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ESTROGEN SULFATASE AND STEROID SULFATASE ACTIVITIES IN INTRAUTERINE TISSUES OF THE PREGNANT GUINEA PIG

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Summary—The possible role of intrauterine estrogen sulfatase and steroid sulfatase around the time of parturition in the guinea pig was investigated. [^{3}H]Estrone sulfate or [^{3}H]pregnenolone sulfate was incubated with intrauterine tissues. Estrogen sulfatase was found in placenta, endometrium, decidua basalis, amnion and chorion. The presence of steroid sulfatase was established in endometrium and decidua basalis but not in placenta or the fetal membranes. Examination of activities in early (days 32–35), mid (days 44–46) and late (within 5 days of parturition) gestation revealed no significant change in estrogen sulfatase specific activity in decidua basalis. However, in chorion and endometrium this activity was seen to increase approx. 12-fold ($P < 0.001$) and 2.8-fold ($P < 0.001$), respectively, from early to late gestation. In placenta, estrogen sulfatase activity appeared to increase 2.4-fold ($P < 0.001$) and in amnion it decreased 2.8-fold ($P < 0.002$). Steroid sulfatase activity in decidua basalis did not change during gestation, while activity in endometrium was found to increase by a factor of 5.3 ($P < 0.001$), from early to late gestation. The increases, both in estrogen sulfatase activity in chorion, endometrium and placenta and in steroid sulfatase activity in endometrium, occurred primarily within the final 3 weeks of gestation. In contrast, the decrease in estrogen sulfatase activity in amnion occurred principally between the fifth and sixth weeks of gestation. Analysis of radiolabelled metabolites indicated that estradiol and progesterone could be produced via estrogen sulfatase and steroid sulfatase activities in certain tissues. Subcellular fractionation of tissues revealed that the greatest specific activity and total activity, in all cases, was associated with the 105,000 g pellet. Significant activity was also detected in the 750 and 10,000 g pellets but not in the 105,000 g supernatant. Radioimmunoassay of endogenous estradiol-17β (estradiol) in chorion extracts revealed a 6.3-fold increase in the hormone from mid to late gestation. Estradiol levels in endometrium and myometrium did not appear to change during this time. It was concluded that increased estrogen sulfatase activity in guinea pig chorion in late gestation occurs along with elevated levels of the hormone estradiol which may be important for parturition in this species.

INTRODUCTION

Attempts to understand the hormonal factors which may be responsible for parturition have often involved monitoring maternal peripheral plasma levels of steroid hormones over the course of gestation and in the days and hours prior to delivery. While this approach has been relatively successful in the sheep [1, 2], studies of other species such as the human [3, 4] and guinea pig [5, 6] have failed to demonstrate dramatic or consistent changes in serum hormone levels at term. This result has led to the concept of local production of steroid hormone and its potential importance in parturition. Changes in steroid hormone availability in intrauterine structures such as the endometrium at term might be secondary to changes in levels or activities of the enzymes responsible for their production. In view of the abundance of steroid sulfates in the maternal and fetal circulation and in the amniotic fluid of many mammals at term, the possible role of sulfatase enzymes in hormone production during gestation has been investigated.

Estrogen sulfatase and steroid sulfatase activities were measured in guinea pig intrauterine tissues at three times during the 62–71 day gestation period. Metabolites of sulfatase activity were isolated and identified. In addition, tissues levels of estradiol-17β (estradiol) were determined at various times in gestation by radioimmunoassay (RIA), in an attempt to relate tissue hormone levels to any observed

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changes in sulfatase activity. The results of these studies and the possible significance of sulfatase enzymes in parturition of the guinea pig are discussed below.

EXPERIMENTAL

Chemicals and reagents

All radiolabelled steroids were obtained from New England Nuclear Canada Ltd, Dorval, Quebec. [6,7-^{3}H(N)]Estrone (45–60 Ci/mmol), [6,7-^{3}H(N)]estradiol (48 Ci/mmol) and [2,4,6,7-^{3}H(N)]estradiol (93 Ci/mmol) were purified by chromatography on Sephadex LH_{20} (Pharmacia, Canada Ltd, Montreal) using the solvent system hexane:chloroform:ethanol (5:5:1) and then stored in methanol at 4°C. [6,7-^{3}H(N)]Estrone-3-sulfate (40 Ci/mmol) was purified on DEAE-Sephadex A_{25} (Pharmacia) employing a 0–0.8 M NaCl gradient as described previously [7]. Fractions containing [^{3}H]estrone sulfate were passed through a Sep-Pak C_{18} cartridge (Waters Associates, Milford, Mass). Steroid sulfate was eluted with and stored in methanol at −20°C.

[7-^{3}H(N)]Pregnenolone-3-sulfate was synthesized from [7-^{3}H(N)]pregnenolone (10–23 Ci/mmol) by a method based on that of Fieser [8]. [7-^{3}H(N)]Pregnenolone was reacted with chlorosulfonic acid in pyridine at 0°C. The product was isolated by extracting into butanol and the crude pregnenolone sulfate was purified by chromatography on DEAE-Sephadex A_{25} using a 0–0.4 M NaCl gradient. Steroid sulfate was concentrated by use of Sep-Pak and stored in methanol at −20°C. The purity of all radiolabelled steroids and steroid sulfates was periodically checked by chromatography as described above. Non-radiolabelled steroid sulfates were synthesized, purified and stored as described elsewhere [9].

Non-radiolabelled steroids. Trizma base and monothioglycerol were obtained from Sigma Chemical Co., St Louis, Mo. and Steraloids, Wilton, N.H. Dulbecco's Modified Eagle Medium (DMEM) and nutrient mixture F-12 (HAM) were obtained from Gibco Laboratories, Life Technologies, Grand Island, N.Y. All other chemicals and organic solvents were purchased from BDH Chemicals Canada Ltd, Toronto, Canlab, Toronto, or Fisher Scientific Co., Toronto, Ontario.

Tissues

The guinea pigs used were of the English Shorthair variety and were bred in our animal quarters. Animals were sacrificed by a single blow to the neck region as approved at the time by the Canadian Council for Animal Care. Tissues were rapidly dissected from guinea pigs in early (days 32–35) mid (days 44–46) and late (days 64–68) gestation. At this latter time, the pubic symphysis is greatly relaxed in preparation for delivery within about 1–4 days.

Whole tissue incubation

Chorion, amnion, endometrium, placenta and decidua basalis were collected and rinsed with ice-cold saline, blotted on moist filter paper and weighed. Whole tissue portions, 300–600 mg in weight, were incubated with 4 nM (approx. 2×10^5 dpm) [6,7-^{3}H]estrone-3-sulfate or [7-^{3}H]pregnenolone-3-sulfate in 2 ml of DMEM + F12(HAM) at 37°C for 2 h. Tissue was removed and 3 ml of double-distilled water were added to the incubation medium prior to extraction of free steroid into 5 ml of diethyl ether. Triplicate aliquots of both the organic and aqueous phases were transferred to mini counting vials and organic aliquots were allowed to evaporate before analysis by liquid scintillation spectrometry [10]. Both phases were counted to confirm total isotope in each incubation. Blank values obtained by substituting tissue boiled for 15 min for intact tissue were of the order of 0 and 2% ether-extractable radioactivity when [6,7-^{3}H]estrone sulfate and [7-^{3}H]pregnenolone sulfate, respectively, were employed as substrates. Blanks measured in the absence of tissue were found to be identical to those mentioned above and therefore this second methodology was used in subsequent studies. Sulfatase activity was calculated as pmol hydrolysed/2 h/g wet wt of tissue.

Isolation and identification of steroid metabolites

In order to specifically identify the free steroidal products of sulfatase action, $8 \times 10^6 - 1.2 \times 10^7$ dpm of [6,7-^{3}H]estrone-3-sulfate were incubated with chorion and endometrium as previously described; 4×10^6 dpm of [7-^{3}H]pregnenolone-3-sulfate were incubated with endometrium only. Subsequent to extraction, the ether phases from a series of similar experiments were combined, evaporated and fractionated on Sephadex LH_{20}. C_{18} metabolites were chromatographed in hexane:chloroform:methanol (5:5:1), while C_{21} steroids were fractionated by the solvent system isooctane:benzene:methanol (85:10:5).

C_{18} metabolites were identified as follows. Column fractions expected to contain labeled estrone were pooled, as were those believed to contain labelled estradiol, and the pooled fractions were reduced in volume by evaporation. Portions of these samples containing 1×10^5–3×10^5 dpm of ^3H were mixed with 100 mg of the appropriate unlabelled carrier steroid and recrystallized to constant specific activity from methanol. To further prove the identities of these metabolites, the estrone and estradiol crystals were acetylated by reaction with acetic anhydride in pyridine. The resulting acetate and diacetate derivatives were recrystallized to constant specific activity from methanol–water.

The C_{21} metabolites of steroid sulfatase activity in endometrium were identified by techniques similar to those described above. In experiments involving pregnenolone sulfate as substrate, column fractions corresponding to labelled progesterone and pregnenolone were pooled, and samples of these containing $2 - 4 \times 10^5$ dpm of ^3H were mixed with 100 mg of the corresponding unlabelled steroid and recrystallized to constant specific activity from methanol–water and methanol, respectively. To further establish their identities, the progesterone sample was reduced by reaction with $NaBH_4$ in methanol at 4°C, and pregnenolone and the reduced progesterone were acetylated and recrystallized to constant specific activity from methanol–water.

Preparation of subcellular fractions

Chorion or enodmetrial tissue was finely minced and homogenized in 10 vol ice-cold, 10 mM Tris–acetate buffer, pH 7.6, containing 0.25 M sucrose, 1 mM Na_2EDTA and 1 mM monothioglycerol, on ice, by 4–8 × 15 s bursts of a Brinkman Polytron at setting 6. The homogenate was centrifuged at 750 ***g*** for 15 min and the resulting pellet was washed with buffer. Wash and supernatant were combined and centrifuged at 10,000 ***g*** for 20 min. The pellet was again washed, wash and supernatant were combined and spun at 105,000 ***g*** for 60 min. Pellets from 750, 10,000 and 105,000 ***g*** spins were resuspended by homogenization in buffer and were stored frozen along with high speed supernatant at −20°C.

Sulfatase assay of subcellular fractions

The assay for estrogen sulfatase activity was based upon a published procedure [11]. Five nmol of estrone-3-sulfate in methanol, containing approx. 2×10^5 dpm of [6,7-^3H]-estrone-3-sulfate were measured into tubes. After evaporation of the solvent, an appropriate volume of enzyme preparation was added, together with 100 mM Tris–acetate, pH 7.6, to give a final volume of 500 μl. Blanks were prepared by omitting the enzyme. Incubation was carried out at 37°C in a shaking water bath for various periods of time and the reaction was terminated by the addition of 2.5 ml of ice-cold, 3.0 M acetate, pH 4.0. Free steroid was extracted into 6 ml of diethyl ether and triplicate aliquots of organic and aqueous phases were taken for isotope counting. In the case of the steroid sulfatase assay labelled pregnenolone-3-sulfate served as substrate. The steroid sulfatase incubation was terminated by the addition of 2.5 ml of ice-cold, double-distilled water [12] and immediate extraction into ether. All other aspects of the assay were as previously described for the estrogen sulfatase assay.

Protein determination

Protein was measured by the method of Bradford [13] employing Biorad bovine serum albumin as a protein standard, and utilizing the BioRad Protein Assay kit purchased from BioRad Laboratories, Richmond, Calif.

Preparation of tissue samples for RIA

Endometrium was carefully dissected from the surrounding myometrium, and along with chorion these tissues were rinsed well with saline. Tissues were homogenized in a volume of methanol equal to the weight in grams of tissue taken (2–6 g), by 2 × 15 s bursts of a polytron. Steroids were extracted for 2 h at 4°C and the homogenate was centrifuged at 10,000 ***g*** for 15 min. The pellet was dried for 48 h at 70°C prior to determination of the pellet mass. To the methanol supernatant were added approx. 2×10^5 dpm of [6,7-^3H]estrone and the solvent was evaporated. The sample was fractionated on Sephadex LH_{20} employing the solvent system heptane:chloroform:methanol:water (500:500:75:3). From the elution profile and previous calibration of the column with [^3H]estrone and [^3H]estradiol, the fractions containing estrone and those containing the extracted estradiol were each pooled. The estradiol sample was dried under nitrogen and stored for RIA.

RIA of estradiol

The antibody used was kindly provided by Dr T. G. Kennedy (Departments of Obstetrics and Gynecology and of Physiology, University of Western Ontario) and had been prepared in rabbits against estradiol-6-CMO-BSA by the method of Dean *et al.* [14, 15]. Lyophilized antiserum was dissolved in 0.1 M phosphate buffer containing 0.15 M NaCl, 0.1% gelatin and 0.1% sodium azide (w/v), to a final concentration of 5 mg/ml. One hundred μl aliquots were frozen at $-20°C$ for storage and diluted with 10 ml of buffer on the day of the assay.

The dried estradiol sample was dissolved in 0.8 ml of the RIA assay buffer mentioned above. Triplicate aliquots of 100 μl were added to 12 × 75 mm borosilicate glass test tubes and mixed with 100 μl of the dilute antiserum. The dilution of antiserum that bound 50–60% of 24,000 dpm (30 pg) of [2,4,6,7-^{3}H]estradiol was utilized in the assay. One hundred μl of assay buffer containing 2.5×10^4 dpm of [2,4,6,7-^{3}H]estradiol were added to give a final volme of 300 μl. A sample blank contained 100 μl of assay buffer in place of 100 μl of antiserum.

A standard curve was constructed over the range 5–640 pg of estradiol. Eight concentration points were utilized, each in triplicate, apart from a zero point which employed buffer in place of unlabelled estradiol. Non-specific binding was assessed by substituting buffer for antiserum.

Sample tubes and standard curve tubes were mixed briefly by vortexing and left at 4°C overnight. Free and bound fractions were separated by dextran–charcoal treatment and supernatants were decanted into scintillation vials for counting.

Statistical analysis

The unpaired Student's t-test was utilized to compare the difference betwen any two times for a single tissue.

RESULTS

Sulfatase activities in intrauterine tissues during gestation

Incubations of whole tissue with nM concentrations of [^{3}H]estrone sulfate revealed the presence of estrogen sulfatase in chorion, amnion, endometrium, placenta and decidua basalis (Fig. 1). Steroid sulfatase activity, measured in terms of pregnenolone sulfate hydrolysis, was substantially lower than that of the estrogen sulfatase in the tissues examined (Fig. 2). During the 2 h incubation period, the hydrolysis of estrone sulfate reached a maximum of 12% in chorion and 25% in endometrium. The highest value for pregnenolone sulfate hydrolysis (endometrium) was 10%. Due to low activity of the enzyme and considerable variability between animals, the presence of steroid sulfatase could only be established in endometrium and decidua basalis under the experimental conditions.

The greatest change in activity observed over the course of gestation was a 12-fold increase in chorion estrogen sulfatase activity from early to late gestation ($P < 0.001$). The activity of this

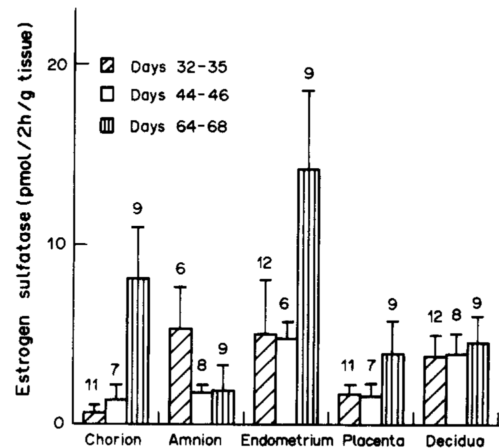

Fig. 1. Estrogen sulfatase activity in guinea pig intrauterine tissues during gestation. 4 nM (approx. 2×10^5 dpm) [6,7-^{3}H]estrone sulfate was incubated with whole tissue in medium for 2 h at 37°C. Medium was extracted with diethyl ether and aqueous phases were analyzed by liquid scintillation spectrometry. Error bars refer to one standard deviation; numbers over error bars indicate the number of animals used.

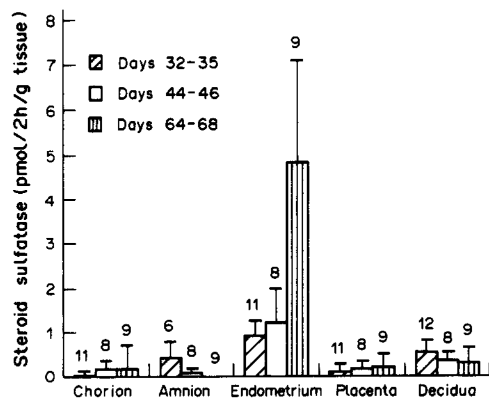

Fig. 2. Steroid sulfatase activity in guinea pig intrauterine tissues during gestation. 4 nM (approx. 2×10^5 dpm) [6,7-^{3}H]pregnenolone sulfate was incubated with whole tissue in medium for 2 h at 37°C. See Fig. 1 for further details.

Table 1. Identification of the free steroidal products of estrogen sulfatase activity (as measured by [³H]estrone sulfate hydrolysis) in chorion

	Specific activity (dpm/mg)			
	Estrone 3000 (calculated)		Estradiol 1370	
Crystallization from methanol	XLS I	3414	XLS I	850
	ML I	4071	ML I	1723
	XLS II	3248	XLAS II	886
	ML II	3115	ML II	996
	XLS III	3102	XLS III	781
	ML III	2979	ML III	822
	Estrone acetate 2685 (calculated)*		Estradiol diacetate 597	
Acetylation; crystallization from methanol-H_2O	XLS IV	2393	XLS IV	629
	ML IV	2588	ML IV	711
	XLS V	2673	XLS V	718
	ML V	2684	ML V	714
	XLS VI	2884	XLS VI	683
	ML VI	2907	ML VI	662

*Calculated specific radioactivities of acetylated forms are based upon that of the last crystals in the free form.

enzyme also increased 2.8-fold in endometrium ($P < 0.001$) and 2.4-fold in placenta ($P < 0.002$) during gestation. In contrast, estrogen sulfatase activity of amnion was significantly higher in early gestation than at term ($P < 0.001$). Activity in amnion during early gestation was also higher than that of chorion at the same time. Activity in the decidua basalis did not change appreciably with time.

Steroid sulfatase activity rose in the endometrium by a factor of 5.3 ($P < 0.001$) during gestation but was unchanged in decidua basalis. In all cases where sulfatase activities appeared to increase over the course of gestation, the greatest increase occurred within the final three weeks. By comparison, the decrease in amnion estrogen sulfatase activity occurred primarily between the fifth and sixth weeks.

Identification of metabolites of sulfatase action

Chromatography of incubation medium extracts on Sephadex LH_{20} indicated the production of both estrone and estradiol in late gestational chorion and endometrium, as a result of estrogen sulfatase (and 17-reductase) activity. In chorion, approx. 12% of the ether-soluble radioactivity appeared to be estradiol, while in endometrium this value was 16%. Recrystallization of samples and subsequent recrystallization of the acetate and diacetate derivatives confirmed these identities (Tables 1 and 2). In both tissues, production of the hormone estradiol was clearly established, although some contamination of the chorion-derived estradiol fraction was evident.

Table 2. Identification of the free steroidal products of estrogen sulfatase activity (as measured by [³H]estrone sulfate hydrolysis) in endometrium

	Specific activity (dpm/mg)			
	Estrone 3000 (calculated)		Estradiol 2000	
Crystallization from methanol	XLS I	2670	XLS I	1648
	ML I	3225	ML I	2136
	XLS II	3074	XLS II	1771
	ML II	3125	ML II	1913
	XLS III	3154	XLS III	1913
	ML III	3207	ML III	1876
	XLS IV	3299		
	ML IV	3283		
	Estrone acetate 2855 (calculated)*		Estradiol diacetate 1459	
Acetylation; crystallization from methanol-H_2O	XLS V	2761	XLS IV	1397
	ML V	3005	ML IV	1519
	XLS VI	2971	XLS V	1470
	ML VI	4530	ML V	1613
	XLS VII	2825	XLS VI	1497
	ML VII	2528	ML VI	1345

*Calculated as described in Table 1.

Table 3. Identification of the free steroidal products of steroid sulfatase activity (as measured by [^{3}H]pregnenolone sulfate hydrolysis) in endometrium

	Specific activity (dpm/mg)			
	Progesterone		Pregnenolone	
	2400 (calculated)		3600	
Crystallization from methanol-H_2O (progesterone) or methanol (pregnenolone)	XLS I	534	XLS I	3642
	ML I	4280	ML I	4775
	XLS II	480	XLS II	3244
	ML II	541	ML II	3510
	XLS III	442	XLS III	3121
	ML III	452	ML III	3177
	Diacetate derivative		Acetate derivative	
	345 (calculated)*		2755	
Reduction (progesterone); acetylation; crystallization from methanol-H_2O	XLS IV	353	XLS IV	2952
	ML IV	i.s.†	ML IV	2718
	XLS V	336	XLS V	2633
	ML V	352	ML V	2798
			XLS VI	2881
			ML VI	i.s.†

*Calculated as described in Table 1.
†Insufficient sample for analysis.

Chromatography of endometrial steroid sulfatase metabolites suggested that pregnenolone and progesterone were responsible for 65 and 35%, respectively, of ether-soluble radioactivity. Recrystallization techniques established the presence of both metabolites (Table 3). However, significant contamination of the progesterone fraction was apparent, indicating production of this hormone was much less than originally anticipated.

Subcellular localization of sulfatase activity

The highest specific activity and total activity for estrogen sulfatase and steroid sulfatase were found in the 105,000 *g* pellet (Table 4). The 750 and 10,000 *g* pellets also possessed a considerable fraction of the total activity, particularly in terms of the chorion estrogen sulfatase. Virtually no activity was present in the high speed supernatant.

The subcellular fraction studies discussed above were carried out within the time and protein concentrations during which the microsomal sulfatase reaction velocities were linear. Estrogen sulfatase activity in the chorion 105,000 *g* pellet was found to be linear up to 60 min and 150 μg protein. In endometrium, the same reaction was linear up to 40 min and 125 μg protein. Steroid sulfatase in this tissue showed a linear response up to 60 min and 300 μg protein.

Search for a soluble inhibitor of sulfatase activity in the endometrial 105,000 g supernatant

It was reported that an inhibitor of guinea pig microsomal DHAS sulfatase had been found in the soluble fraction of non-pregnant guinea pig liver [16]. In order to determine if the lower sulfatase activities found in early gestational endometrium were the result of a soluble inhibitor, microsomal estrogen sulfatase and steroid sulfatase activities were compared in the presence and absence of high speed supernatant from endometrium at days 32 and 65. No effect of either supernatant on endometrial microsomal sulfatase activities was observed.

Reliability of the estradiol RIA

Estrone (cross-reactivity 17%) and estriol (cross-reactivity 1.1%), as well as other

Table 4. Sulfatase activity in subcellular fractions of chorion and endometrium obtained from guinea pig in late (days 64–68) gestation

Tissue/substrate	Subcellular fraction	Specific activity (pmol/mg protein/min)	Total activity* (nmol/min)
Chorion/estrone sulfate	750 *g* pellet	220	10.0
	10,000 *g* pellet	145	2.8
	105,000 *g* supernatant	5	0.6
	105,000 *g* pellet	438	10.5
Endometrium/estrone sulfate	750 *g* pellet	365	22.7
	10,000 *g* pellet	345	17.8
	105,000 *g* supernatant	0	0.0
	105,000 *g* pellet	1062	69.9
Endometrium/pregnenolone sulfate	750 *g* pellet	65	4.0
	10,000 *g* pellet	63	3.2
	105,000 *g* supernatant	3	1.0
	105,000 *g* pellet	187	12.3

*Based upon total protein in each subcellular fraction; total activities should not be compared between tissues.

relatively polar steroids such as 16-epiestriol were readily separated from estradiol by chromatography. Estradiol-17α is not distinguishable from its β isomer by this technique and was found to possess a cross-reactivity of only 3.6%. Due to its low affinity for the antibody and a lack of strong recent evidence for its presence in guinea pig [17, 18], estradiol-17α was not anticipated to interfere strongly in the RIA. Progesterone, cortisol, testosterone and androstenedione all exhibited negligible cross-reactivity (<0.01%).

The recovery value for each sample through the entire procedure was checked by the use of [^{3}H]steroid as internal standard. Recoveries varied considerably between samples; mean = 72.3 ± 12.5% (SD). Blank values were of the order of 0.6 pg estradiol per assay tube. The values quoted for endogenous estradiol have been corrected for recovery and for the blank in each case. In addition, the values have been adjusted for the weight of labelled steroid added to check recovery. Based upon the standard binding curve, it was concluded that 10 pg of estradiol per tube was the lower limit of detection. The intra-assay and inter-assay coefficients of variation were 8.8 and 16.6%, respectively. The accuracy of the procedure, as determined by recovery of known weights of added unlabelled estradiol, indicated overestimation at the lower range of 50 pg or less per tube.

Endogenous estradiol in tissues from mid and late gestation

No significant changes in estradiol levels were found in endometrium or myometrium from mid to late gestation (Fig. 3). In chorion the mean estradiol value of 1.2 ± 1.1 (SD) ng/g pellet from mid gestation increased 6-fold to 7.6 ± 2.6 ng/g pellet in late gestation. These mid-gestational values corresponded to about 50 pg estradiol/assay tube; the late values to about 150 pg/assay tube. At the lower level there is almost certainly a problem with accuracy, and overestimation is likely. Thus the estradiol increase from mid to late gestation is probably >6-fold.

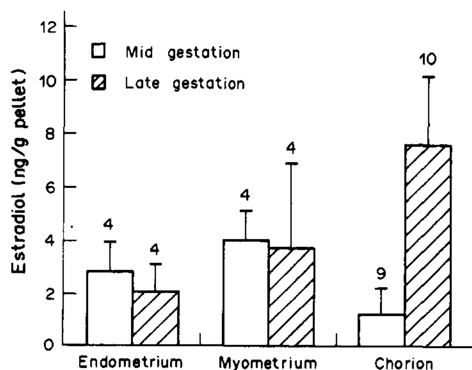

Fig. 3. Endogenous levels of estradiol in extracts of guinea pig endometrium, myometrium and chorion at mid (43–45 days) and late (64–68 days) gestation. Error bars refer to one standard deviation; numbers over error bars indicate the number of animals used.

DISCUSSION

In the present investigation, the possible involvement of sulfatase enzymes in local production of steroid hormone during gestation of the guinea pig was explored. The presence of estrogen sulfatase in various tissues of this species has been previously documented [11], although studies of the activity in intrauterine tissues with respect to gestation have been largely limited to the uterus itself [11, 19]. In one such investigation, uterine estrone sulfatase activity was found to increase by 20% in homogenates from late gestational guinea pigs compared with those of non-pregnant animals [11]. Moutaouakkil *et al.* reported a 2.8-fold increase in activity in term guinea pig uterus relative to the same tissue from non-pregnant animals [19]. These latter findings are similar to those of the present study which have identified an increase in endometrial estrogen sulfatase activity between early and late gestation. Perhaps more intriguing is the dramatic 12-fold increase in chorion estrogen sulfatase activity observed from early to late gestation. Analysis of radiolabelled metabolites by chromatography and recrystallization techniques established production of the hormone estradiol as a result of sulfatase (and 17-reductase) activity in chorion and endometrium at term, when a low concentration of estrone sulfate was used as substrate. In view of this, it was anticipated that an increase in the endogenous estradiol level of these tissues during gestation might be detectable by RIA. While a considerable increase in chorion-associated estradiol was found, no change was apparent in the hormone level of endometrium or myometrium towards term. It is possible that the latter finding was a consequence of the reduced accuracy of the radioimmunoassay at the lower levels of estradiol associated with these tissues. It is of interest that uterine estradiol has been reported to be higher in guinea pigs at mid gestation than in those at late pregnancy [20]. One possible explanation for the apparent contradiction may relate

to the different and much broader definitions of mid (26–34 days) and late (56–64 days) gestation used by the latter investigators.

The considerable increase in chorion estrogen sulfatase activity and in estradiol associated with this tissue in late gestation may have particular relevance with regard to an anatomical change which occurs in the uterine compartment. We have observed that, prior to day 50 of gestation, guinea pig chorion has no apparent association with the endometrium and is, in fact, separated from it by the uterine lumen. However, between approx. day 50 and day 55, the chorion forms a strong physical attachment with the endometrium and this remains intact throughout the remainder of gestation until term. This feature would seem to imply a particularly close communication between the tissues at term, and suggests the possible transfer of estradiol formed in the chorion to other intrauterine tissues such as the endometrium and/or myometrium. The stimulatory effects of this hormone on mammalian myometrium, particularly in the sheep, are well documented [21–24].

In attempting to interpret the observed increase in chorion-associated estradiol in late gestation, it is important to recognize that the static nature of the RIA, i.e. measurement at one point in time, did not take into account kinetic factors such as the turnover rate of the hormone. This is also true of the data for endometrium and myometrium. For this reason the physiological implications of the findings are unclear. However, the data do indicate the potential for an increased local production of estradiol late in gestation.

While estrogen sulfatase activity was evident in all tissues examined in our study, the presence of steroid sulfatase, measured by pregnenolone sulfate hydrolysis, was established only in endometrium and decidual basalis. This increase in endometrial pregnenolone sulfatase activity, along with the reportedly unchanging DHAS sulfatase activity of pregnant guinea pig uterus [19], once again raises the question of the number of enzymes responsible for the activity termed steroid sulfatase. No inhibitor of steroid sulfatase activity could be identified in the soluble fraction from endometrium. Others have reported the presence of such an inhibitor in non-pregnant guinea pig liver [16]. Although endometrial steroid sulfatase activity was found to increase in late gestation and to produce some progesterone, previous studies have not established any significant difference in the endogenous progesterone level of guinea pig uterus from mid and late gestation [20]. In fact, evidence exists to suggest that progesterone neither prolongs gestation nor inhibits myometrial activity in the pregnant guinea pig [25–27], unlike the situation in the rabbit [28]. In view of these findings, the possible role of the increased steroid sulfatase activity of guinea pig endometrium at term remains obscure.

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REFERENCES

1. Bassett J. M., Oxborrow T. J., Smith I. D. and Thorburn G. D.: The concentration of progesterone in the peripheral plasma of the pregnant ewe. *J. Endocr.* **45** (1969) 449–457.
2. Challis J. R. G.: Sharp increase in free circulating oestrogen immediately before parturition in sheep. *Nature. Lond.* **229** (1971) 208.
3. Anderson P. J. B., Hancock K. W. and Oakey R. E.: Non-proteinbound oestradiol and progesterone in human peripheral plasma before labour and delivery. *J. Endocr.* **104** (1985) 7–15.
4. Tulchinsky D., Hobel C. J., Yeager E. and Marshall J. R.: Plasma estrone, estradiol, progesterone and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am. J. Obstet. Gynec.* **112** (1972) 1095–1100.
5. Challis J. R. G., Heap R. B. and Illingworth D. V.: Concentration of oestrogen and progesterone in the plasma of non-pregnant, pregnant and lactating guinea-pigs. *J. Endocr.* **51** (1971) 333–345.
6. Illingworth D. V., Challis J. R. G., Ackland N., Burton A. M., Heap, R. B. and Perry J. S.: Parturition in the guinea-pig; plasma levels of steroid hormones, steroid-binding proteins, and oxytocin, and the effect of corticosteroids, prostaglandins and adrenocorticotrophin. *J. Endocr.* **63** (1974) 557–570.
7. Hobkirk R., Musey, P. and Nilsen M.: chromatographic separation of estrone and 17β-estradiol conjugates on DEAE-Sephadex. *Steroids* **14** (1969) 191–206.
8. Fieser L. F.: Naphthaquinone antimalarials. XVI. Water-soluble derivatives of alcoholic and unsaturated compounds. *J. Am. Chem. Soc.* **70** (1948) 3232–3237.
9. Hobkirk R., Nilsen M. and Blahey P. R.: Conjugation of urinary phenolic steroids in the nonpregnant human female with particular reference to estrone sulfate. *J. Clin. Endocr. Metab.* **29**. (1969) 328–337.
10. Hobkirk R.: Heterogeneity of guinea pig chorion and liver estrogen sulfotransferases. *J. Steroid Biochem.* **29** (1988) 87–91.
11. Hobkirk R., Cardy C., Nilsen M. and Saidi F.: Estrone sulfatase activity in guinea pig tissues. *J. Steroid Biochem.* **17** (1982) 71–76.
12. Hobkirk R., Renaud R. and Raeside J. I.: Partial characterization of steroid sulfohydrolase and steroid sulfotransferase activities in purified porcine Leydig cells. *J. Steroid Biochem.* **32** (1989) 387–392.
13. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72** (1976) 248–254.

14. Dean P. D. G., Exley D. and Johnson M. W.: Preparation of 17β-oestradiol-6-(o-carboxymethyl)oxime-bovine serum albumin conjugate. *Steroids* **18** (1971) 593–603.
15. Exley D., Johnson M. W. and Dean P. D. G.: Antisera highly specific for 17β-oestradiol. *Steroids* **18** (1971) 605–620.
16. Moutaouakkil M. and Adessi G. L.: A dehydroepiandrosterone sulfatase inhibitory activity in soluble proteins of guinea pig liver. *Steroids* **47** (1986) 401–411.
17. Church R. L. and Eleftheriou B. E.: Tentative identification of plasma, ovarian and placental oestrogens in the guinea-pig. *J. Reprod. Fert.* **12** (1966) 369–372.
18. Hobkirk R., Nilsen M. and Mori J.: 16-Hydroxylation of estrone-3-sulfate and estrone in the guinea pig *in vivo*. *Endocrinology* **103** (1978) 1227–1233.
19. Moutaouakkil M., Prost O., Dahan N. and Adessi G. L.: Estrone and dehydroepiandrosterone sulfatase activities in guinea-pig uterus and liver: estrogenic effect of estrone sulfate. *J. Steroid Biochem.* **21** (1984) 321–328.
20. Batra S., Sjöberg N.-O. and Thorbert G.: Sex steroids in plasma and reproductive tissues of the female guinea pig. *Biol. Reprod.* **22** (1980) 430–437.
21. Liggins G. C., Fairclough R. J., Grieves S. A., Kendall J. Z. and Knox B. S.: The mechanism of initiation of parturition in the ewe. *Recent Prog. Horm. Res.* **29** (1973) 111–150.
22. Windmoller R., Lye S. J. and Challis J. R. G.: Oestradiol modulation of ovine myometrial activity. *Can. J. Physiol. Pharmac.* **61** (1983) 722–728.
23. Porter D. G. and Lye S. J.: Partial reversal of the myometrial progesterone 'block' in the non-pregnant ewe *in vivo* by oestradiol-17β. *J. Reprod. Fert.* **67** (1983) 227–234.
24. Lye S. J., Wathes D. C. and Porter D. G.: Oestradiol 17β both inhibits and stimulates myometrial activity in ewes. *J. Reprod. Fert.* **67** (1983) 335–341.
25. Zarrow M. X., Anderson N. C. and Callantine M. R.: Failure of progestogens to prolong pregnancy in the guinea pig. *Nature (Lond).* **198** (1963) 690–692.
26. Schofield B. M.: Myometrial activity in the pregnant guinea-pig. *J. Endocr.* **30** (1964) 347–354.
27. Porter D. G.: The failure of progesterone to affect myometrial activity in the guinea-pig. *J. Endocr.* **46** (1970) 425–434.
28. Csapo A. I. and Takeda A. H.: Effect of progesterone on the electric activity and intra-uterine pressure of pregnant and parturient rabbits. *Am. J. Obstet. Gynec.* **91** (1965) 221–231.