Growth and in-vitro metabolism of placental tissues of cows from Day 100 to Day 250 of gestation

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Summary. Weight of placental tissues of cows increased exponentially from Day 100 to Day 250 of gestation, but at much slower relative and absolute rates than fetal weight. In addition, growth rate of fetal placental tissues was less than that of maternal placental tissues. Concentrations of DNA, RNA and protein, however, increased in fetal placental but not in maternal placental tissues. Fetal placental tissues therefore exhibited hyperplasia, which probably contributes to increased functional capacity of the placenta during late gestation. The rate of O₂ uptake *in vitro* was greatest for maternal placental tissues, suggesting that the maternal portion of the placenta accounts for most of the large rate of placental O₂ utilization *in vivo*. Compared with other placental tissues, rate of secretion of macromolecules by intercaruncular endometrium was high, but decreased from Day 100 to 250, suggesting that uterine glandular secretory activity may decrease as gestation advances. Rate of secretion of macromolecules also was high for intercotyledonary tissues and increased with day of gestation, suggesting a role for secretory products of chorioallantois in gravid uterine function.

Keywords: placenta; growth; metabolism; cow; gestation

Introduction

The mammalian placenta consists of fetal and maternal tissues and is the site of exchange of respiratory gases, nutrients and waste substances between the fetal and maternal systems (Barcroft, 1946; Ramsey, 1982). Normal growth and development of the placenta are therefore vital to normal fetal growth and development. It is well known that treatments which reduce fetal growth (e.g. maternal genotype, reduced uterine surface area per fetus, maternal nutrient deprivation, environmental heat stress) are associated with decreased placental size (Wallace, 1948; McKeown & Record, 1953; Eckstein *et al.*, 1955; Joubert & Hammond, 1958; Alexander, 1964a, b; Bell *et al.*, 1987). In addition, in normal pregnancy placental and fetal weights are highly correlated (Eckstein *et al.*, 1955; Alexander, 1964a; Anthony *et al.*, 1986). Size of the placenta and its blood supply are primary determinants of the rate of physiological exchange between the fetal and maternal systems (Reynolds *et al.*, 1985a, b, 1986; Bell *et al.*, 1986, 1987; Reynolds & Ferrell, 1987). Conditions associated with reduced rate of fetal growth also are associated with reduced rates of placental blood flow and reduced fetal O₂ and nutrient uptakes (Wootton *et al.*, 1977; Reynolds *et al.*, 1985a, b; Bell *et al.*, 1987; Ferrell & Reynolds, 1987). Therefore, factors which influence placental growth and development will probably have a substantial influence on fetal growth and development. Before the relationships between fetal and placental growth can be determined, however, an understanding of normal placental growth must be obtained.

The ruminant placenta is divided into placentomes comprising maternal caruncular endometrium and fetal cotyledon, and also into interplacentomal areas comprising intercaruncular endometrium

and intercotyledonary fetal membrane (Ramsey, 1982). Growth of these placental tissues, however, has not been well characterized in cows. Prior & Laster (1979) measured weights and surface areas for cotyledons and placenta (defined as fetal membranes with cotyledons removed) of cows, but did not report weights or surface areas of maternal caruncles. Ferrell *et al.* (1976a) reported weights of fetal membranes (presumably including fetal cotyledons) at several stages of gestation, but found growth rate of fetal membranes to be about half that observed by Prior & Laster (1979). Anthony *et al.* (1986) reported a 2-fold increase in total placental weight from Day 200 to 260 of gestation. Additionally, we are not aware of any reports which describe gestational changes in cellular composition (DNA, RNA and protein contents) of placental tissues in cows. An important consideration in describing the relationship between fetal and placental growth is the high metabolic rate exhibited by the placenta. Indeed, major portions (60–80% at midgestation) of O_2 and nutrients taken up by the gravid uterus are utilized by the placenta, which has a high rate of metabolism compared with other gravid uterine or maternal tissues (Meschia *et al.*, 1966; Reynolds *et al.*, 1986; Bell *et al.*, 1986). Which of the placental tissues accounts for this high metabolic activity, however, has not been determined.

The objectives of the present study were to determine the relationship between fetal and placental growth, and to characterize cellular growth and metabolism of individual components of the placenta at several stages of gestation in cows.

Materials and Methods

Hereford and Hereford-cross cows were mated with Hereford bulls (Day 0 of gestation) and fed alfalfa/grass hay at a level calculated to maintain body weight, with free access to salt and mineral supplements. The 21 cows were assigned randomly to slaughter on approximately Days 100 (103 ± 0.7 , $N = 4$), 150 (151 ± 0.5 , $N = 5$), 200 (200 ± 1.2 , $N = 6$) and 250 (250 ± 0.5 , $N = 6$) of gestation. For each cow, the entire gravid uterus (from mid-cervix to both utero-tubal junctions) was obtained. For 9 cows (2 at Day 150, 4 at Day 200 and 3 at Day 250), a sample (≈ 2 g) of maternal liver was also obtained. Gravid uteri and maternal liver samples were immediately transported to the laboratory (within 10 min after collection and within 30 min after slaughter).

Weights of gravid uterine tissues. At the laboratory, the entire gravid uterus of each cow was weighed and fetus, fetal membranes and uterus were separated. Cotyledons were carefully dissected from fetal membranes. Caruncles were dissected from the uterine luminal surface, taking care not to include intercaruncular tissue. Any cotyledonary villi remaining in caruncular crypts were removed with forceps and included with cotyledons. Weights of fetus, uterus (minus caruncles), total caruncles, total cotyledons and total fetal membranes (chorioallantois plus amnion) were determined. Weight of fetal fluids was calculated as gravid uterine weight minus total weights of gravid uterine tissues (fetus + uterus + caruncles + cotyledons + fetal membranes). Placental weight was calculated as the sum of caruncular and cotyledonary weights. Weights of tissue samples obtained for evaluation of cellular growth, metabolic rate and [3H]leucine incorporation (see below) were included with the appropriate tissue weight. Curved crown-rump lengths of fetuses were determined as described by Ferrell *et al.* (1976a).

DNA, RNA and protein concentrations. To evaluate cellular growth of placental tissues, samples (≈ 2 g) of caruncular, intercaruncular, cotyledonary and intercotyledonary (chorioallantoic) tissues were obtained from the middle portion (near the fetus) of the gravid uterine horn of each cow and stored frozen at -80°C . Approximately 1 g of each tissue sample was homogenized in PBS-EDTA buffer (0.01 M-sodium phosphate, 0.14 M-NaCl, 3 mM- NaN_3 , 1 mM-EDTA, pH 7.3) by using a Polytron (Brinkmann, Westbury, NY, USA). Tissue homogenates were analysed for concentrations of DNA and RNA by using diphenylamine and orcinol procedures, respectively, as described previously (Reynolds *et al.*, 1985a). Standards were DNA Type I from calf thymus and RNA Type IV from calf liver (both from Sigma, St Louis, MO, USA). Concentrations of protein in tissue homogenates were determined by the method of Lowry *et al.* (1951) with bovine serum albumin (Fraction V, Sigma) as standard. Concentration and total content of DNA were used as indexes of hyperplasia, and ratios of RNA:DNA and protein:DNA were used as indexes of tissue hypertrophy (Enesco & Leblond, 1962; Rattray *et al.*, 1975). For caruncular and cotyledonary tissues, total content of DNA, RNA and protein were determined by multiplying concentrations in tissue samples by total tissue weight.

Oxygen consumption. To evaluate metabolic rate of placental tissues, samples (≈ 1 g) of caruncular, intercaruncular, cotyledonary and intercotyledonary (chorioallantoic) tissues were obtained from the middle portion (near the fetus) of the gravid uterine horn of each cow, and samples of maternal liver were obtained from 9 of the cows. Tissue samples were sliced by using a Stadie-Riggs microtome (Thomas, Philadelphia, PA, USA), and slices (0.5 mm thick, 66.6 ± 1.4 mg) were placed into 35-mm Petri dishes containing 3 ml Krebs-Ringer buffer (37°C) of the following composition (mM): NaCl, 118.1; NaHCO_3 , 25.0; KCl, 4.7; CaCl_2 , 2.5; KH_2PO_4 , 1.2; MgSO_4 , 1.2; glucose,

11:1 (Reynolds & Ford, 1984). Tissue slices were weighed on a torsion balance (Roller-Smith, Bethlehem, PA, USA), and placed into a stirred-cell O_2 consumption monitor with a Clark-type polarographic electrode (model 5300, Yellow Springs Instruments, Yellow Springs, OH, USA). Oxygen consumption of tissue slices was monitored for 5 min in 1 ml of O_2 consumption buffer (Krebs⁻-Ringer buffer containing 5.0 mM-sodium pyruvate, 5.0 mM-sodium glutamate, 4.5 mM-sodium acetate and 4.5 mM-malic acid; Ferrell *et al.*, 1976b) at 37°C and recorded on a chart recorder. To minimize time effects, all tissues were analysed within 4 h after collection and in random order. Available O_2 in solution was assumed to be 224 nmol/ml Krebs⁻-Ringer buffer at 760 mmHg and 37°C (Umbriet *et al.*, 1972) and was adjusted daily for barometric pressure, which was determined in the laboratory by using a mercury barometer. Oxygen consumption was calculated as percentage utilization of available O_2 and is reported as $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}$ tissue⁻¹.

Incorporation of [³H]leucine into macromolecules. To evaluate rate of synthesis/secretion of macromolecules, samples (≈ 1 g) of caruncular, intercaruncular, cotyledonary and intercotyledonary (chorioallantoic) tissues were obtained, as above, from the middle portion of the gravid uterine horn of each cow. Explants (175.7 ± 7.4 mg) of these tissues were incubated for 24 h in 10-ml low-leucine Eagle's MEM containing ≈ 20 μCi (17.6 ± 0.3 μCi ; 293.7 ± 4.3 pmol) [³H]leucine (1-[4,5-³H(N)]leucine: New England Nuclear, Boston, MA, USA), as described previously (Bartol *et al.*, 1985a, b; Millaway *et al.*, 1989). After incubation, duplicate 0.25 ml aliquants of media were diluted with 1.75 ml distilled H_2O and dialysed (SpectraPor 6, 1000 M_r cutoff, Spectrum, Los Angeles, CA, USA) against 1000 volumes phosphate-buffered saline (pH 7.4) at 4°C, as described by Millaway *et al.* (1989). Radioactivity remaining after dialysis was determined by liquid scintillation counting of 0.5 ml samples of retentates. To determine rate of synthesis/secretion of macromolecules, duplicate explants (185.7 ± 17.1 mg) of placental tissues were obtained from 2 additional cows (Days 197 and 202 of gestation) and incubated as described above. Samples (0.25 ml) of media from these explant incubations were obtained at 8, 16 and 24 h of incubation, diluted, dialysed and counted for radioactivity as described above. Data are reported as pmol [³H]leucine incorporated into non-dialysable macromolecules per unit time per gram of tissue.

Statistical analysis. To describe growth of gravid uterine tissues, an exponential growth model was used:

$$W = W_0 e^{(b_1 + b_2 t)}$$

where W = weight in grams, W_0 = initial weight in grams at Day 0 of gestation, b_1 = initial growth rate, b_2 = change in growth rate and t = day of gestation (Koong *et al.*, 1975; Ferrell *et al.*, 1976a; SAS, 1985). This exponential model also was used to describe total contents of DNA, RNA and protein in caruncular and cotyledonary tissues, except that content in milligrams was used instead of weight in grams. For curved crown-rump length, linear regression on day of gestation was used. Least-squares (General Linear Models) analysis of variance was used to determine differences in DNA, RNA and protein concentrations and ratios, O_2 consumption rates, and [³H]leucine incorporation rates of placental tissues, with day of gestation, tissue and day $\times$ tissue interaction included in the model (SAS, 1985). When a main effect or interaction F-test was significant ($P < 0.05$), differences between specific means were determined by using Bonferroni's t test (Kirk, 1968). Data are reported as means $\pm$ s.e.m.

Results

Weights of gravid uterine tissues

Weights of gravid uterine tissues and fetal fluids, and also curved crown-rump lengths, are given in Table 1. Weights of all the gravid uterine tissues increased exponentially ($P < 0.01$) from Day 100 to Day 250 of gestation, and the model used to describe growth of these tissues explained a large proportion of the variation in weights ($R^2 = 0.80$ – 0.98 ; Table 2, Fig. 1a and 1b). Although weight of fetal fluids also increased exponentially ($P < 0.02$), the model explained only 26% of variation in fetal fluid weight (Table 2). Curved crown-rump length increased as a linear function of day of gestation [curved crown-rump (cm) = $-21.032 + 0.457$ (day of gestation), $R^2 = 0.96$, $N = 21$, $P < 0.01$].

The initial rate of increase in fetal weight was 8.02% per day, and this rate decreased by 0.014% per day as gestation advanced (Fig. 1a). This model agrees with the data of Koong *et al.* (1975) for sheep and Ferrell *et al.* (1976a) for cattle, who also reported a decrease in relative rate (%/day) of fetal growth as gestation progressed. Although relative rate decreased, absolute rate (kg/day) of fetal growth continued to increase throughout gestation because of increasing fetal mass. Thus, in the present study, increase in fetal weight from Days 100 to 150, 150 to 200 and 200 to 250 of gestation was 816, 407 and 220% on a relative basis but 2.72, 9.53 and 15.12 kg on an absolute basis (Table 1). The initial rate of increase in placental (caruncular + cotyledonary) weight was 6.16% per day, which decreased by 0.012% per day thereafter (Fig. 1a). Because the relative rate of

Table 1. Weights (means $\pm$ s.e.m.) of gravid uterine tissues of cows

Tissue wt (kg)	Day of gestation			
	100	150	200	250
Gravid uterus	3.61 $\pm$ 0.16	12.73 $\pm$ 0.56	28.79 $\pm$ 1.59	47.34 $\pm$ 3.64
Uterus	—	1.99 $\pm$ 0.13	3.75 $\pm$ 0.20	4.28 $\pm$ 0.32
Fetus	0.38 $\pm$ 0.01	3.10 $\pm$ 0.11	12.63 $\pm$ 0.38	27.75 $\pm$ 2.13
Fetal membranes	0.25 $\pm$ 0.02	0.46 $\pm$ 0.03	1.15 $\pm$ 0.77	1.45 $\pm$ 0.92
Fetal fluids	—	6.01 $\pm$ 0.45	7.86 $\pm$ 1.07	9.05 $\pm$ 0.85
Caruncles	0.13 $\pm$ 0.001	0.71 $\pm$ 0.05	2.12 $\pm$ 0.14	3.11 $\pm$ 0.29
Cotyledons	0.16 $\pm$ 0.01	0.46 $\pm$ 0.03	1.28 $\pm$ 0.11	1.69 $\pm$ 0.28
Curved crown-rump length (cm)	23.0 $\pm$ 1.0	48.5 $\pm$ 0.8	75.6 $\pm$ 1.3	90.1 $\pm$ 2.4

Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 of gestation for all tissue weights and also for curved crown-rump length.

Table 2. Relationships between weights (g) of some components of the gravid uterus and day of gestation (t) in cows

Component	Exponential equation	Coefficient of determination (R^2)	Coefficient of variation (%)
Gravid uterus	$W = 71.228e^{(0.0468 - 0.0000838t)}$	0.98	1.5
Uterus	$W = 11.257e^{(0.0503 - 0.0001064t)}$	0.80	2.0
Fetal membranes	$W = 19.387e^{(0.0289 - 0.0000462t)}$	0.92	3.0
Fetal fluids	$W = 3257.09e^{0.00405t}$	0.26	2.9

Significance of exponential regressions: $P < 0.01$ for gravid uterus, uterus and fetal membranes, and $P < 0.02$ for fetal fluids. Numbers of observations were 21 for gravid uterus and fetal membranes, and 17 for uterus and fetal fluids. Regressions of uterine and fetal fluid weights include only data from Day 150 to Day 250 since data for these components were not obtained on Day 100 (Table 1).

increase of fetal weight was greater than that of placental weight, fetal weight increased 73-fold whereas placental weight increased only 16-fold from Day 100 to 250 of gestation (Table 1; Fig. 1a). Thus, although fetal and placental weights were similar at Day 100 of gestation (0.38 and 0.29 kg, respectively), by Day 250 fetal weight was 6-fold greater than placental weight (Table 1, Fig. 1a). Similarly, because of a difference in relative rate of increase in weight of maternal caruncles and fetal cotyledons, these components of the placenta exhibited differential growth (Fig. 1b). Although their weights were similar at Day 100, caruncular weight was 2-fold greater than cotyledonary weight by Day 250 of gestation (Table 1, Fig. 1b). Although caruncular and cotyledonary tissues cannot be separated with 100% efficiency, our experience suggests that only a relatively small amount of fetal cotyledonary tissue remains in maternal caruncular crypts when these tissues are carefully separated, and we have substantiated this observation histologically (Reynolds & Redmer, 1988). It therefore seems unlikely that the 2-fold difference between caruncular and cotyledonary weights at Day 250 could be explained by poor efficiency of separation of these tissues.

DNA, RNA and protein concentrations

Concentrations and ratios of DNA, RNA and protein in placental tissues are given in Table 3. A significant effect of day of gestation was found only for tissue protein concentrations, which

Fig. 1. Relationships of tissue weights (g) with day of gestation (t) in cows. Exponential regressions: **(a)** fetal weight = $0.463e^{(0.0802 - 0.0001452t)t}$, $R^2 = 0.99$, $N = 21$, C.V. = 1.6%, $P < 0.01$; and placentomal weight = $1.757e^{(0.0616 - 0.0001203t)t}$, $R^2 = 0.97$, $N = 21$, C.V. = 2.5%, $P < 0.01$. **(b)** caruncular weight = $0.532e^{(0.0682 - 0.0001343t)t}$, $R^2 = 0.98$, $N = 21$, C.V. = 2.6%, $P < 0.01$; and cotyledonary weight = $3.680e^{(0.0458 - 0.0000859t)t}$, $R^2 = 0.91$, $N = 21$, C.V. = 4.2%, $P < 0.01$. **(c)** caruncular DNA content = $5.347e^{(0.0589 - 0.0001115t)t}$, $R^2 = 0.94$, $N = 21$, C.V. = 3.4%, $P < 0.01$; and cotyledonary DNA content = $3.422e^{(0.0515 - 0.0000891t)t}$, $R^2 = 0.89$, $N = 21$, C.V. = 5.6%, $P < 0.01$.

increased ($P < 0.01$) from Day 100 to 250. When averaged across all days of gestation, concentrations of DNA, RNA and protein (mg/g tissue) were greater ($P < 0.01$) in caruncular (4.31 ± 0.22 , 3.88 ± 0.12 and 51.1 ± 1.4) than in intercaruncular (2.70 ± 0.22 , 1.85 ± 0.14 and 37.2 ± 2.2) or cotyledonary (2.54 ± 0.21 , 1.79 ± 0.19 and 36.4 ± 2.9) tissues, and were greater ($P < 0.01$) in intercaruncular and cotyledonary than in intercotyledonary (1.62 ± 0.21 , 1.24 ± 0.16 and 22.7 ± 1.9) tissues (Table 3). Additionally, significant ($P < 0.01$) day $\times$ tissue interactions were observed for DNA, RNA and protein concentrations of placental tissues. Concentrations of DNA, RNA and protein remained constant across day of gestation in caruncular and intercaruncular tissues. For cotyledonary and intercotyledonary tissues, however, concentrations of DNA, RNA and protein increased ($P < 0.01$) from Day 100 to 250 of gestation. As mentioned above, rate of increase in cotyledonary weight was less than that of caruncular weight. However, since concentrations of DNA increased 2-fold in cotyledonary tissues while remaining constant in caruncular tissues, the rate of increase of total DNA was similar for cotyledonary and caruncular tissues (Fig. 1c). The ratio of cotyledonary to caruncular DNA therefore remained constant across day of gestation, averaging 0.39 ± 0.02 (Fig. 1c). Similarly, because concentrations of RNA and protein in cotyledonary tissues increased 3-fold across gestation, rates of increase of total tissue RNA and protein also were comparable for cotyledonary and caruncular tissues (data not shown). These differences in cellular growth of maternal and fetal placental tissues also were observed for interplacental tissues, with intercotyledonary tissues exhibiting 3–4-fold increases

in DNA, RNA and protein concentrations, whereas concentrations of DNA, RNA and protein in intercaruncular tissues remained constant from Day 100 to 250. Ratios of RNA:DNA and protein:DNA remained constant across day of gestation for all placental tissues, and no day $\times$ tissue interaction was observed. When averaged across all days of gestation, ratio of RNA:DNA was greatest ($P < 0.05$) in caruncular (0.94 ± 0.05), intermediate in intercotyledonary (0.82 ± 0.08) and least ($P < 0.05$) in intercaruncular and cotyledonary (0.73 ± 0.05 and 0.74 ± 0.09) tissues (Table 3). Protein:DNA ratio was greater ($P < 0.05$) in intercotyledonary (16.7 ± 1.5) than in caruncular (12.4 ± 0.6) tissues, and was intermediate in intercaruncular and cotyledonary (15.3 ± 1.5 and 15.3 ± 1.4) tissues (Table 3).

Table 3. Mean $\pm$ s.e.m. DNA, RNA and protein concentrations and ratios in placental tissues of cows

Tissue	Day of gestation	DNA (mg/g)	RNA (mg/g)	Protein (mg/g)	RNA:DNA	Protein:DNA
Caruncle	100	4.88 ± 0.35	4.16 ± 0.26	49.9 ± 6.2	0.86 ± 0.05	10.1 ± 0.7
	150	4.55 ± 0.52	4.09 ± 0.18	51.9 ± 2.3	0.94 ± 0.09	11.9 ± 1.1
	200	3.77 ± 0.37	3.46 ± 0.20	47.5 ± 2.2	0.97 ± 0.11	13.2 ± 1.3
	250	4.25 ± 0.44	3.92 ± 0.27	54.7 ± 0.8	0.96 ± 0.11	13.5 ± 1.2
Intercaruncular endometrium	100	2.66 ± 0.53	2.10 ± 0.46	31.6 ± 10.2	0.90 ± 0.14	11.7 ± 4.5
	150	2.23 ± 0.27	1.64 ± 0.34	36.7 ± 2.5	0.75 ± 0.14	17.9 ± 3.4
	200	2.87 ± 0.34	1.90 ± 0.15	39.2 ± 3.1	0.70 ± 0.10	14.6 ± 2.2
	250	2.94 ± 0.60	1.80 ± 0.29	39.4 ± 2.1	0.65 ± 0.08	16.1 ± 3.0
Cotyledon	100	1.56 ± 0.32	0.76 ± 0.20	17.1 ± 2.0	0.49 ± 0.10	11.6 ± 1.4
	150	2.48 ± 0.24	1.30 ± 0.13	29.1 ± 1.0	0.53 ± 0.06	12.1 ± 1.1
	200	2.39 ± 0.40	2.03 ± 0.29	40.4 ± 2.9	1.02 ± 0.29	19.7 ± 3.8
	250	3.39 ± 0.31	2.65 ± 0.26	51.3 ± 1.3	0.78 ± 0.04	16.0 ± 1.9
Intercotyledonary chorioallantois	100	0.66 ± 0.28	0.36 ± 0.10	7.8 ± 1.4	0.79 ± 0.26	18.4 ± 5.7
	150	1.40 ± 0.18	1.30 ± 0.30	23.2 ± 2.0	0.91 ± 0.17	18.3 ± 3.4
	200	2.05 ± 0.51	1.47 ± 0.24	24.8 ± 1.6	0.82 ± 0.16	14.6 ± 2.1
	250	2.03 ± 0.32	1.55 ± 0.28	30.0 ± 1.6	0.76 ± 0.08	16.4 ± 2.2

Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 for all tissues.

Oxygen consumption

Day of gestation had no significant effect on O_2 consumption of placental tissues, and no day $\times$ tissue interaction was found. Oxygen consumption differed, however, among placental tissues (Table 4). Across all days of gestation, O_2 uptake ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$) of intercaruncular tissues (0.37 ± 0.02) was greater ($P < 0.01$) than that of other placental tissues. In addition, O_2 uptake of caruncular tissues across gestation (0.30 ± 0.02) was greater than that of cotyledonary and intercotyledonary tissues (0.10 ± 0.01 and 0.15 ± 0.01) which were similar.

Incorporation of [^3H]leucine into macromolecules

For all tissues, rate of incorporation of [^3H]leucine into non-dialysable macromolecules was linear from 8 to 24 h of culture (regressions, where $y = \text{pmol/g tissue}$ and $x = \text{time in hours}$: caruncular incorporation = $-0.587 + 0.1312x$, $R^2 = 0.44$, $P < 0.05$; intercaruncular incorporation = $1.682 + 1.8169x$, $R^2 = 0.34$, $P < 0.10$; cotyledonary incorporation = $-1.965 + 0.5936x$, $R^2 = 0.52$, $P < 0.01$; and intercotyledonary incorporation = $0.756 + 0.3478x$, $R^2 = 0.29$, $P < 0.10$). Incorporation of [^3H]leucine by placental tissues did not vary across day of gestation. Significant differences ($P < 0.01$) in incorporation of [^3H]leucine into secreted macromolecules were found, however, among placental tissues, and a day of gestation $\times$ tissue interaction ($P < 0.01$) was also observed. When averaged across all days of gestation, rates of secretion

Table 4. Mean $\pm$ s.e.m. oxygen consumption ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g tissue}^{-1}$) of placental tissues of cows

Tissue	Day of gestation			
	100	150	200	250
Caruncle	0.20 $\pm$ 0.07 (3)	0.29 $\pm$ 0.03 (5)	0.32 $\pm$ 0.04 (6)	0.33 $\pm$ 0.06 (6)
Intercaruncular endometrium	0.30 $\pm$ 0.08 (3)	0.39 $\pm$ 0.04 (5)	0.40 $\pm$ 0.04 (6)	0.37 $\pm$ 0.04 (6)
Cotyledon	0.14 $\pm$ 0.04 (2)	0.08 $\pm$ 0.01 (5)	0.10 $\pm$ 0.01 (6)	0.10 $\pm$ 0.01 (6)
Intercotyledonary chorioallantois	0.14 $\pm$ 0.06 (2)	0.11 $\pm$ 0.01 (5)	0.14 $\pm$ 0.02 (6)	0.21 $\pm$ 0.02 (5)

Maternal liver samples averaged $0.48 \pm 0.06 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g tissue}^{-1}$ ($n = 9$). Number of observations is indicated in parentheses.

of macromolecules ($\text{pmol}\cdot 24 \text{ h}^{-1}\cdot\text{g tissue}^{-1}$) by intercaruncular and intercotyledonary tissues (31.3 ± 4.8 and 31.3 ± 3.0) were greater ($P < 0.01$) than that of cotyledonary tissues (21.0 ± 1.9 ; Table 5). In addition, secretory rate of cotyledonary tissues was greater ($P < 0.01$) than that of caruncular tissues (6.2 ± 0.8 ; Table 5). Rate of secretion of macromolecules by caruncles and cotyledons remained constant across day of gestation. Rate of secretion of macromolecules by intercaruncular endometrium, however, decreased ($P < 0.01$), whereas that of intercotyledonary chorioallantois increased ($P < 0.05$) from Day 100 to Day 250 of gestation (Table 5).

Table 5. Mean $\pm$ s.e.m. incorporation of [^3H]leucine into macromolecules by placental tissues of cows*

Tissue	Day of gestation			
	100	150	200	250
Caruncle	7.2 $\pm$ 2.0	8.0 $\pm$ 2.0	4.1 $\pm$ 1.0	6.0 $\pm$ 1.3
Intercaruncular endometrium	49.0 $\pm$ 22.8	35.2 $\pm$ 6.1	26.8 $\pm$ 4.0	20.8 $\pm$ 1.5
Cotyledon	18.8 $\pm$ 5.6	21.2 $\pm$ 4.3	20.7 $\pm$ 4.2	22.5 $\pm$ 2.5
Intercotyledonary chorioallantois	16.5 $\pm$ 6.4	33.4 $\pm$ 5.1	35.7 $\pm$ 5.4	35.2 $\pm$ 4.9

*Non-dialysable [^3H]leucine in culture media expressed as $\text{pmol}\cdot 24 \text{ h}^{-1}\cdot\text{g tissue}^{-1}$. Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 for all tissues.

Discussion

Although placentomal weight increased exponentially throughout gestation, the absolute rate of increase was much less than that of fetal weight. In the ewe, placental weight ceases to increase or even decreases after Day 90 of gestation (Barcroft, 1946; Wallace, 1948; Alexander, 1964a). A similar pattern of continued growth of the fetus, but limited placental growth during the last half of gestation has been observed in several other mammalian species (Ibsen, 1928; Warwick, 1928; Hammond, 1935). In addition, a positive correlation between fetal and placental weight has been reported for several species (Ibsen, 1928; Warwick, 1928; Hammond, 1935; McKeown & Record,

1953; Alexander, 1964a). These observations led Huggett & Hammond (1952) to propose that "the size to which the fetal placenta grows during the early stages of pregnancy may determine, other things being equal, the amount of nutrition that is at the disposal of the fetus for growth during the later stages of pregnancy". However, Huggett & Hammond (1952) also pointed out that this proposal may not be entirely valid since placentar weight may not be indicative of placental function. Indeed, placental clearance of antipyrine, sodium, urea and H_2O increases with gestational age in several species (Flexner & Gellhorn, 1942; Meschia *et al.*, 1966, 1967). In cows, transplacental diffusion of H_2O not only increases with gestational age, but remains constant when expressed as $\text{ml}\cdot\text{min}^{-1}\cdot\text{kg fetus}^{-1}$ (Reynolds & Ferrell, 1987). In the present study, DNA, RNA and protein concentrations of fetal placentomal tissues increased whereas those of maternal placentomal tissues remained constant from Day 100 to Day 250 of gestation. Because DNA, RNA and protein increased at about the same rates, growth of fetal placental tissues did not result from cellular or tissue hypertrophy, but rather from substantial hyperplasia. A portion of this hyperplasia may have resulted from increased density of vascular endothelial cells, which has been shown to occur primarily in fetal compared with maternal placentomal tissues of cows and ewes during late gestation (Hammond, 1927; Barcroft, 1946; Teasdale, 1976). In support of this proposal, umbilical blood flow and fetal O_2 and glucose uptakes increased 20-fold whereas uterine blood flow and uterine O_2 and glucose uptakes increased only 5-fold from mid- to late gestation in cows (Reynolds *et al.*, 1986). Based upon these observations, it seems that, during the second half of gestation, fetal placental functional capacity increases at a greater rate than mass, due, in part, to hyperplasia of fetal placental tissues.

In the present study, the in-vitro rate of oxygen consumption by bovine placental tissues was similar to that reported by us and others for ovine placental tissues (Bell *et al.*, 1987a; Reynolds & Redmer, 1987). The large proportion of O_2 taken up by the gravid uterus and utilized to support placental metabolism varies from $\approx 80\%$ at mid-gestation to $\approx 40\%$ during late gestation in cows and ewes (Battaglia & Meschia, 1981; Bell *et al.*, 1986; Reynolds *et al.*, 1986). In addition, in-vivo studies have shown a relatively high rate of O_2 consumption for the placenta compared with the fetus in cows (0.46 versus $0.25 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$; Reynolds *et al.*, 1986) and ewes (0.63 versus $0.32 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$; Meschia *et al.*, 1966). In the present study, in-vitro uptake of O_2 by maternal placental tissues was comparable to that reported for placental tissues *in vivo* and was 2–3-fold greater than O_2 uptake of fetal placental tissues. Similar observations have been reported for sheep placental tissues (Reynolds & Redmer, 1987). Therefore, maternal placental tissues probably account for a major portion of the high metabolic demand of the placenta. Intercaruncular endometrium, which is the site of uterine glands (Ramsey, 1982), exhibited a greater metabolic rate than did any of the other placental tissues. Uterine glands continue to develop throughout gestation in cows and ewes and secrete large amounts of histotrophe, thereby providing a portion of the nutritional demands of the fetus (Hammond, 1927; Bazer *et al.*, 1979; Ramsey, 1982; L. P. Reynolds & D. A. Redmer, unpublished observations), which may account for the high metabolic rate of intercaruncular endometrium. Fetal placental tissues had relatively low growth and metabolic rates, yet, as discussed above, probably continue to increase their functional capacity throughout gestation. Thus fetal placental tissues appear to provide for the metabolic demands of fetal growth while their own metabolic demands are minimal.

As further indication of the importance of uterine glandular secretions in supporting fetal growth, intercaruncular tissues exhibited a relatively high rate of production of macromolecules. Secretory activity of intercaruncular endometrium, however, decreased from Day 100 to Day 250 of gestation. Assuming that all non-dialysable [^3H]leucine was incorporated into macromolecules and that in-vitro secretory rate reflects secretory activity *in vivo*, decreased secretory activity of intercaruncular endometrium may indicate reduced importance of histotrophic secretion during late gestation. Uterine-specific proteins secreted by intercaruncular endometrium of cows throughout gestation may also have other functions in addition to providing for fetal nutrition (Bazer & First, 1983; Bartol *et al.*, 1985a). Reynolds & Redmer (1988) found that, from Day 100 to 250 of

gestation, intercaruncular endometrium secreted a factor(s) which may regulate growth of blood vessels (angiogenesis) in the placenta. In contrast with intercaruncular tissues, intercotyledonary tissues increased their rate of secretion of macromolecules from Day 100 to 250. The role of secretory products of the chorioallantois in gravid uterine function is not known. Bartol *et al.* (1985b) found a glycoprotein of high molecular weight (735 000) secreted by bovine chorion up to Day 69 of gestation, but did not evaluate later stages and reported no function for this glycoprotein. Reynolds & Redmer (1988) reported that intercotyledonary tissues also secreted a factor(s) which may modulate placental angiogenesis. Additionally, products of intercotyledonary chorioallantois may regulate growth of non-vascular components of placenta. That intercotyledonary tissues can affect endometrial function is indicated by the observation that 'adventitious placentomes', which are smaller and more diffuse than normal placentomes but exhibit similar arrangement of fetal and maternal tissues, frequently form in interplacentomal areas during late pregnancy in cows (Hammond, 1927; L. P. Reynolds & D. A. Redmer, unpublished observations). Therefore, as stated by Hammond (1927), "it is apparent that the power, not only of developing the dormant caruncles of the uterus, but also of initiating the formation of new adventitious placentomal growths rests with the foetal membranes".

We thank C. T. Wold for technical assistance; D. W. Zaeske for assistance at slaughter; and J. Berg for typing the manuscript. Supported, in part, by PHS grant HD22559 to D.A.R. and L.P.R. Journal Article No. 1783 of the North Dakota Agricultural Experiment Station, Project 1782.

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Received 25 August 1989