CHRONIC HEAT STRESS AND PRENATAL DEVELOPMENT IN SHEEP: I. CONCEPTUS GROWTH AND MATERNAL PLASMA HORMONES AND METABOLITES

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ABSTRACT

Pregnant ewes were chronically exposed to thermoneutral (TN; 20°C, 30% relative humidity) or hot (H; 40°C 9 h/d, 30°C 15 h/d, 40% relative humidity) environments between d 64 and 136 to 141 of pregnancy. They were sampled for blood at 14-d intervals during this period for measurement of plasma metabolites and hormones, then slaughtered and dissected to measure conceptus weights, dimensions and fetal organ weights. Rectal temperatures of H ewes were elevated .3 to 1.0°C above those of TN ewes throughout the experiment. Voluntary feed intakes were not altered by heat exposure except after 120 d of pregnancy, when feed intake was about 25% lower (P < .10) by H than by TN ewes. Blood 3-hydroxybutyrate concentrations were not affected by heat, but plasma glucose concentrations were greater in H than in TN animals after 120 d (P < .05). Placental weight, reduced by 54% (P < .001) by heat exposure of ewes, was correlated positively with fetal weight and correlated negatively with fetal/placental weight ratio, fetal brain/liver weight ratio and fetal relative heart weight. Late in pregnancy, plasma concentrations of progesterone, cortisol and placental lactogen were reduced (P < .01) in H ewes, whereas triiodothyronine levels were markedly lower (P < .03) at all stages of pregnancy. Plasma concentrations of prolactin were elevated dramatically (P < .01) and a modest increase (P < .03) in somatotropin levels was recorded in H ewes. These results are consistent with our hypothesis that heat-induced fetal growth retardation is secondary to a primary reduction in placental growth; this could be mediated partly by reduced peripheral activity of thyroid hormones. Heat-induced reductions in secretion of progesterone and ovine placental lactogen more likely were a consequence than a cause of placental stunting. (Key Words: Heat Stress, Fetus, Placenta, Plasma Hormones, Sheep.)


Introduction

Pregnant ewes exposed to heat for lengthy periods often produce small lambs of poor viability (Moule, 1954; Shelton, 1964; Hopkins et al., 1980). These field observations are consistent with experimental evidence that prolonged heat exposure retards fetal growth, perhaps being mediated by a primary inhibition of placental growth and functional capacity (Alexander and Williams, 1971; Bell et al.,
The placenta is stunted to a greater degree than is the fetus. The endocrine regulation of normal placental growth and functional development in sheep and other livestock species is poorly understood; it is presumed to involve hormones and growth factors of maternal and fetal origin. Postulated mediators include steroids, thyroid hormones, insulin and a number of peptide growth factors (Alexander, 1978; Bell et al., 1987a). Heat stress caused chronic changes in plasma concentrations of progestins, estrone sulfate and thyroid hormones in pregnant dairy cows and decreased calf birth weight (Collier et al., 1982), but placental weights were not recorded. The objective of the present study was to determine the effects of heat stress on maternal endocrine and metabolic status in mid and late pregnancy, maternal metabolic adaptation and consequences on fetal and placental growth.

Materials and Methods

Animals and Feeding. Thirteen mature, pregnant Suffolk ewes of known mating date were used. They weighed 83 to 112 kg at the start of the experiment (approximately 60 d of gestation), when pregnancy was confirmed and litter size was determined by real-time ultrasonic scanning. All ewes were fed individually once daily a ration of pelleted alfalfa hay (ME content of 2.15 Mcal/kg DM, CP content of 210 g/kg DM) to estimated energy requirements based on body weight, stage of pregnancy and litter size (NRC, 1985).

Experimental Design and Procedure. Ewes were weighed, ultrasonically scanned and assigned to thermoneutral (TN, n = 7, dry bulb temperature = 18 to 20°C, relative humidity = 30%) or heat (H, n = 6, dry bulb temperature = 40°C for 9 h/d, 30°C for 15 h/d, relative humidity 40°C) treatment at approximately 60 d of pregnancy. At this time litter sizes were estimated as: TN, 1 single, 6 twins; H, 6 twins. Ewes were maintained in their allotted environments from d 64 until slaughter at d 136 to 141 of gestation, during which time they were weighed and their blood was sampled (jugular venipuncture) every 2 wk. Blood samples were first taken 1 d after treatments began. On each sampling day, three blood samples (each = 10 ml) were taken at 20-min intervals during the 60-min period before daily feeding (i.e., 23 to 24 h after the preceding day’s feed was offered). These samples were stored on ice and pooled after each collection. Aliquots of whole blood were taken for measurement of 3-hydroxybutyrate concentration, and the remainder was centrifuged at 2,000 x g and 4°C to separate plasma. Whole blood was immediately deproteinized with HClO4 (1 M) and neutralized with KOH (5 M). Plasma and neutralized, deproteinized extracts from whole blood were stored at -20°C until analysis.

Rectal temperature of heated ewes was measured three times a week, just before the chamber temperature was raised to 40°C (i.e., after ewes had been exposed to 30°C for 14 to 15 h) and again just before it was decreased to 30°C (i.e., after ewes had been exposed to 40°C for 8 to 9 h). Temperatures of TN ewes were measured once or twice weekly.

Dissection Procedure. Ewes at 136 to 141 d of pregnancy were weighed, stunned with a captive bolt pistol and exsanguinated. The pregnant uterus was separated from the rest of the tract at the cervix, rapidly removed, weighed and dissected into its main components, fetus(es), fetal membranes, placentomes and myoendometrium, each of which was weighed. Placental weight was defined as the total mass of placentomes, trimmed of membranes and endometrium, associated with each fetus. Fetuses were towel-dried and weighed, and their lengths from crown to rump were measured. Fetal brain, liver, heart, skeletal muscles (longissimus, biceps femoris, semimembranosus, semitendinosus from right side) and right femur were removed and weighed. From these direct measurements, fetal ponderal index was calculated as: fetal wt (g)/crown-rump length (cm)3 x 100, and average placental weight was calculated as: total placental wt/no. of placentomes.

Measurements. Blood samples were analyzed for 3-hydroxybutyrate (3HB) concentration. Plasma samples were analyzed for concentrations of glucose, urea, creatinine, progesterone, estradiol, cortisol, total thyroxine (T4), total triiodothyronine (T3), insulin, prolactin, somatotropin and ovine placental lactogen (oPL). Methods are detailed below.

Blood 3HB was assayed by the enzymatic method of Kientsch-Engel and Siess (1985)

6Technicare 210DX, Johnson & Johnson, New Brunswick, NJ.
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TABLE 1. FETAL AND PLACENTAL SIZE IN EWES EXPOSED TO THERMONEUTRALITY (TN) OR HEAT (H) DURING MID AND LATE PREGNANCY

<table>
<thead>
<tr>
<th>Item</th>
<th>TN</th>
<th>H</th>
<th>SEb</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal wt, kg</td>
<td>4.07</td>
<td>3.39</td>
<td>.43</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Fetal crown-rump length, cm</td>
<td>48.0</td>
<td>46.4</td>
<td>1.7</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Ponderal index, (wt/length 3)</td>
<td>3.64</td>
<td>3.35</td>
<td>.13</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>398</td>
<td>185</td>
<td>37</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Avg placentome wt, g</td>
<td>8.06</td>
<td>4.59</td>
<td>.91</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Fetal/placental wt</td>
<td>10.7</td>
<td>18.8</td>
<td>1.2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

TN, means of 12 fetuses from 7 ewes; H, means of 7 fetuses from 5 ewes.
bPooled standard error of treatment means.

Using 3-hydroxybutyrate dehydrogenase. Plasma glucose was determined by the glucose oxidase method, using a commercial kit. Plasma urea and creatinine were measured colorimetrically in a Coulter Dacos autoanalyzer involving the urease reaction for urea and reaction with picric acid for creatinine.

Plasma hormone concentrations were measured by radioimmunoassay, using procedures detailed by Fitzgerald and Butler (1982) for progesterone and estradiol-17β, Krey et al. (1975) for cortisone, Butler et al. (1972) for prolactin and Gorewit (1981) for somatotropin. Plasma T₄, T₃ and insulin were assayed with diagnostic kits, validated for use with heparinized ovine plasma. For each hormone, all samples were analyzed in a single assay, for which the coefficient of variation was <10%.

Plasma concentrations of oPL were measured by a specific double antibody radioimmunoassay, using oPL purified from simple extracts of ovine placenomes by anion and cation exchange chromatography, chromatofocusing and high resolution molecular filtration. The preparation was homogenous when assessed by SDS-PAGE (Laemmli, 1970) followed by silver staining (Blum et al., 1987) and was 1.6 times more potent than bovine somatotropin (Miles bGH Lot 13, 1.2 IU/mg) when used in radioreceptor assays for somatotropin-like activity (Tsushima and Friesen, 1973). Rabbits were immunized with oPL and serum was collected 10 d after booster injections. Serum was used at a final dilution of 1:48,000, which provided 45% specific binding of approximately 20,000 cpm of ¹²⁵I-labeled oPL, prepared using Iodo-Gen. Antiserum, 20,000 cpm ¹²⁵I-oPL and either standard amounts of oPL (190 pg to 50 ng) or unknown plasma samples, in a total volume of 400 µl phosphate buffered saline (containing .1% gelatin, 12.5 mM EDTA and 1:1,600 dilution of non-immune rabbit serum) were incubated overnight at 4°C. An appropriate amount of ovine antirabbit gamma globulin was added and incubation was continued for an additional 24 h. Antibody-bound ¹²⁵I-oPL was collected by centrifugation after diluting the contents of each tube with 3 ml ice-cold PBS-gelatin. To permit direct comparison of unknown samples with the standards, an equivalent volume of plasma from nonpregnant sheep was added to all standard tubes. The assay detected 190 pg oPL, distinct from no added oPL, with binding equivalent to 95% that obtained with the zero standard corresponding to 250 pg. Logit-log transformations of standard curves were linear over all standards. Using 25-µl sample volume, the assay was conservatively reliable in the range of 10 to 2,000 ng/ml. There was no cross-reaction with either ovine somatotropin or prolactin at concentrations up to 1,000 ng/ml. Samples, measured in a single assay, had a cv of 5.1%.

Statistical Analysis. Treatment means in Tables 1 and 2 were compared by unpaired t-test. Treatment effects on plasma metabolite and hormone concentrations over time of pregnancy were assessed by analysis of variance using the repeated measurements procedure of Gill (1986), with main effects being treatment, animals, time and treatment x time.
Where appropriate, variance attributable to time or treatment × time was partitioned to allow contrasts at different stages of pregnancy. Relations between placental weight and other variables were described by least squares linear or curvilinear regression analysis. More complex models were chosen only if they improved the R² value significantly.

**Results**

**Rectal Temperature.** Average rectal temperatures of individual TN ewes ranged from 39.1 to 39.5°C (overall mean 39.3 ± 1°C). Average rectal temperatures of individual H ewes ranged from 40.2 to 40.5°C at the end of 9 h daily exposure to 40°C, and from 39.5 to 39.7°C at the end of 15 h daily exposure to 30°C.

**Litter Size.** After ultrasonic scanning at 60 d of pregnancy, litter sizes of the two groups were estimated as: TN, 1 single, 6 twins, H, 6 twins. One H ewe died for reasons unrelated to the experiment. At slaughter, litter sizes were: TN, 2 singles, 5 twins, H, 3 singles, 2 twins. Based on the small number of placentomes per fetus and other evidence of early fetal death (necrotic remains of fetal placental tissue attached to enlarged caruncles, incomplete resorption of fetal remains) we concluded that each of the H ewes carrying a single live fetus at 136 to 141 d originally had been pregnant with twins, at least to 60 d of pregnancy. In contrast, postmortem inspection indicated that diagnostic error was responsible for our failure to identify one TN as a singleton.

**Feed Intake and Live Weight Gain of Ewes.** Mean feed DM intakes by TN and H ewes between d 64 and 120 of pregnancy were 17.5 ± .2 and 16.7 ± .7 g·kg⁻¹·d⁻¹, respectively, which were not different (P > .10). Mean intake over the subsequent 16- to 21-d period until slaughter tended to be greater in TN ewes (22.4 ± 1.9 g·kg⁻¹·d⁻¹) than in H ewes (17.7 ± 1.8 g·kg⁻¹·d⁻¹) (P < .10). Mean live weight gain between d 64 and slaughter was greater (P < .05) in TN (22.6 ± 2.2 kg) than in H ewes (15.8 ± 1.7 kg) ewes. About half this difference was due to the greater (P < .05) mass of the pregnant uterus of the TN ewes (12.4 ± .9 vs 9.0 ± 1.0 kg), so that the estimated gain in weight of nonpregnant tissues was not affected (P = .3) by heat treatment (10.1 ± 2.0 vs 6.8 ± 2.2 kg).

**Fetal and Placental Weights.** Mean fetal weight was 17% lower in H than in TN ewes, but this difference was not statistically significant (P = .14). In contrast, total placental weight was reduced by 54% (P < .001) in H ewes, mainly due to decreased (P < .01) weight of individual placentomes (Table 1).

Fetal weight was curvilinearly related to placental weight (Figure 1a; r = .61, P < .01). The ratio of fetal to placental weights was increased (P < .001) by heat stress (Table 1) and was correlated negatively with placental weight (Figure 1b; r = −.89, P < .001). Fetal crown-rump length was unaffected by heat, but fetal ponderal index was lower (P < .05) in H ewes (Table 1).

**Table 2. Fetal Organ Weights in Ewes Exposed to Thermoneutrality (TN) or Heat (H) During Mid and Late Pregnancy**

<table>
<thead>
<tr>
<th>Item</th>
<th>TN</th>
<th>H</th>
<th>SEb</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ wt, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>49.0</td>
<td>45.5</td>
<td>2.2</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Liver</td>
<td>108.9</td>
<td>76.7</td>
<td>11.7</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Heart</td>
<td>27.8</td>
<td>28.5</td>
<td>3.0</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Skeletal musclec</td>
<td>85.7</td>
<td>66.0</td>
<td>9.8</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Femur</td>
<td>29.9</td>
<td>26.6</td>
<td>3.5</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Brain/liver</td>
<td>.48</td>
<td>.61</td>
<td>.05</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Organ wt, g/kg fetal wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>12.5</td>
<td>13.9</td>
<td>1.1</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Liver</td>
<td>27.0</td>
<td>22.9</td>
<td>2.1</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Heart</td>
<td>6.8</td>
<td>8.6</td>
<td>.5</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Skeletal musclec</td>
<td>21.2</td>
<td>19.3</td>
<td>.8</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Femur</td>
<td>7.3</td>
<td>7.9</td>
<td>.3</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

aTN, means of 12 fetuses from 7 ewes; H, means of 7 fetuses from 5 ewes.

bPooled standard errors of treatment means.

cAggregate of longissimus, biceps, femoris, semimembranosus and semitendinosus.
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Figure 1. Relations between (a) fetal weight (F) and placental weight (P); \( F = 1.27 \ln P - 3.38, r = .61 \) (P < .01) (b) fetal placental weight ratio (F/P) and placental weight (P); \( F/P = -0.06 \ln P + 65.0, r = -0.89 \) (P < .001). Circles = singles; triangles = twins; open symbols = thermoneutral; closed symbols = heat.

Figure 2. Relations between (a) fetal brain/liver weight ratio (B/L) and placental weight (P), B/L = -0.0083 P + .80, \( r = -.85 \) (P < .001) (b) fetal heart weight (H) and placental weight (P), H = -0.0000801 P^2 + .000801 P + 13.75, \( r = -.87 \) (P < .001). Circles = singles; triangles = twins; open symbols = thermoneutral; closed symbols = heat.

Figure 3. Changes in (a) blood 3HB concentrations, (b) plasma urea concentrations, (c) plasma creatinine levels, and (d) plasma progesterone concentrations over the experimental period (P < .001) (Figure 3c). In contrast, plasma creatinine levels were unchanged within groups but were greater (P < .01) in H than in TN ewes throughout the experimental period (Figure 3d).

Blood 3HB concentrations were unaffected by heat at any stage of environmental treatment (Figure 3b). Mean concentrations increased from .4 to .5 mmol/liter before 120 d of gestation to .7 to .8 mmol/liter at 135 d in TN and H ewes (P < .001).

Plasma urea concentrations were similar in TN and H ewes but increased over the experimental period (P < .001) (Figure 3c). In contrast, plasma creatinine levels were unchanged within groups but were greater (P < .01) in H than in TN ewes throughout the experimental period (Figure 3d).

Plasma Hormones. Plasma concentrations of progesterone increased steadily from 4.00 ± .47 to 8.66 ± .75 ng/ml during the latter half of pregnancy in TN ewes but were unchanged during this period in the H group (treatment x time interaction, P < .001) (Figure 4a). Thus, at 135 d, its mean level was 73% greater (P < .01) in TN than in H ewes.

Plasma levels of estradiol-17β increased steadily (P < .001), but at no time during the experimental period were they affected by heat (Figure 4b).
Figure 3. Gestational changes in concentrations of (a) plasma glucose, (b) blood 3-hydroxybutyrate (3HB), (c) plasma urea, (d) plasma creatinine in ewes exposed to thermoneutral (open circles) or hot (closed circles) environments.

Figure 4. Gestational changes in plasma concentrations of (a) progesterone (b) estradiol-17β, (c) cortisol in ewes exposed to thermoneutral (open circles) or hot (closed circles) environments.

Plasma concentrations of cortisol were relatively constant between d 64 and 135 of gestation in TN ewes, but in H ewes cortisol declined sharply between d 64 and 78; thereafter values were significantly lower than in TN ewes (treatment x time interaction, P < .01) (Figure 4c). Although blood samples were taken by venipuncture, values for plasma cortisol consistently were within the range observed in pregnant ewes sampled by catheter.

Plasma concentrations of T₄ suffered a steady, modest decline (P < .001) throughout the experimental period and were not significantly affected by heat (Figure 5a). In contrast, T₃ levels persistently were 30 to 50% lower in H than in TN ewes (P < .025) over the whole treatment period (Figure 5b).

Effects on several peptide hormones are summarized in Figure 6. Plasma concentrations increased (P < .001) between mid and late gestation but were not affected by heat (Figure 6a). Prolactin levels remained very low in TN ewes through most of the experimental period but increased linearly throughout the period of heating in H ewes to a mean value of 126 ng/
ml at 135 d (treatment main effect, $P < .01$; treatment × time interaction, $P < .001$) (Figure 6b). Plasma somatotropin levels did not vary with stage of gestation but were elevated ($P < .025$) in $H$ ewes (Figure 6c). Somatotropin concentrations in all ewes were low and close to the sensitivity limit of the assay. Plasma oPL concentrations increased steadily through the latter half of gestation in the TN group. In $H$ ewes the rate of increase was attenuated before 103 d and levels were unchanged thereafter (treatment × time interaction, $P < .001$). Thus, mean concentration over the period 120 to 135 d was lower ($P < .01$) in $H$ ewes, being only 44% of that in TN ewes (Figure 6d).

**Relations Between Plasma Constituents and Placental Weight.** Relations between mean values for plasma concentrations of glucose, progesterone and oPL at 120 and 135 d gestation and total placental weight (i.e., aggregate of both placentae in ditocous ewes) are illustrated in Figure 7. Plasma glucose declined with increasing placental weight ($r = -.60$, $P < .05$; Figure 7a), whereas plasma progesterone increased ($r = .75$, $P < .01$; Figure 7b), as did plasma oPL ($r = .77$, $P < .01$; Figure 7c). One ewe pregnant with twins, with a total placental weight of 540 g, had unusually low plasma glucose (2.70 μmol/ml) and high levels of progesterone (10.5 ng/ml) and oPL (488 ng/ml). When these values were treated as outliers and excluded from the analysis, $r$ values were −.85 ($P < .001$), .92 ($P < .001$) and .88 ($P < .001$), respectively, for glucose, progesterone and oPL vs placental weight.
Figure 7. Relations between (a) plasma glucose (G) and placental weight (P), \( G = -0.00080 \, P + 3.92, \ r = -0.60 \) \((P < 0.05)\), (b) plasma progesterone (PG) and placental weight (P), \( PG = 0.0053 \, P + 4.21, \ r = 0.75 \) \((P < 0.01)\), (c) plasma placental lactogen (PL) and placental weight (P), \( PL = 0.40P + 64.6, \ r = 0.77 \) \((P < 0.01)\). Plasma concentrations are mean values measured at 120 and 135 d gestation. Circles = singles; triangles = twins; open symbols = thermoneutral; closed symbols = heat.

Discussion

The heating regimen used in this study was sufficient to maintain chronic hyperthermia during the treatment period of over 10 wk without causing severe or prolonged inapetence in heated ewes. The absence of nutritional stress in these animals is indicated by their weight gain (corrected for pregnant uterus) and maintenance of normal glycemia and ketonemia during mid and late pregnancy. Contrary to the suggestion of Cartwright and Thwaites (1976), it seems unlikely that observed effects of heat on conceptus growth were indirectly mediated via inadequate mater-
ewes with placentae experimentally embolized during gestation (Charlton and Johengen, 1987). The significant negative correlations of brain/liver weight ratio and relative heart weight with placental weight further suggest primary involvement of placental impairment in the heat-induced changes in these variables. The increase in brain/liver ratio, originally suggested by Dawkins (1964) as an index of human fetal growth retardation, was due primarily to retarded hepatic growth, presumably related to the reduced capacity of the heat-stunted placenta to deliver nutrients (Bell et al., 1987b). The relative hypertrophy of the fetal heart in H ewes could have been caused by the chronic hypoxemia we observed previously in heated fetuses, the magnitude of which was related inversely to placental size (Bell et al., 1987b), because short-term fetal hypoxia causes a redistribution of cardiac output in favor of the heart (Cohn et al., 1974). However, increased relative heart weight was not observed in chronically hypoxemic, small fetuses from carunclectomized ewes (Robinson et al., 1979).

The preceding discussion is weighted in favor of placental mediation of heat-induced changes in fetal organ growth. An alternative possibility is that fetal hyperthermia more directly affects relative growth of fetal tissues, including the trophoblastic component of the placenta. This possibility merits investigation. However, we remain impressed by the remarkable similarity between fetal growth responses to heat and those to direct manipulation of placental size by pre-mating carunclectomy of ewes (Alexander, 1974).

Most progesterone and all oPL circulating in maternal blood during late pregnancy is of placental origin in sheep (Porter et al., 1982). Heat-induced changes in plasma concentrations of these hormones and of glucose further reinforce the idea that fetal growth retardation in H ewes was caused primarily by placental insufficiency rather than by an inadequate maternal nutrient supply. In particular, maternal plasma glucose concentrations increased in H but not in TN ewes during the period of most rapid fetal growth, and maternal glycemia at this time was correlated negatively with placental weight. We cannot ascertain whether increased glucose levels were a direct consequence of reduced placental glucose transport capacity, and thus of decreased demand on the maternal glucose pool (Bell et al., 1987b), or whether they were more indirectly mediated by reduced placental secretion of progesterone and/or oPL in H ewes. Evidence for a specific effect of progesterone on ovine glucose metabolism is equivocal (Luthman et al., 1972; Samad and Ford, 1981). With regard to oPL, present results are consistent with the elevation of plasma glucose levels in carunclectomized ewes with small placentae and proportionately reduced secretion rates and circulating levels of oPL (Falconer et al., 1985). Neither set of observations is consistent with the postulated induction of insulin resistance in women by human placental lactogen during late pregnancy (Grumbach et al., 1968), but it must be emphasized that this theory remains unproven in humans or any other species.

Heat-induced reductions in plasma progesterone and oPL presumably were consequences, rather than causes, of placental stunting. Similarly, decreased plasma concentrations of estrone sulfate in chronically heat-stressed cows were ascribed to decreased placental secretion, although placental size was not measured (Collier et al., 1982). Because placental weight is related directly both to fetal weight and to maternal plasma concentrations of progesterone and oPL, it is not surprising that lamb birth weight has been correlated positively with circulating levels of each of these placental hormones (Taylor et al., 1980; Caton et al., 1983) during late pregnancy.

Other heat-induced changes in plasma hormone concentrations included decreased T₃ and cortisol, greatly increased prolactin and a small, probably biologically insignificant increase in somatotropin. Of these, the reduction in T₃ may have had the greatest modulating effect on placental metabolism and growth. This suggestion is not necessarily incompatible with previous findings (Shelton, 1965; Alexander and Williams, 1971) that supplementation of heat-stressed ewes with T₄ did not alleviate fetal or placental stunting. In the present study, T₄ levels were unchanged by heat, indicating that reduced T₃ levels were due to decreased peripheral deiodination of T₄ rather than to reduced secretion of thyroid hormones. This contrasts with the Collier et al. (1982) finding of decreased plasma T₄ and unchanged T₃ in naturally heat-exposed pregnant cows. However, chronic, thermally induced changes in T₄ secretion appear to be mediated mainly by changes in feed intake (Evans and Ingram, 1977), which were relatively minor in the
present experiment. Specific placental responses to thyroid hormones in TN and H ewes remain to be studied, but it is notable that weight-specific oxygen uptake of fetal placental tissue in vitro was reduced in H ewes (McBride et al., 1988).

Decreased plasma cortisol and greatly increased plasma prolactin levels have been observed previously in chronically heat-stressed cows (Christison and Johnson, 1972) and ewes (Schillo et al., 1978), respectively. These hormonal changes presumably could underlie adaptive changes in tissue calorigenesis (cortisol) or osmoregulation (prolactin), but their consequences, if any, for placental development in the pregnant ewe are unknown.

Finally, much of the regulation of placental and fetal growth may be mediated by factors derived within the conceptus itself, with maternal hormones acting as modulators, rather than as primary stimuli (Bassett, 1986; Bell et al., 1987a). The possibility that secretion of or sensitivity to trophic factors in the conceptus were affected by hyperthermia was not tested in this study and will be examined in future experiments. Our present interpretation is that heat-induced fetal growth retardation is secondary to a primary reduction in placental size. We are currently studying the metabolic and cytological bases for this negative influence on placental growth.

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