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Alterations in fetal and placental deoxyribonucleic acid synthesis rates after chronic fetal placental embolization

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OBJECTIVE: Fetal growth and development are closely related to normal placental growth and function. We performed a study to determine the effect of a 10-day period of fetal hypoxemia induced by umbilical-placental hypoperfusion on tissue deoxyribonucleic acid synthesis rates in the 0.84 to 0.91 of gestation ovine fetus and placenta.

STUDY DESIGN: Daily fetal placental embolization was performed in four chronically catheterized sheep fetuses until fetal arterial oxygen content decreased by ~30% compared with preembolization values. Five control fetuses received vehicle only. On experimental day 10, the deoxyribonucleic acid synthesis rate was determined by injecting tritiated thymidine (1 mCi/kg) intravenously approximately 8 hours before the end of the study.

RESULTS: Fetal arterial oxygen decreased from 3.2 ± 0.1 (SEM) mmol/L preembolization to 2.2 ± 0.2 mmol/L on day 10 ($p < 0.001$) and remained unchanged in controls. On day 10 deoxyribonucleic acid synthesis rates were significantly reduced in embolized fetuses compared with controls, by 38% in cotyledons (83.0 ± 15.1 vs 133.7 ± 9.9 disintegrations/min/μg deoxyribonucleic acid, $p < 0.05$), 28% in the left ventricular wall (36.8 ± 3.7 vs 51.0 ± 4.7 disintegrations/min/μg deoxyribonucleic acid, $p < 0.05$), and 45% in the quadriceps muscle (15.4 ± 4.0 vs 28.1 ± 3.0 disintegrations/min/μg deoxyribonucleic acid, $p < 0.05$). Tritiated thymidine autoradiography demonstrated that cotyledonary deoxyribonucleic acid synthesis occurred exclusively in the fetal trophoblasts cells.

CONCLUSION: We concluded that a reduction in cotyledonary, quadriceps muscle, and left ventricular myocardium deoxyribonucleic acid synthesis rates are the earliest adaptive mechanisms of fetal growth associated with development of umbilical-placental insufficiency. We speculate that alteration in the myocardial deoxyribonucleic acid synthesis rate could be a major contributing factor in the deterioration of fetal myocardial function associated with increased placental vascular resistance. (Am J Obstet Gynecol 1995;172:1451-8.)

Key words: Placental insufficiency, fetal hypoxia, fetal growth

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Fetal growth and development are closely related to normal placental growth and function. It has been shown that fetal placental embolization by latex microspheres is associated with progressive fetal hypoxemia and changes in umbilical artery Doppler flow velocity waveforms suggestive of an increase in placental vascular resistance similar to that observed in pregnancies complicated with intrauterine growth retardation (IUGR).[1, 2] It is not clear yet how and to what extent fetal and placental growth would be altered during the development of umbilical-placental hypoperfusion.[1, 3] Changes in cellular growth

can be assessed in vivo by measuring tritiated thymidine incorporation into deoxyribonucleic acid (DNA), which allows measurement of the DNA synthesis rate in tissues.[4, 5] To improve our understanding of the growth and development of the fetal-placental unit during the early stage of placental insufficiency, we performed a study to determine the effect of chronic fetal placental embolization on tissue DNA synthesis rates in the late-gestation ovine fetus and placenta.

Material and methods

Surgical procedures. Nine singleton fetal lambs of mixed breed ($n = 4$ in the embolized group, $n = 5$ in the control group) were prepared surgically between 118 and 121 days of gestation (term 147 days). After induction of anesthesia with 600 mg of intravenous thiopenthal sodium (Abbott Laboratories, Montreal, Canada) ewes were intubated and maintained on a closed-circuit anesthesia system with 0.5% to 1.5% halothane (Halocarbone Laboratories, North August, S.C.) and a 50:50 (vol/vol) mixture of oxygen and nitrous oxide, with a flow rate between 2 and 3 L/min. The uterus was exposed and a hind limb was exteriorized. Polyvinyl catheters (V4, Bolab, Lake Havasu City, Ariz.) were inserted through the fetal femoral artery into the descending abdominal aorta, with the catheter tip approximately 2 cm below the renal arteries and 1 to 2 cm above the common umbilical artery, and into the inferior vena cava through the femoral vein. The correct position of the fetal catheters was confirmed at the postmortem examination. A polyvinyl catheter (V11, Bolab) was sutured to the exterior of the fetal hind limb to record amniotic pressure. Teflon-coated stainless steel wire electrodes (Cooner, Chatsworth, Calif.) were sewn into the myometrium for continuous recording of uterine electromyographic activity, as previously described.[6] All catheters were exteriorized through the flank of the ewe, and the abdomen was closed in layers. Polyvinyl catheters (V11, Bolab) were placed into the femoral artery and vein of the ewe. At surgery and for 3 days thereafter the ewes received intramuscular injections of 4 ml of 200,000 IU of sodium penicillin G and 250 mg of dihydrostreptomycin/ml (Pen-di-Strep, Rogar, London, Canada). In addition 1 ml of 1,000,000 IU of penicillin G (Crystapen, Ayerst, Montreal) was injected daily for 3 days into the fetal femoral vein and into the amniotic sac.

After surgery the ewes were housed in individual metabolic cages, with hay and water available ad libitum. They were maintained on a 12-hour light-dark cycle and were allowed at least 4 days to recover from surgery before the experiments began. The study was approved by the Animal Care Committees of St. Joseph's Health Centre and the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care.

Experimental protocol. On the fourth day after recovery (range 122 to 125 days) animals were assigned randomly to either an embolized ($n = 4$) or a control group ($n = 5$). The chronic fetal placental embolization was performed according to the protocol we recently described.[1] Briefly, nonradiolabeled carbonized latex 15 μm microspheres suspended in dextran and diluted with sterile saline solution (0.1 ml of microspheres in 0.9 ml of saline solution = 1.83×10^6 microspheres/mL) were sonicated and injected through the descending aorta into the experimental fetuses every 15 minutes in boluses over a 2-hour period (10 AM to 12 noon). Microspheres were injected daily for 10 days, and the number of microspheres injected was adjusted to decrease the fetal arterial oxygen content (Cao_2, in millimoles per liter) by 30% to 35% of the preembolization value. Control fetuses were injected over the same period with the vehicle diluted in sterile saline solution. Paired maternal (3.0 ml) and fetal (3.0 ml) femoral arterial blood samples were taken daily at 9 AM for measurement of glucose, lactate, oxygen content, Po_2, Pco_2, pH, and hematocrit. During the 2-hour daily embolization period 0.5 mL of fetal arterial blood was taken every 15 minutes between successive embolizations to measure arterial gases and oxygen content until fetal Cao_2 was 30% to 35% below control values. The animals were then allowed to recover until the next day. A fetal arterial blood sample was taken at 4 PM each day to evaluate recovery.

On day 10, tritiated thymidine (New England Nuclear, Dupont, Mississauga, Ontario) was injected into the fetal femoral vein at a dose of 1 mCi/kg of estimated fetal weight, as previously described.[4] Eight hours later the ewe was killed, and the uterus and its contents were removed, dissected, weighed to the nearest 0.001 gm with an electronic weight scale (model PC440, Mettlar, Zurich, Switzerland), and calculated in grams per kilogram of body weight. The following fetal tissues were analyzed: brain (frontal cortex), adrenals (whole gland), kidneys (upper half), right and left ventricles (lower half of each ventricular wall), small intestine (jejunum), lungs (right middle lobe), quadriceps muscle (middle portion), right (beside the base of the gall bladder) and left (middle portion) lobes of the liver, thymus (whole gland), and cotyledons (six large cotyledons centrally located near the main branch of the umbilical cord). Tissues were immediately flash-frozen in liquid nitrogen and stored at −70° C for biochemical analysis. For histologic studies and tritiated thymidine autoradiography selected tissues were fixed by immersion in a fixative (4% paraformaldehyde and 0.2% glutaraldehyde in 70 mmol/L phosphate buffer, pH 7.0), processed, and embedded in paraffin by standard techniques.

Analytic measurements. Maternal and fetal arterial blood samples were drawn into heparinized syringes and placed on ice. Fetal arterial Po_2, Pco_2, and pH were

measured in a blood gas analyzer (ABL3, Radiometer, Copenhagen) with measurements corrected to a maternal and fetal temperature of 39° C and 39.5° C, respectively. Arterial oxygen saturation and hemoglobin were measured in duplicate with an OSM_2 hemoximeter device (Radiometer). Oxygen content was calculated with a capacity of 1.34 ml of oxygen per gram of hemoglobin. Whole blood glucose and lactate measurements were made in triplicate with membrane-bound glucose oxidase and D-lactate dehydrogenase, respectively (model 23A, Yellow Springs Instrument, Yellow Springs, Ohio).

Determination of fetal tissue DNA and protein concentration. Tissues (0.5 gm) were homogenized in phosphate buffer (2 mol/L sodium chloride, 50 mmol/L ethylenediaminetetraacetic acid, pH 7.4), centrifuged at 2500 revolutions/min for 5 minutes, and supernatant was assayed for protein and DNA. The protein concentration was assayed with the BioRad method and expressed as milligrams of protein per gram of tissue wet weight. The concentration of DNA was assayed with the fluorometric method[7] and expressed as milligrams of DNA per gram of tissue wet weight.

Tritiated thymidine incorporation. Tissue DNA synthesis rates were estimated by a previously described method.[4] Briefly, fetal tissue samples were homogenized in 5% trichloroacetic acid and then an aliquot of each homogenate was centrifuged at 2500 revolutions/min for 5 minutes. The pellet was washed by fresh 5% trichloroacetic acid, and after centrifugation the pellet was dissolved in perchloric acid (0.5 mol/L) at 90° C for 15 minutes. The supernatant was added to 5 ml of liquid scintillation fluid (American Chemical Society) to determine the amount of radioactivity (disintegrations per minute per milliliter of supernatant). The concentration of DNA was measured by the fluorometric technique.[7] The mass of DNA per gram of tissue was calculated according to the dilution of each sample aliquot and to the mass of tissue originally homogenized. The relative incorporation of the tritiated thymidine into DNA was determined from the concentration of radioactivity (disintegrations per milliliter) and from the concentration of DNA (micrograms per milliliter) in each sample aliquot (disintegrations per microgram of DNA).

Cotyledonary tissue histologic studies, immunohistochemistry, and tritiated thymidine autoradiography. Tissue sections (0.5 μm) of cotyledons were stained with hematoxylin and eosin and visualized under bright field microscopy to localize microspheres and assess tissue damage. Tissue sections were stained with an antiserum against cytokeratin (pancytokeratin, DAKO, Glostrup; 1:2500), specific to trophoblast cells, by use of the avidin-biotin-peroxidase method befor coating with photoemulsion.[8] Tritiated thymidine autoradiography was determined with cotyledonary tissue

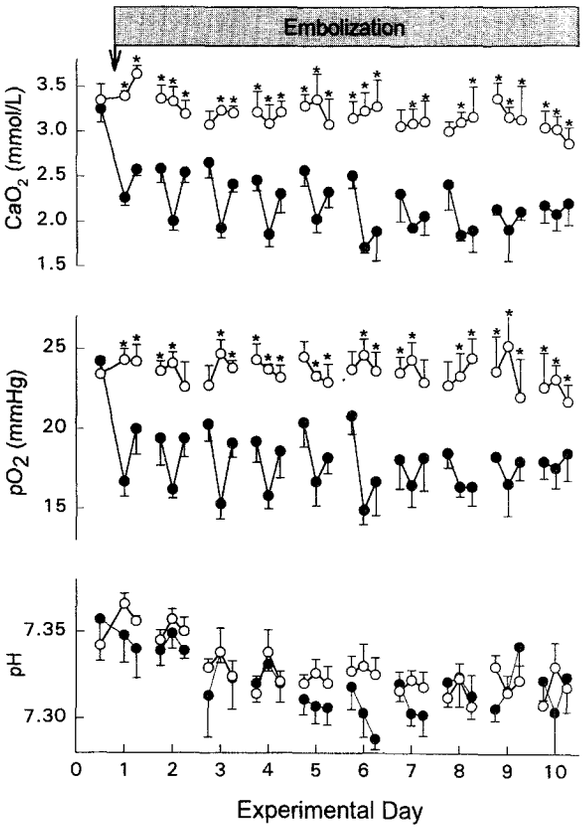

Fig. 1. Fetal femoral Cao_2, Po_2, and pH (mean ± SEM) before and during daily fetal placental embolization (*closed circles*, $n = 4$) or controls (*open circles*, $n = 5$). For each experimental day *first symbol* indicates preembolization value (~9 AM), *second symbol* end-of-embolization value (12 noon), and *third symbol* recovery (~ 4 PM). *Asterisk*, Control values significantly greater than in embolized group ($p < 0.05$).

sections (0.5 μm) coated with photoemulsion (NTB-3 nuclear track emulsion) and exposed for 4 weeks at 4° C, developed with D-19, fixed, stained with hematoxylin and eosin, and mounted with Permount. Tissues were visualized under both bright and dark field microscopy.

Data analysis. Changes in fetal blood gases, glucose, and lactate were analyzed with a two-way correlation matrix analysis of variance with repeated measures (BMDP 2V, BMDP Statistical Software, Los Angeles) comparing the effect of time, group (embolized vs control), and interaction between group and time. If a significant effect of group or time was observed ($p < 0.05$), within-animal comparisons were conducted by multiple comparison Tukey's t test[9] and between-group comparisons with unpaired t tests with BMDP 3D. Comparisons of body and tissue weights, tissue DNA contents, and relative tissue DNA synthesis rates between embolized and control fetuses were made with unpaired t tests. All results are presented as means ± SEM for the number of fetuses studied.

Table I. Maternal and fetal arterial gases, glucose, and lactate concentrations

	Day 1 (preembolization)		*Day 10 (postembolization)*	
	Embolized (n = 4)	*Control (n = 5)*	*Embolized (n = 4)*	*Control (n = 5)*
Maternal				
Cao_2 (mmol/L)	6.23 ± 0.47	5.95 ± 0.16	5.40 ± 0.41	5.47 ± 0.68
Po_2 (mm Hg)	115.2 ± 8.8	110.9 ± 5.9	109.6 ± 9.9	116.3 ± 2.1
Pco_2 (mm Hg)	31.9 ± 1.1	33.4 ± 1.5	37.5 ± 1.4	33.8 ± 1.6
pH	7.46 ± 0.02	7.49 ± 0.01	7.42 ± 0.01	7.43 ± 0.01
Glucose (mmol/L)	2.63 ± 0.21	2.65 ± 0.13	2.57 ± 0.08	2.57 ± 0.15
Lactate (mmol/L)	1.03 ± 0.34	0.90 ± 0.21	1.22 ± 0.63	0.86 ± 0.12
Fetal				
Cao_2 (mmol/L)	3.23 ± 0.15	3.35 ± 0.18	2.18 ± 0.19*	3.04 ± 0.19
Po_2 (mm Hg)	24.2 ± 1.0	23.4 ± 1.1	18.0 ± 1.0†	22.6 ± 1.1
Pco_2 (mm Hg)	47.0 ± 2.1	50.6 ± 1.7	50.5 ± 0.8	52.4 ± 0.3
pH	7.36 ± 0.02	7.34 ± 0.01	7.31 ± 0.01‡	7.31 ± 0.01‡
Glucose (mmol/L)	1.04 ± 0.18	1.10 ± 0.12	0.93 ± 0.07	1.04 ± 0.21
Lactate (mmol/L)	1.91 ± 0.54	1.55 ± 0.14	1.39 ± 0.10	1.98 ± 0.26
Hemoglobin (gm/dl)	8.6 ± 0.3	9.3 ± 0.5	9.3 ± 0.9	10.6 ± 1.0

*$p < 0.01$, compared with control.
†$p < 0.05$, compared with control.
‡$p < 0.05$, compared with day 1.

Table II. Fetal tissue weights

	Tissue weights (gm)		*Tissue weights (gm/kg body weight)*	
Tissue	*Embolized (n = 4)*	*Control (n = 5)*	*Embolized (n = 4)*	*Control (n = 5)*
Brain	53.2 ± 2.5	48.2 ± 3.6	11.5 ± 0.7	11.6 ± 1.3
Heart	37.8 ± 5.4	30.9 ± 1.2	8.0 ± 0.7	7.4 ± 0.5
Thymus	4.6 ± 0.3	5.3 ± 0.9	3.0 ± 0.4	4.3 ± 0.2
Lungs	121.1 ± 2.5	130.2 ± 16.6	26.2 ± 1.7	30.2 ± 1.9
Thyroid gland	0.9 ± 0.1	0.9 ± 0.1	0.19 ± 0.0	0.23 ± 0.03
Liver	131.0 ± 7.0	126.6 ± 6.0	28.6 ± 3.1	29.6 ± 2.2
Adrenals (combined)	0.60 ± 0.01	0.42 ± 0.08	0.14 ± 0.01	0.10 ± 0.02
Kidneys (combined)	27.3 ± 2.4	27.3 ± 1.6	5.0 ± 0.3	6.7 ± 0.7
Cotyledons	375.4 ± 24.1	373.0 ± 9.8	84.0 ± 2.0	89.8 ± 6.9

Results

Fetal blood gases, glucose, and lactate. After the onset of fetal placental embolization the mean fetal Cao_2 decreased from preembolization value of 3.23 ± 0.15 mmol/L to 2.27 ± 0.08 mmol/L at the end of embolization period on day 1, which was lower than the 3.40 ± 0.06 mmol/L in controls ($p < 0.001$, Fig. 1, *A*). Although embolized fetuses always became hypoxemic with chronic repetitive embolization (analysis of variance, group effect $p < 0.001$, time effect $p < 0.001$), there was partial recovery in fetal oxygenation the following morning. Changes in fetal arterial Po_2 paralleled changes observed in Cao_2 (Fig. 1, top and middle). There was a modest but significant fall in fetal arterial pH over time in both control and embolized fetuses (analysis of variance, time effect $p < 0.001$, Fig. 1, bottom) from a mean pH of 7.36 ± 0.02 on day 1 to 7.31 ± 0.01 on day 10, and there was no significant difference between the embolized and control fetuses. Fetal arterial Pco_2, maternal blood gases, and fetal and maternal glucose and lactate concentrations remained unchanged throughout the study period (Table I). Fetal lactate levels were variable between animals. Over the 10-day study period in the embolized group fetal plasma lacate levels increased in two fetuses and decreased in two. In the control group fetal lactate levels increased in three animals and decreased in the other two animals.

Fetal weight and tissue weights. The mean gestational age of fetuses studied on day 10 after the onset of embolization was 134.4 ± 0.5 days in controls and 133.3 ± 0.9 days in embolized animals (term 147 days). The mean fetal body weight was 4.3 ± 0.3 kg in control fetuses, which was not significantly different from 4.7 ± 0.3 kg in embolized fetuses. Fetal organ weights and organ weight/fetal body weight ratios (grams per kilogram of body weight) are summarized in Table II. Although adrenal gland weights and adrenal gland/body weight ratios of hypoxemic fetuses were 40% greater than those of controls and thymus gland/body weight ratios were 30% smaller, the difference did not reach statistical significance because of the small number of animals studied.

Tissue DNA content and DNA synthesis rate. Tis-

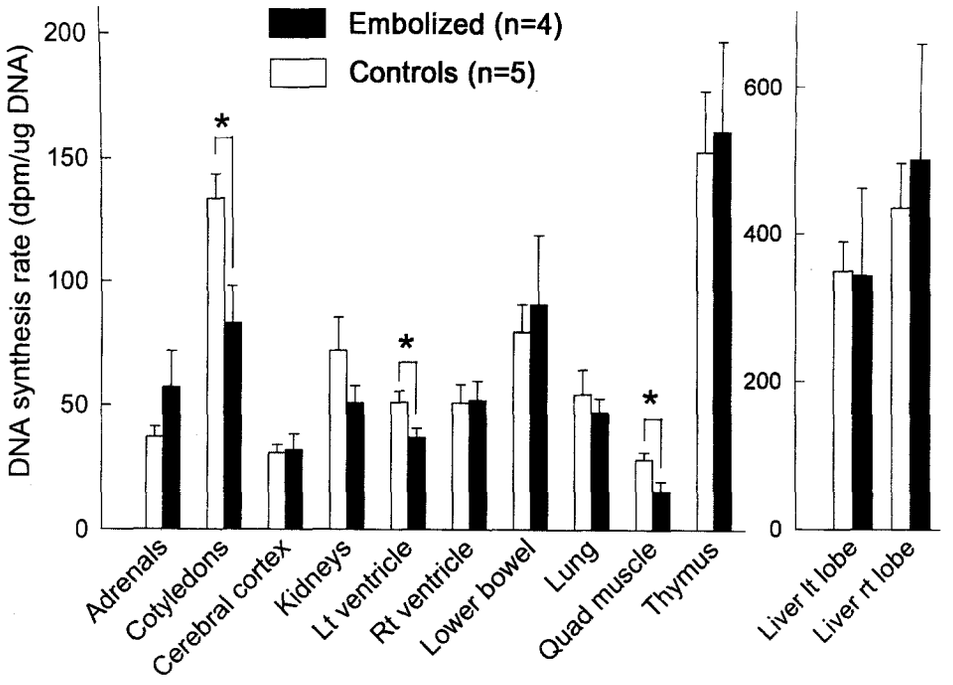

Fig. 2. Tissue DNA synthesis rates collected from fetuses after either 10-day period of daily fetal placental embolization *(closed bars)* or 10-day control period *(open bars). Asterisks,* DNA synthesis rates in tissues from embolized fetuses that are significantly less than control ($p < 0.05$).

sue DNA contents (milligrams of DNA per gram of tissue) were not significantly different in tissues from embolized fetuses compared with those from control fetuses (Table III). However, DNA synthesis rates were significantly reduced in embolized fetuses by 38% in cotyledons (83.0 ± 15.1 vs 133.7 ± 9.9 disintegrations/min/μg DNA, $p < 0.05$), 28% in the left ventricular wall (36.8 ± 3.7 vs 51.0 ± 4.7 disintegrations/min/μg DNA, $p < 0.05$), and 45% in the quadriceps muscle (15.4 ± 4.0 vs 28.1 ± 3.0 disintegrations/min/μg DNA, $p < 0.05$) (Fig. 2). There was no significant effect of embolization on DNA synthesis rates in any of the other tissues studied (control values were as follows: 37.0 ± 4.3 disintegrations/min/μg DNA in adrenals, 30.6 ± 3.3 disintegrations/min/μg DNA in the cerebral cortex, 72.1 ± 13.5 disintegrations/min/μg DNA in the kidneys, 51.0 ± 7.4 disintegrations/min/μg DNA in the right ventricular wall, 79.7 ± 10.9 disintegrations/min/μg DNA in the small intestine, 349.5 ± 40.1 disintegrations/min/μg DNA in the left lobe of the liver, 435.6 ± 61.0 disintegrations/min/μg DNA in the right lobe of the liver, 54.5 ± 9.9 disintegrations/min/μg DNA in the lungs, and 152.6 ± 24.9 disintegrations/min/μg DNA in the thymus).

Cotyledonary histologic studies and tritiated thymidine autoradiography. Fig. 3 is a typical example of histologic changes at the syndesmochorial fetomaternal interface in sheep cotyledons of a control fetus (Fig. 3, *A*) and embolized cotyledon (Fig. 3, *C*). In the four embolized animals studied all microspheres were lodged in the fetal side of the placental microcirculation, and there was no evidence of inflammatory reaction or infarcts induced by the microspheres (Fig. 3, *C*). There were no microspheres on the maternal side of the placenta. In addition, tritiated thymidine autoradiography of the same tissue sections (Fig. 3, *B* through *D*) demonstrated that tritiated thymidine incorporation occurred almost exclusively in the trophoblast cells, as indicated by positive immunoreactivity to cytokeratin in both control and embolized fetuses. The apparent reduction in radiolabeled nuclei was distributed evenly

Table III. Fetal tissue DNA content (milligrams of DNA per gram of tissue)

Tissue	*Embolized (n = 4)*	*Control (n = 5)*
Adrenals (combined)	5.71 ± 0.57	4.79 ± 0.40
Cotyledons	4.13 ± 0.24	4.19 ± 0.18
Cerebral cortex	1.14 ± 0.03	1.11 ± 0.02
Kidneys (combined)	5.45 ± 0.36	5.29 ± 0.34
Left ventricle	3.62 ± 0.07	3.20 ± 0.17
Right ventricle	2.91 ± 0.05	3.38 ± 0.27
Small intestine	5.87 ± 0.29	6.05 ± 0.34
Liver, left lobe	5.09 ± 0.69	5.24 ± 0.50
Liver, right lobe	5.03 ± 0.81	4.95 ± 0.29
Lungs	5.62 ± 0.67	5.40 ± 0.61
Quadriceps muscle	2.99 ± 0.28	2.79 ± 0.09
Thymus	27.03 ± 0.60	27.33 ± 1.75

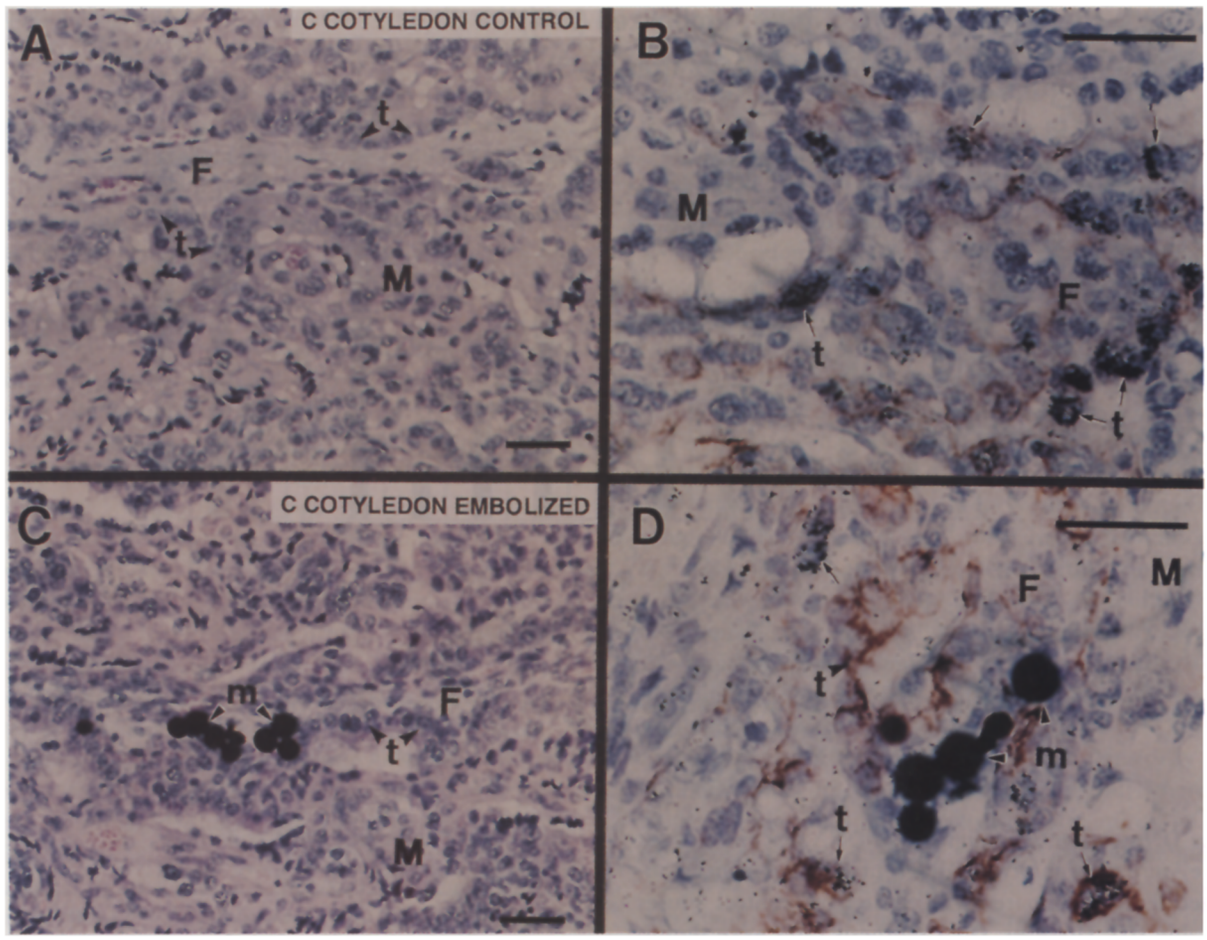

Fig. 3. Photomicrographs of syndesmochorial fetomaternal interface in sheep cotyledons of control fetus **(A)** and embolized cotyledon **(C).** Tritiated thymidine autoradiography **(B, D)** illustrates that radiolabeled nuclei are identified exclusively in trophoblast cells *(small arrows).* Tritiated thymidine incorporation appears to be reduced in cotyledon of embolized animal **(D)** compared with control **(B).** Identity of trophoblast cells was confirmed with cytokeratin immunoreactivity. *F,* Fetal compartment; *M,* maternal compartment; *m,* microspheres; *t + arrows,* trophoblast cells with tritiated thymidine incorporation; *t + arrowhead,* trophoblast cells without tritiated thymidine incorporation.

throughout the cotyledon, and the same pattern was observed in the four embolized fetuses. These observations suggested that the decrease in the cotyledonary DNA synthesis rate was most likely because of a reduction in DNA synthesis in the trophoblasts, resulting from a decrease in umbilical-placental perfusion and oxygen delivery to the trophoblast cells.

Comment

The current experimental model was designed to produce a decrease in fetal oxygenation by decreasing umbilical-placental perfusion. Clapp et al.[10] embolized the uterine circulation in the pregnant ewe to induce IUGR. They observed a significant reduction in fetal growth only in the animals where a decrease in umbilical-placental blood flow occurred, suggesting that umbilical-placental hypoperfusion is a prerequisite to a decrease in fetal growth.[10] Although it is not known what causes umbilical-placental hypoperfusion and IUGR, several studies on placental histologic characteristics have demonstrated that the only consistent finding in pregnancies complicated with IUGR and abnormal umbilical artery Doppler flow velocity waveforms is a decrease in the number and surface area of the small arterioles in the tertiary stem villi.[11, 12] Large placental infarcts and extensive tissue damage occurred only in a small proportion of growth-retarded fetuses.[12]

In the current study placental histologic studies demonstrated that microspheres obliterated only the small arterioles of the fetal side of the syndesmochorial sheep placenta (Fig. 3) and did not produce placental infarcts, inflammatory reaction, or tissue damage, therefore creating conditions analogous to the human placenta in the majority of pregnancies complicated with IUGR

and placental insufficiency. Whether recovery in fetal oxygenation after daily embolization was because of opening of placental blood vessels that are normally present but hemodynamically inactive or a remodelling of the placental microcirculation induced by the microspheres remain to be determined, because umbilical blood flow was not measured. The progressive fall in fetal Cao_2 and Po_2 with partial recovery until day 9 of embolization (Fig. 1) was similar to our previous study[1] and supports the concept of a placental reserve that might play an important role to prevent deterioration of the fetal metabolic status in the early stage of placental insufficiency.

Fetal organ weights, placental weight, placental/body weight ratio, or fetal organ/body weight ratios were not significantly altered after 10 days of embolization. Similar observations have also been made by Trudinger et al.[2] In an experimental model in which fetoplacental hypoperfusion was created by a prolonged reduction in maternal uterine blood flow for 21 days Lang et al.[13] have also demonstrated that the reduction in fetal weight and fetal organ weights occur only if the restriction in uteroplacental blood flow is initiated at approximately 115 days' gestation and not at 125 days' gestation. It is therefore becoming evident that the process leading to placental insufficiency must develop over the course of several weeks and early during gestation, before changes in fetal weight and organ weights become detectable. Our data indicated that the degree of fetal hypoxemia was mild to moderate (Po_2 4 to 6 mm Hg less than controls) and reached a steady state for approximately 48 hours before measurements of DNA synthesis rates, a duration probably too short to affect fetal weight or DNA content.

By use of a more sensitive method to assess fetal growth, such as tritiated thymidine incorporation, we were able to demonstrate a reduction in DNA synthesis rates and therefore tissue growth predominantly in cotyledons (38% less), quadriceps muscle (45% less), and, to a lesser extent, in the left ventricular wall (28% less). A reduction in quadriceps muscle DNA synthesis rate has also been reported after 24 hours of restriction of uteroplacental blood flow in the pregnant ewe,[4] and a reduction in skeletal muscle DNA concentration has been described in human fetuses with IUGR.[14] Therefore a reduction in the DNA synthesis rate in limb skeletal muscle appears to be one of the earliest fetal growth adaptive mechanisms associated with the development of placental insufficiency and prolonged hypoxemia.

Although in theory injection of the microspheres into the descending aorta of the fetus would have partially embolized the hind limb muscles,[2] we recently used radioactive microspheres to measure hind limb skeletal muscle and cotyledonary blood flows during acute embolization sufficient to reduce umbilical blood flow by 50% (Gagnon, unpublished observations). Under these experimental conditions and in spite of a reduction in umbilical blood flow by 50%, hind limb skeletal muscle blood flow remained unchanged, indicating that this technique of embolization would significantly alter only umbilical blood flow. These recent observations suggest that the reduction in quadriceps DNA synthesis rate is a response to chronic hypoxemia similar to that seen in the myocardium, also a skeletal muscle, rather than to changes in blood flow.

Because hypobaric hypoxia for 3 weeks does not alter placental or cotyledonary weight significantly,[15] as well as DNA content, the reduction in the DNA synthesis rate in cotyledons may therefore have been related to a reduction in cotyledonary blood flow. Tritiated thymidine autoradiographic studies combined with cytokeratin immunocytochemistry demonstrated clearly that the tritiated thymidine incorporation occurred exclusively in the fetal trophoblasts of the cotyledons of the ovine pregnancy at this gestation. DNA synthesis in fetal trophoblasts reflects the repair, replacement, or regeneration of these cells undergoing natural or programmed cell death. Because fetal trophoblasts were the cells identified to synthesize DNA almost exclusively, the reduction in the DNA synthesis rate observed in cotyledons occurred in these cell types. If these experimental conditions were maintained for longer periods of time, a decrease in placental weight may have occurred.

The decrease in DNA synthesis rate observed after 10 days of embolization in the left ventricular wall may represent an adaptation to chronic repeated stress and hypoxemia. Block et al.[3] with similar experimental conditions reported that myocardial blood flow was not different in sheep fetuses after 9 days of fetal placental embolization in spite of an approximate 35% decrease in arterial oxygen content, similar to the current study. Although they did not differentiate between the left and right ventricles, their data indicated that the average oxygen delivery to the myocardium was 1080 $\mu mol \cdot min^{-1} \cdot 100\ gm^{-1}$ in control fetuses and 756 $\mu mol \cdot min^{-1} \cdot 100\ gm^{-1}$ in embolized fetuses, which was approximately 30% less than controls. Because there is a close coupling between myocardial blood flow and oxygen consumption and because myocyte proliferation in the left ventricular myocardium exceeds that of the right ventricle,[16] to maintain oxygen consumption by the developing myocardium in the presence of chronic hypoxemia induced by increasing umbilical-placental vascular resistance, metabolic requirements must decrease. Our data suggested that a decrease in the DNA synthesis rate (28% less), as observed in the left ventricular wall during placental insufficiency, may be an important adaptive mechanism to maintain myo-

cardial oxidative metabolism and function at the expense of myocyte proliferation in the presence of chronic hypoxemia resulting from placental insufficiency. However, eventually this adaptive mechanism may have deleterious effects. A decrease in the myocardial DNA synthesis rate could contribute significantly to the progressive deterioration in myocardial function reported in the human fetus during the progression of placental insufficiency, when there is redistribution of the fetal cardiac output from a right ventricular predominance to a left ventricular predominance.[17] In addition, recent epidemiologic studies from Barker and Osmond[18] indicate an increased risk of ischemic heart disease in adults who had a low birth weight, suggesting that a decreasing placental function could have consequences on myocardial function and development beyond the fetal and neonatal periods.

In summary, after 10 days of fetal placental embolization and progressive fetal hypoxemia, DNA synthesis rates were markedly decreased in trophoblast cells of cotyledons, quadriceps muscle, and the left ventricular myocardium before any changes in tissue DNA content or weight were detectable. These were most likely adaptive mechanisms to fetal hypoxia resulting from umbilical-placental hypoperfusion. The cellular mechanisms by which DNA synthesis is selectively reduced in fetal tissues during progressive placental insufficiency remain to be determined. However, norepinephrine, which increased progressively during fetal placental embolization,[1] is known to increase insulin-like growth factor binding protein-1 messenger RNA levels in the liver of the late-gestation ovine fetus.[19] It is also established that insulin-like growth factor binding protein-1 is an inhibitor of insulin-like growth factor biologic action.[20] It is therefore possible that chronic elevation of this catecholamine in the fetus, with the development of placental insufficiency, could be responsible, at least in part, through its action on insulin-like growth factor binding protein-1 gene expression, for the reduction in the DNA synthesis rate seen in the fetus and placenta.

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