 380 mosmols) and a flow rate of 1.8 ml/min. Maximum flow pressures usually reached 20 to 30 mmHg after which we continued perfusion for several minutes. The perfused placenta and fetus were then removed from the uterus and each weighed separately. The fetus was measured for crown-rump length.

After using a sharp razor blade to make a vertical section through the placenta near the umbilical cord, we took a 1 mm thick slice from one of the exposed halves. This section was trimmed to a 4 to 5 mm wide strip and further sectioned into approximately 4 x 4 x 1 mm squares. The tissues were placed in the phosphate-buffered 2.2% glutaraldehyde for two hours, washed with a phosphate buffer (pH = 7.2 to 7.3, 145 mosmols), immersed in 1% osmium tetroxide (pH = 7.3, 215 mosmols) for two hours, washed with a 1:1 ratio of one part sugar phosphate buffer (0.2 M phosphate plus 0.2 M sucrose) to one part distilled water,

dehydrated by passage through a graded series of alcohol, and embedded in Epon as described by Luft ('61).

The blocks of tissue were sectioned at 1 μm (Porter-Blum Ultramicrotome MT-2, Ivan Sorval, Inc.), stained at 70°C with a 4:1 ratio of 1% toluidine blue: 1% pyronin G basified with several drops of NaOH per 10 ml of stain.

We used random selection of tissues for both embedding and sectioning. Five out of the 10 to 12 pieces of tissue per placenta were embedded in Epon. Usually two of these five blocks were sectioned and photographed (Zeiss Photomic 3, 40X oil-immersion objective). We selected the area to be photographed by focusing on an edge of the interlobium and then further moving the micrometer stage precisely 1 mm to bring the peripheral labyrinth (zone of gas exchange) into view. Usually two pictures per block were taken, making a total of four pictures per placenta. We averaged the measurements from the four pictures to give the values for each placenta.

We used a computerized image analyzer (Zeiss Videoplan, Carl Zeiss, Inc.) to make the stereological measurements. After identifying and marking maternal and fetal vessels on the photographs, we traced their perimeters with a stylus connected to the image analyzer, recording maternal and fetal vessels separately for each placenta. The Videoplan computed the areas contained within the tracings and the sum of the areas, the percent of vessel area measured as compared to the total picture area, the perimeters and the sum of the perimeters of the vessels, and the number of vessels measured.

We considered the percent areas of the maternal and fetal vessels, averaged for the four pictures, to be equal to the maternal and fetal percent volumes respectively (Weibel et al., '66).

Absolute surface area per unit volume (mm^2/mm^3) was determined for each placenta using the following stereological equation from the Videoplan Operation Manual:

$$SV = \frac{(U)(4)(mm)}{(AT)(\pi)(mm)}$$

where U is the total perimeter of maternal or fetal vessels averaged for the four pictures and expressed in mm, (4) is a constant to correct for vessel orientation within the tissues, and AT is the average total area in mm^2 of the four pictures. Since the peripheral labyrinth composes 70% of placental volume (Kaufmann and Davidoff, '77), total surface area was determined by multiplying the absolute surface area in m^2/cm^3 by 70% of placental weight in grams.

Diffusion distance or average membrane thickness was found by dividing the tissue volume (average total area percent volume minus maternal and fetal area percent volumes per picture) by one-half the sum of the fetal and maternal surface areas (Kaufmann and Davidoff, '77).

We used the Student's-t test and linear correlation to evaluate differences between the control and CO hypoxia groups.

RESULTS

Of the 64 animals only 31 were actually pregnant at the time of study. Of these, two control and six CO mothers delivered before surgery. We did not use the placenta from the one mother who died before perfusion was completed. This left a total of 13 control and 9 CO hypoxia guinea pigs.

Table 1 presents the data for maternal weights, fetal weight, placental weight, fetal length, and the ratio of placental to fetal weight.

Figure 1 shows a representative section from a control placenta and is illustrative of the photomicrographs from which our measurements were taken. Table 2 presents these stereological measurements of vessel volumes, surface areas, average duffusion distance, and the number of vessels.

We found no significant differences between control and CO hypoxia animals in initial maternal weight, term maternal weight, or fetal length. However, a marginal 10% decrease in fetal weight, a 12% increase in perfused placental weight, and a 25% increase ($p < 0.01$) in the ratio of placental to fetal weight occurred in the CO hypoxia group.

Stereological data for maternal values showed a slight decrease in the absolute maternal surface area (7.6%) as well as in the maternal lacunal percent volume (15%) for the CO hypoxia group. However, total maternal surface area and the number of maternal lacunae remained basically unchanged.

Fetal surface area increased for both the absolute surface area (16.5%) ($p < 0.05$) and the total surface area (29.6%) ($p < 0.05$) (fig. 2).

The number of fetal capillaries also increased (35%) ($p < 0.01$) (fig. 3). However, fetal capillary percent volume did not change. Thus fetal capillaries in the CO hypoxia group were smaller, maintaining the same total percent volume while showing an increase in surface area.

Tissue percent volume increased 12%. We qualitatively observed that this increase seemed to be in the connective tissue associated with the fetal capillaries. Diffusion distance also increased 13%.

Fetal weight was highly correlated with placental weight (fig. 4) and with total maternal surface area (fig. 5) for both the control and the CO hypoxia groups.

We compared the data we obtained for control maternal and fetal percent volumes, percent tissue volume, and maternal and fetal surface areas to values obtained in earlier studies (Table 3). Measurements were made with similar techniques and pressures. However, the data from Smith et al. are for placentas perfused in animals which have been given euthanasia 5 to 10 minutes earlier. Our values incorporate the constant '4' in the surface area equation while the other studies use a modified form of '3.46' (Stelter, '79).

The range of values for each measurement indicates the variability which can result even with similar techniques. The differences may be due to individual variations in actual perfusion technique, blockage of perfusion flow within the vessels, time delay in perfusion, or handling of the tissues immediately after perfusion.

DISCUSSION

Morphometry is a valuable tool for equating physiological functions with anatomical structures. Several studies on the human placenta (Aherne, '75, '66; Aherne and Dunnill, '66b; Bhargava et al., '76; Clavero and Llusia, '63) quantitatively analyzed normal and/or pathological tissues. Some data is also available for several experimental animal placentas. Baur ('77), Firth and Farr ('77), and Kaufmann and Davidoff ('77) described age-dependent changes and/or normal term structural features in quantitative terms. However, there are few studies such as Tominaga and Page ('55) which combine morphometry and physiology under experimental conditions in an attempt to evaluate changes in function rather than limiting the study to description.

Our results indicate that guinea pig placental tissue is modified by changes in physiological conditions due to carbon monoxide exposure. We found that carbon monoxide modifies vessel numbers, surface area and vessel volumes of the placenta.

Effects on the fetus A study of pregnant rats exposed to 150 ppm of carbon monoxide continuously throughout gestation (Fechter and Annau, '77) shows only a slight 3.3% decrease in birthweights. The 10% decrease which we found is closer to that given by Astrup et al. ('72) for young born to rabbits exposed 30 days to a continuous concentration of 90 ppm of carbon monoxide. However, rabbits in that study showed a 20% decrease in birthweights after exposure to 180 ppm of carbon monoxide.

Fetal length (crown-rump) is considered by Kaufmann and Davidoff

('77) to be a more accurate indicator of fetal development than fetal weight. The similar results between the two groups which we obtained for both fetal length and fetal weight seem to indicate that exposure to the given level of carbon monoxide did not result in a significant retardation of intrauterine growth.

Effects on the placenta Though Thompson et al. ('69) felt that placental weight is not a valuable method for indicating placental functional adequacy, Aherne ('66) and Aherne and Dunnill ('66a) felt it important due to the proportionality they found between human chorionic villous surface area and fetal weight and between chorionic villous surface area and placental weight. This agrees with the high correlation which we found between fetal weight and placental weight and between fetal weight and total maternal surface area, indicating a placental response to fetal metabolic requirements.

The mean placental weight increase in the CO hypoxia animals is opposed to the mean fetal weight decrease, though both changes are slight. This gives a higher ratio of placental to fetal weight in the CO hypoxia animals. Increases in placental to fetal weight ratio are also reported by Gilbert et al. ('79) for hypoxic hypoxia guinea pigs. The increase in placental weight in our animals may be due to edema as has been suggested for the increased brain weights of pregnant rats exposed to carbon monoxide during gestation (Garvey and Longo, '78), increased blood volume or an increase in total tissue. Thompson et al. ('69) suggested from Fox's ('64) report of increased Langhans cells in the human placenta during preeclampsia that chronic hypoxia may cause

some placental hypertrophy. Although our measurements do not show a significant increase in the amount of tissue present in the CO group, it might be worthwhile to make nuclei counts of the two groups to verify that no differences actually exist.

The fetal capillary response of the CO hypoxic guinea pigs is similar to the increase in muscle capillaries which Cassin et al. ('71) reported for rats under chronic hypobaric conditions. They demonstrated through endothelial cell nuclei count that the increase is due to the opening of preexisting capillaries rather than to an increasing vascularity. However, our study indicates the increased capillary number to be due to an increase in vascularity.

The increase in the number of fetal capillaries did not, however, seem to result in a closer proximity between maternal and fetal vessels, for we found a nonsignificant increase in diffusion distance from 3.69 μm to 4.14 μm. Our results at first seem opposed to those of Tominaga and Page ('66) who found cytoplasmic thinning of syncytiotrophoblastic tissue and fetal vessel dilation in human placentas exposed to hypoxic hypoxia. They suggested that these results represent placental accomodation to reduce diffusion distance. However, the guinea pig placenta does contain thin syncytial lamellae similar to the epithelial platelets in the human placenta (Enders, '65; Kaufmann and Davidoff, '77). Kaufmann studied representative samples of our tissues with the electron microscope and reported (personal communication) that these thin syncytial lamellae, though of normal shape and extension in the control group, were extremely thin over short distances in the CO tissues.

Changes in diffusion distance in the guinea pig may be limited to these zones.

Placental diffusing capacity and placental changes Smith et al. (unpublished) performed stereological measurements on placentas of guinea pigs subjected to varying degrees of exercise and reported that maternal surface area seemed to be the placental parameter most closely correlated with diffusing capacity.

Gilbert et al. (unpublished) measured carbon monoxide diffusing capacity in guinea pigs subjected to 180 ppm of carbon monoxide throughout gestation. They found a 5.6% decrease in the mean diffusing capacity for the CO hypoxia group from a control value of 0.302 ± 0.059 (SE of mean) ml/min·Torr to 0.285 ± 0.019 (SE of mean) ml/min·Torr. This slight decrease is similar to that which we found for absolute maternal surface area. Since we measured placental parameters on animals different from those used for diffusing capacities, we can only suggest that placental maternal surface area is correlated with placental diffusing capacity after carbon monoxide exposure. The increased number of fetal capillaries and increased fetal surface area seem to demonstrate a fetal compensation under hypoxic stress. Whether or not fetal compensation modifies placental diffusing capacity is still unknown.

Comparison of the 5.6% decreases in placental diffusing capacity of CO hypoxia animals with the 14% increase found in hypoxic hypoxia guinea pigs suggests that the different physiological states produced by the two types of hypoxia may affect placental surface area differently and thus

would be reflected in different placental diffusing capacities.

Carbon monoxide does not affect maternal arterial oxygen tensions by any appreciable amount (Longo, '76). However, maternal arterial oxygen tensions do decrease under hypoxic hypoxia conditions. Clavero and Llusia ('63) found increased villous surface area in placentas from women with severe cardiac failure. They suggested that the increase in surface area was due to the decrease in maternal arterial oxygen tensions. Thus the lack of an appreciable amount of change in CO hypoxia maternal placental tissue may be due to the relatively stable maternal arterial oxygen tensions.

Morphometric analysis of the guinea pig placenta shows that though exposure to carbon monoxide does not markedly affect fetal development it does effect changes in placental tissues, especially the fetal components.

Relating our data and previous studies (Clavero and Llusia, '63; Longo, '76) to placental function and to general hypoxia, we suggest that the placental diffusing capacity may reflect the response of maternal placental surface area to changes in maternal arterial oxygen tension.

TABLE 1. Maternal and fetal body weights, placental weight, fetal length, and placental to fetal weight ratio.

	CONTROL		CO		% CHANGE
	n = 13		n = 9		
Maternal weight (gm)					
initial	793.76	± 34.60	761.22	± 18.56	-4
term	1284.18	± 54.81	1328.41	± 44.08	3
Fetal weight (gm)	92.87	± 4.08	83.16	± 3.93	-10
Placental weight (gm)	6.26	± 0.37	7.02	± 0.50	12
Fetal length (mm)	123.23	± 1.94	122.66	± 1.66	<1
Placental wt/fetal wt gm/gm	.067	± .003	.084	± .004*	25

Values are mean ± SE of mean

placental weights are for perfused placentas

*significantly different from control ($p < 0.05$)

TABLE 2. Morphometric analysis of the peripheral labyrinth

	CONTROL	CO	% CHANGE
	n = 13	n = 9	
Maternal lacunae percent volume	43.39 ± 2.58	36.68 ± 1.59	-15
Fetal capillary percent volume	12.44 ± 1.26	13.13 ± 1.26	6
Tissue percent volume	44.44 ± 2.50	50.25 ± 2.00	12
Absolute maternal surface area (mm^2/mm^3)	143.25 ± 4.97	132.31 ± 6.37	8
Total maternal surface area (m^2 per placenta)	0.64 ± 0.05	0.65 ± 0.05	2
Absolute fetal surface area	98.13 ± 5.22	114.04 ± 4.53*	17
Total fetal surface area (m^2 per placenta)	0.43 ± 0.04	0.56 ± 0.04	30
Maternal lacunae (per mm^2)	1373.99 ± 101.84	1409.08 ± 83.13	3
Fetal capillaries (per mm^2)	2325.13 ± 183.67	3140.81 ± 100.53**	35
Diffusion distance (μm)	3.69 ± 0.25	4.14 ± 0.29	13

Values are mean ± SE of mean

* significantly different from control ($p < 0.05$)

** significantly different from control ($p < 0.01$)

TABLE 3. Comparative morphometric analysis of the peripheral labyrinth of control animals in near term guinea pigs.

	Kaufmann and Davidoff ('77)	[1]Kaufmann and Davidoff ('77)	Stelter ('79)	Smith et al. (unpublished)	Woolsey et al.
Maternal lacunae percent volume	42.5	35.0	43.4	31.8	43.4
Fetal capillary percent volume	14.8	17.0	16.3	21.8	12.4
Tissue percent volume	42.8	48.0	40.8	46.3	44.4
Absolute maternal surface area mm^2/mm^3	149.8	110.5	113.4	137.3	143.2
Absolute fetal surface area mm^2/mm^3	113.8	87.3	93.5	133.17	98.1

[1]Mean values for measurements made using flow pressures ranging from 20 mmHg to 80 mmHg; all other values are from flow pressures of 20 to 30 mmHg.

Figure 1. Light microscope representation of the normal peripheral labyrinth in a term guinea pig placenta. The various components are labeled: (END) endothelial cell nuclei, (CT) connective tissue surrounding fetal capillaries, (F) fetal capillary, (M) maternal lacunae, (T) trophoblast. (X 1280)


F.
CT
M

Figure 2. Surface area in mm^2/mm^3 for maternal and fetal vessels.
(* significantly different from control, $p < 0.05$)

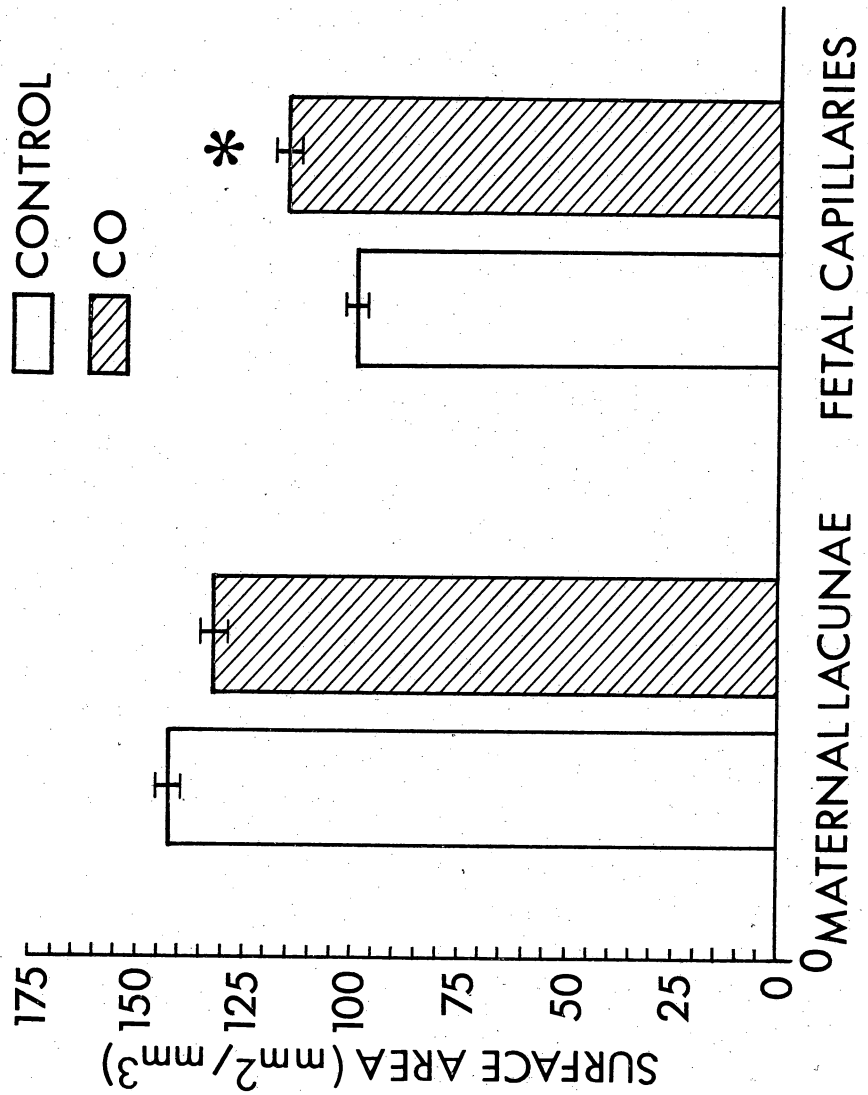

CONTROL
CO
*
SURFACE AREA (mm^2/mm^3)
175
150
125
100
75
50
25
0
MATERNAL LACUNAE
FETAL CAPILLARIES

Figure 3. Number of maternal and fetal vessels per mm^2 in the placental labyrinth. (* significantly different from control, $p < 0.01$)

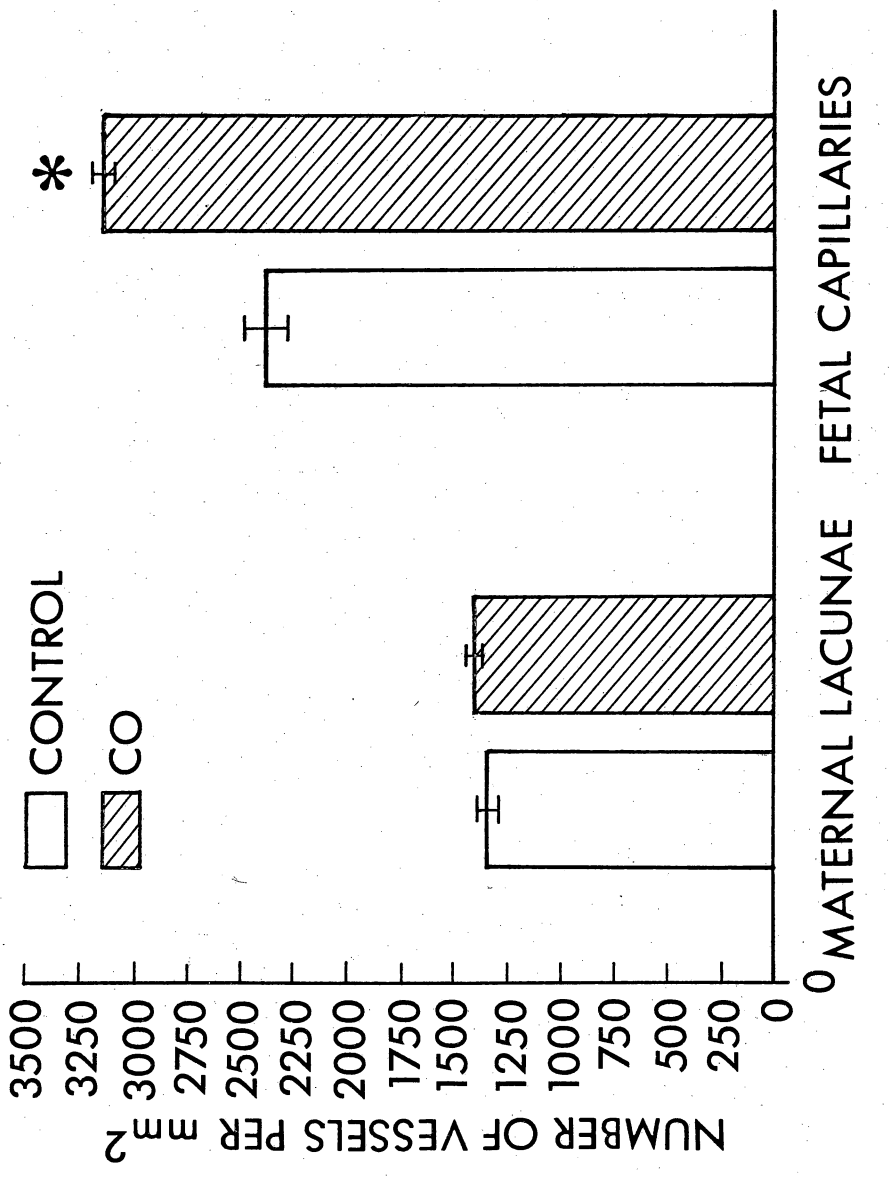

NUMBER OF VESSELS PER mm2
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
CONTROL
CO
*
0
MATERNAL LACUNAE
FETAL CAPILLARIES

Figure 4. Correlation of fetal weight and placental weight. Control animals ($r = 0.72$, $p < 0.01$). CO animals ($r = 0.82$, $p < 0.01$).

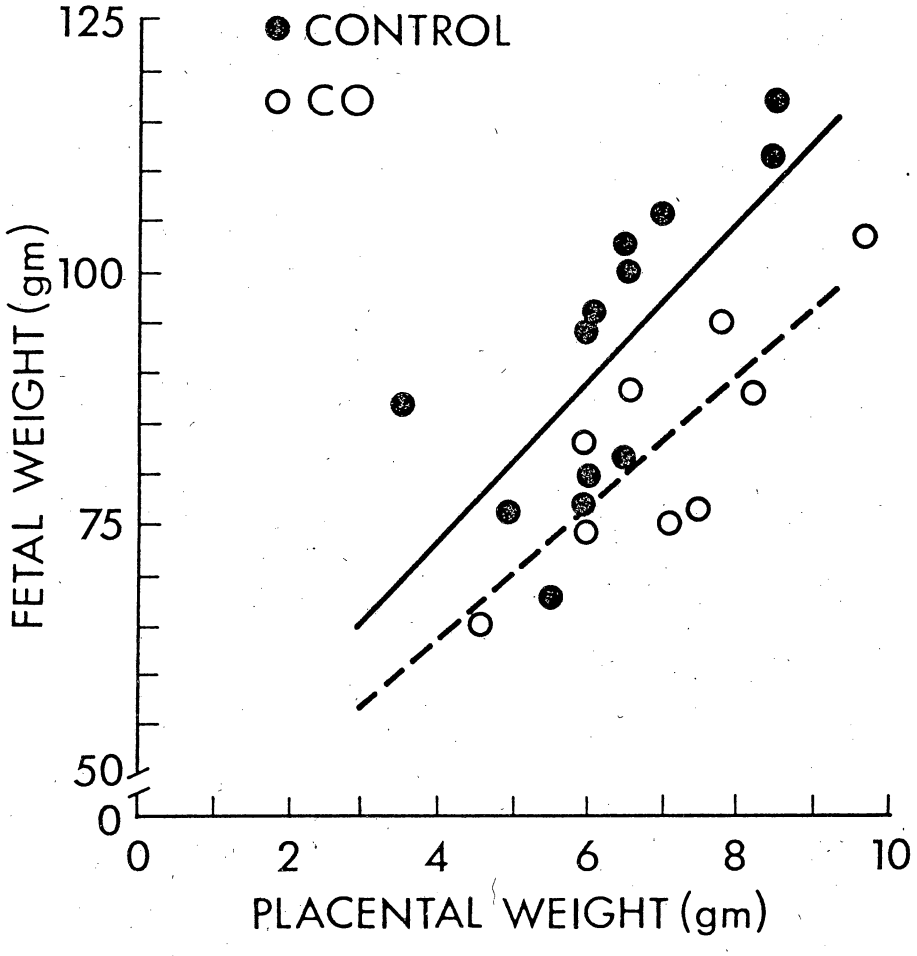
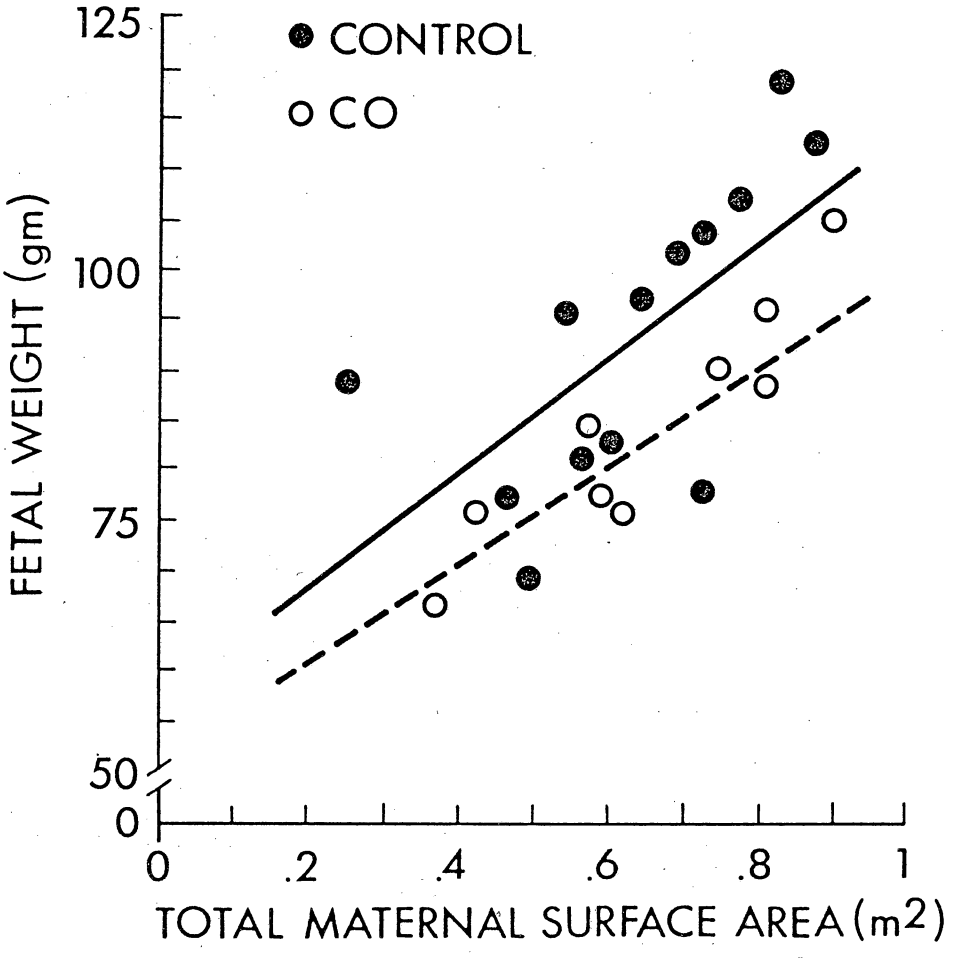

CONTROL
CO
125
100
75
50
0
FETAL WEIGHT (gm)
0
2
4
6
8
10
PLACENTAL WEIGHT (gm)

Figure 5. Correlation of fetal weight and total maternal surface area. Control animals ($r = .66$, $p < 0.02$), CO animals ($r = .78$, $p < 0.02$).


125
100
75
50
0
FETAL WEIGHT (gm)
CONTROL
CO
0
.2
.4
.6
.8
1
TOTAL MATERNAL SURFACE AREA (m2)

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APPENDIX 1

Appendix 1 contains the formulas for the various fixation, washing, and buffering solutions.

A. Glutaraldehyde for perfusion 2.2% (pH = 7.28 to 7.33, 338-344 mosmols) (osmolarity varies between batches of glutaraldehyde)

Sorensen's phosphate buffer 91.2 ml (100 ml)

25% glutaraldehyde 8.8 ml (9.65 ml)

Before use, mix and filter

B. Sorensen's phosphate buffer (pH = 7.2 to 7.3, 145 mosmols)

Solution A: 9.073 gm KH_2PO_4

Solution B: 17.91 gm $Na_2HPO_4 \cdot 7H_2O$

Just before use, mix 29.6 ml of Solution A with 70.4 ml of Solution B. When kept separate in the refrigerator, Solutions A and B will last six to eight weeks.

C. 0.2 M phosphate buffer

$NaH_2PO_4 \cdot 1H_2O$ 2.76 gm

$Na_2HPO_4 \cdot 7H_2O$ 21.44 gm

add distilled water to 500 ml

substitute chemicals for the 21.44 gm $Na_2HPO_4 \cdot 7H_2O$

28.67 gm $Na_2HPO_4 \cdot 12H_2O$

14.21 gm $Na_2HPO_4 \cdot 2H_2O$

11.36 gm $Na_2HPO_4 \cdot H_2O$ (anhydride)

D. Immersion fixation with 1% osmium tetroxide (pH = 7.3, 215 mosmols)

4% osmium tetroxide: one part

phosphate buffer with sucrose: one part

mix together under the hood just before use

For the 4% osmium tetroxide, use one ampul (1 gm) per 25 ml of distilled water. First place the sealed ampul in hot water and let the osmium tetroxide dissolve and collect in one end. Then place it in cold water so it will solidify. Use a file to make a scratch on the glass if the ampul is not pre-nicked. Drop the ampul into 25 ml of water in a ground glass stoppered bottle. Allow 24 to 48 hours for the osmium tetroxide to completely dissolve. Keep it in the refrigerator, preferably frozen, when not in use.

For the 0.2 M phosphate buffer with 0.2 M sucrose add 6.846 gm sucrose to 100 ml 0.2 M phosphate buffer. This solution keeps 14 days in the refrigerator.

E. Washing Solution

Use after glutaraldehyde and before osmium tetroxide. Mix one part 0.2 M phosphate buffer containing 6.846 gm of sucrose with one part distilled water immediately before use.

Tissues should not be kept longer than eight days in this solution.

APPENDIX 2

Table A.1 Individual control body weights, placental weight, fetal length and placental to fetal weight ratio.

Placenta	Maternal wt (gm) initial	Maternal wt (gm) term	Fetal wt (gm)	Placental wt (gm)	Fetal length (gm)	Placental wt/ Fetal wt (gm/gm)
9980A	769.9	1228.0	96.05	6.06	120	.063
9980B	754.5	1082.2	75.99	4.92	115	.065
9980C	909.5	1612.6	117.12	8.51	123	.073
9980D	627.5	1391.6	105.93	6.96	135	.066
9980E	883.5	1356.3	76.76	5.96	111	.077
91180A	737.0	1205.4	94.50	6.00	129	.063
91180B	672.0	935.0	111.50	8.50	135	.074
91180C	623.0	1198.5	81.50	6.50	120	.080
91180D	669.0	1318.0	100.50	6.50	120	.065
91180E	806.0	1076.5	102.50	6.50	127	.063
91180F	886.5	1241.3	80.00	6.00	122	.067
91180G	865.5	1566.0	87.00	3.50	120	.040
91180H	1065.0	1483.0	68.00	5.50	125	.081

Table A.2 Individual carbon monoxide hypoxia body weights, placental weight, fetal length, and placental to fetal weight ratio.

Placenta	Maternal wt (gm) initial	Maternal wt (gm) term	Fetal wt (gm)	Placental wt (gm)	Fetal length (gm)	Placental wt/ Fetal wt (gm/gm)
91680A	785.0	1385.0	103.50	9.65	126	.093
91680B	735.0	1397.0	88.40	6.55	120	.074
91680C	771.0	1135.1	82.94	5.92	116	.072
91680D	760.5	1326.3	76.26	7.46	125	.098
91680E	868.5	1539.4	65.22	4.55	120	.070
91780A	780.0	1444.0	94.77	7.77	130	.082
91780B	691.5	1341.0	74.99	7.10	116	.095
91780C	778.5	1210.0	87.99	8.20	128	.093
91780D	681.0	1178.0	74.40	5.97	124	.080

Table A.3 Individual morphometric data for control placentas.

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
9980A	42.5	14.8	42.7	2907.35	2318.01	45	85
	50.5	17.9	31.6	3503.32	2547.21	41	87
	29.4	8.0	62.6	2551.05	1573.87	33	69
	30.6	9.8	59.6	2750.67	1638.27	36	56
MEAN	38.2	12.6	49.2	2928.07	2019.34	39	74
9980B	52.2	6.7	41.1	2996.14	1626.81	36	61
	44.1	12.1	43.8	2741.76	1971.01	40	71
	29.2	6.5	64.3	1855.71	1544.42	33	85
	23.4	3.3	73.3	2917.17	1391.88	36	69
MEAN	37.2	7.2	55.6	2627.70	1633.53	36	72
9980C	35.2	29.3	35.5	2486.64	2610.99	37	37
	30.8	22.8	46.4	2434.46	2684.82	20	49
	39.1	24.8	36.1	3175.23	2638.95	35	61
	39.8	21.2	39.0	2586.15	2138.98	27	36
MEAN	36.2	24.5	39.0	2670.62	2518.44	30	46
9980D	59.4	9.7	30.9	3305.14	1739.56	44	64
	63.8	5.7	30.5	2753.06	1068.93	25	35
	56.0	8.8	34.0	3097.52	1539.90	40	59
	60.5	11.0	28.5	3089.73	1680.20	39	50
MEAN	59.9	8.8	31.0	3061.36	1507.15	37	52

Table A.3 cont'd

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
9980E	55.0	11.9	33.1	2253.33	1740.26	19	52
	50.0	10.0	40.0	2959.06	2055.02	34	95
	61.0	10.6	28.4	5252.17	2035.58	107	75
	60.0	11.9	28.1	2970.69	2089.18	22	56
MEAN	56.5	11.1	32.4	3358.81	1980.01	46	70
91180A	28.9	10.5	60.6	2602.76	2056.61	29	82
	38.6	6.4	55.0	2844.38	1276.62	44	61
	46.8	17.1	41.5	2483.92	1736.95	26	38
	41.3	17.2	41.1	2060.66	1843.81	24	50
MEAN	38.9	12.8	49.6	2497.93	1728.50	31	58
91180B	37.3	6.9	55.8	2836.25	1279.58	38	44
	38.5	10.2	51.3	3026.09	1581.10	46	53
	39.3	8.4	52.3	2741.56	1746.36	37	75
	29.7	10.7	59.6	2666.17	2177.90	43	89
MEAN	36.2	9.05	54.8	2817.52	1696.24	41	66
91180C	47.9	7.0	45.1	2465.35	1000.42	18	22
	41.9	9.5	49.0	2157.65	1336.45	18	30
	47.9	10.4	41.7	3016.73	1811.28	37	63
	45.1	8.4	46.5	2634.90	1437.75	24	46
MEAN	45.7	8.8	45.6	2568.66	1396.48	24	40

Table A.3 cont'd

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
91180D	41.6	17.5	40.9	2602.41	2314.64	30	68
	54.6	13.0	32.4	3191.93	1818.60	26	58
	42.8	12.4	44.8	2979.31	2079.31	31	69
	54.3	12.8	32.9	2947.81	1041.66	26	45
MEAN	48.3	13.9	37.8	2930.36	1813.55	28	60
91180E	43.8	13.3	42.9	3148.26	2280.15	49	78
	46.6	11.9	40.1	3103.92	1852.47	48	61
	33.8	15.0	54.3	2929.27	2361.38	51	76
	42.4	13.9	43.7	3144.75	2405.97	49	87
MEAN	41.6	13.5	45.2	3081.55	2225.00	49	76
91180F	59.7	10.4	29.9	2299.00	1410.94	9	36
	54.4	9.6	36.0	2380.97	1295.95	12	32
	52.4	12.7	34.9	2858.96	1766.15	25	48
	54.2	10.6	35.2	2911.75	1461.60	22	41
MEAN	55.2	10.8	34.0	2612.67	1483.66	17	39
91180G	33.8	9.4	56.8	2106.34	1552.97	28	52
	35.9	11.8	52.2	1966.64	1929.39	17	67
	27.1	11.4	61.5	1938.64	1930.26	28	64
	26.3	11.8	61.9	2001.90	2370.67	32	78
MEAN	30.8	11.1	58.1	2003.38	1945.82	26	65

Table A.3 cont'd

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
91180H	33.0	11.3	55.7	2521.33	2207.15	40	78
	31.8	19.2	49.0	2342.94	3141.73	32	90
	52.3	17.3	30.4	3045.00	2485.89	27	77
	31.1	22.6	46.3	2013.57	2050.65	26	36
MEAN	37.0	17.6	45.4	2480.71	2471.36	31	70

Table A.4 Individual morphometric data for carbon monoxide hypoxia placentas.

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
91680A	33.9	14.9	51.2	2909.27	2394.86	48	68
	39.4	11.6	49.0	2403.54	2075.80	32	76
	33.2	8.2	58.6	2698.66	1638.63	45	74
	24.2	8.4	67.4	2198.61	1857.51	41	91
MEAN	32.7	10.8	56.6	2552.52	1991.70	42	77
91680B	24.5	4.1	71.4	2947.29	1415.77	27	54
	41.4	10.8	47.8	2843.68	1635.95	33	52
	55.8	8.5	35.7	3499.58	1980.15	42	100
	46.6	9.5	43.9	3275.90	2118.84	42	112
MEAN	42.1	8.2	49.7	3141.61	1769.68	36	80
91680C	39.4	13.9	46.7	2639.87	2360.31	43	86
	41.7	12.9	45.9	2909.61	2232.53	46	84
	35.9	12.9	49.3	2766.17	2532.87	39	86
	35.5	14.2	50.3	2616.73	2450.32	37	75
MEAN	38.1	13.5	48.0	2733.10	2394.01	41	83
91680D	37.7	25.0	35.3	3216.43	2651.49	30	66
	35.0	18.1	46.9	2420.41	2428.07	35	68
	34.3	17.6	48.1	2083.11	2132.45	27	61
	30.3	23.0	46.7	1985.51	2782.38	27	78
MEAN	34.3	20.9	44.2	2201.36	2498.60	30	68

Table A.4 cont'd

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
91680E	30.0	13.4	56.6	2399.88	2581.02	35	102
	36.4	13.7	49.9	2268.30	2634.33	29	95
	43.2	10.0	46.8	2204.72	2170.19	18	77
	36.6	12.9	50.5	2049.46	2169.12	25	78
MEAN	36.6	12.5	51.0	2230.59	2388.66	27	88
91780A	31.6	17.5	50.9	2538.59	2508.56	44	84
	47.0	19.7	33.3	2720.33	1815.05	34	43
	46.4	16.2	37.4	3372.85	2594.43	45	89
	38.2	12.9	48.9	2797.68	2602.90	43	115
MEAN	40.8	16.6	42.6	2857.36	2380.24	42	83
91780B	21.5	14.3	64.2	1517.01	2178.64	27	71
	37.7	12.5	49.8	2552.82	2187.99	29	71
	34.9	14.0	51.1	2343.83	2594.43	23	78
	45.7	12.6	41.7	3176.55	2200.43	33	75
MEAN	35.0	13.4	51.7	2397.55	2290.37	28	74
91780C	33.8	13.0	53.2	2257.27	2008.23	24	57
	40.8	14.3	44.9	2825.42	2192.35	36	57
	47.9	11.5	40.6	2743.74	2177.79	38	86
	45.1	6.9	48.0	2965.30	1603.70	41	73
MEAN	41.9	11.4	46.7	2697.93	1995.52	35	68

Table A.4 cont'd

Placenta	Maternal percent volume	Fetal percent volume	Tissue percent volume	Total maternal perimeter (mm)	Total fetal perimeter (mm)	Maternal vessels per picture ($0.04\ mm^2$)	Fetal vessels per picture ($0.04\ mm^2$)
91780D	29.8	13.7	56.5	1872.08	2063.10	25	59
	36.2	13.2	50.6	2158.19	2059.57	20	50
	20.4	7.9	71.7	1739.77	2070.76	31	97
	24.6	7.1	68.3	2137.17	1538.50	42	67
MEAN	27.8	10.5	61.8	1976.80	1932.98	30	68

Table A.5 Individual control surface areas and diffusion distance.

Placenta	Absolute maternal surface area (mm^2/mm^3)	Total maternal surface area (m^2)	Absolute fetal surface area (mm^2/mm^3)	Total fetal surface area (m^2)	Diffusion distance (μm)
9980A	153.01	.649	105.52	.446	3.81
9980B	137.31	.473	85.36	.294	5.02
9980C	139.55	.831	131.60	.784	3.05
9980D	159.97	.779	78.76	.384	2.59
9980E	175.52	.732	103.47	.432	2.32
91180A	130.53	.548	90.32	.379	4.49
91180B	147.23	.876	88.64	.527	4.64
91180C	134.23	.611	72.97	.332	4.40
91180D	153.13	.697	94.77	.431	2.50
91180E	161.03	.733	116.27	.529	3.26
91180F	136.53	.573	77.53	.326	4.22
91180G	104.69	.256	101.68	.249	3.30
91180H	129.63	.499	129.14	.497	4.49

Table A.6 Individual carbon monoxide surface areas and diffusion distance.

Placenta	Absolute maternal surface area (mm^2/mm^3)	Total maternal surface area (m^2)	Absolute fetal surface area (mm^2/mm^3)	Total fetal surface area (m^2)	Diffusion distance (μm)
91680A	133.38	.901	104.08	.703	4.77
91680B	164.17	.753	92.48	.424	3.87
91680C	142.82	.592	125.10	.518	3.58
91680D	115.03	.601	130.57	.682	3.60
91680E	116.56	.371	124.82	.398	4.22
91780A	149.31	.812	124.38	.676	3.11
91780B	125.29	.623	119.68	.595	4.22
91780C	140.98	.809	104.28	.598	3.81
91780D	103.30	.432	101.01	.422	6.05