DEMONSTRATION OF AN ACTIVE C₁₇–C₂₀ LYASE IN THE SHEEP PLACENTA

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Studies on the sheep have provided more information concerning the hormonal changes that precede parturition than those on any other mammal. The implications of some of the findings are uncertain but a composite picture is gradually emerging.

The ability of sheep placenta to convert C₁₉-steroids to oestrogens (Ainsworth & Ryan, 1966; Pierrepoint, Anderson, Griffiths & Turnbull, 1970a) and their sulphates (Pierrepoint, Anderson, Harvey, Turnbull & Griffiths, 1971) has been well demonstrated although neither a source nor the species of such potential precursors has been found.

Two of the most active C₂₁-steroid-metabolizing enzymes in the sheep placenta are the 20 α -hydroxysteroid dehydrogenase and the 3 β -hydroxysteroid dehydrogenase- Δ^4 - Δ^5 -isomerase with the consequent formation of large amounts of progesterone and 20 α -dihydroprogesterone (Ainsworth & Ryan, 1967; Pierrepoint *et al.*, 1970b; Pierrepoint, Anderson, Turnbull & Griffiths, 1973). Steroid 17 α -hydroxylase and C₂₁-steroid C₁₇–C₂₀ lyase activity have been shown, although the products of their combined actions amounted to less than 1% of the precursors (Pierrepoint *et al.*, 1970b, 1973).

Because of these findings and the current acceptance that the 'desmolase' enzyme cannot act without the prior 17 α -hydroxylation of the C₂₁-steroid (Slaunwhite & Samuels, 1956; Lynn & Brown, 1958; Ellis & Berliner, 1965), the ability of the sheep placenta to metabolize 17 α -hydroxylated C₂₁-steroid substrates was investigated, with particular regard to the formation of C₁₉- and C₁₈-products.

Placental tissue was removed at hysterotomy from a ewe at 119 days of gestation and transported to the laboratory on ice. A minced preparation (4 g) was added to 24 ml Krebs–Ringer bicarbonate glucose solution containing 10 μ Ci [7 α -³H]17 α -hydroxypregnenolone (179.5 Ci/mol) and 2 μ Ci [4-¹⁴C]17 α -hydroxyprogesterone (35.9 Ci/mol), both supplied by Amersham Radiochemical Centre, Bucks. The mixture was shaken in an atmosphere of 95% O₂:5% CO₂ for 2 hr in a water bath at 39.5°C. After the incubation period, the reaction was stopped by the addition of 30 ml acetone and refrigeration.

Metabolism was assessed by the reverse-isotope dilution method. Steroids to be investigated (17 α -hydroxypregnenolone 17 α -hydroxyprogesterone, dehydroepiandrosterone (DHA), androstenedione, testosterone, epitestosterone, oestradiol-17 β , oestradiol-17 α , oestrone, DHA sulphate, oestrone sulphate and

oestradiol-17 β -3-sulphate) were added in 300- μ g amounts in ethanol. The method of extraction, purification and identification of the metabolites has previously been described (Pierrepont *et al.*, 1970a, 1971). The solvent systems employed (v/v or by vol.) in the thin-layer chromatographic procedures were: (I) chloroform:acetone, 37:3; (II) cyclohexane:ethyl acetate, 1:1; (III) cyclohexane:ethyl acetate, 9:11; (IV) tertiary butanol:ethyl acetate:5 N-ammonium hydroxide, 41:50:20; (V) hexane:ethyl acetate, 1:1; (VI) cyclohexane:ethyl acetate, 3:1; (VII) benzene:ethyl acetate, 9:1; (VIII) hexane:ethyl acetate, 3:2.

Table 1. Evidence for the identification of the steroids isolated following incubation of sheep placenta with labelled 17 α -hydroxylated C₂₁-steroids

Steroid investigated and derivative formed	Solvent system*	Specific activities (d/min/nmol)		% of initial radioactivity associated with the investigated steroids	
		³ H	¹⁴ C	³ H	¹⁴ C
17 α -Hydroxypregnenolone	I, III	998.8	0	2.00	0
17 α -Hydroxypregnenolone acetate	VI	917.5			
17 α -Hydroxyprogesterone	I, III, VIII	10.06	78.94	0.03	1.90
17 α ,20 β -Dihydroxypregn-4-en-3-one	I	12.08	70.11		
17 α -Hydroxypregn-4-en-3-one-20 β -acetate	I	15.07	76.42		
Androstenedione	I, II	26.33	1.60	0.06	0.05
Testosterone	I, III	27.01	1.58		
Testosterone acetate	VI	25.91	1.84		
Testosterone	I, III, II†	5.54	0	0.01	0
Androstenedione	I, II	5.58			
Testosterone	VI	5.91			
Epitestosterone	I, II†	69.91	0.86	0.16	0.02
Androstenedione	I, II	65.45	0.81		
Epitestosterone acetate	VI	67.81	0.83		
DHA	I, III, VIII	44.57	0	0.01	0
Androst-5-ene-3 β ,17 β -diol	II	46.94			
DHA acetate	VI	43.68			
Oestrone	I, II	140.8	4.19	0.34	0.14
Oestrone acetate	VI	147.0	5.08		
Oestradiol-17 β -3-methyl ether	VII	153.1	3.62		
Oestradiol-17 β	I, III, VI	13.35	0.29	0.03	0.01
Oestradiol-17 β -diacetate	VI	12.87	0.35		
Oestradiol-17 β -3-methyl ether	VII	12.23	0.37		
Oestradiol-17 α	I, III, VI	47.31	1.55	0.12	0.04
Oestradiol-17 α -diacetate	VI	49.19	1.43		
Oestradiol-17 α -3-methyl ether	VII	49.86	1.58		
DHA sulphate	IV		0	0.05	0
DHA	I, III, VIII	25.71			
Androst-5-ene-3 β ,17 β -diol	II	25.55			
Oestrone sulphate	IV			6.07	2.43
Oestrone	II	2273.3	77.29		
Oestrone acetate	VI	2483.5	79.90		
Oestrone-3-methyl ether	VII	2378.0	73.07		
Oestradiol-17 β -3-sulphate	IV			0.07	0.03
Oestradiol-17 β	I, III, VI	37.02	1.40		
Oestradiol-17 β -diacetate	VI	40.89	1.67		
Oestradiol-17 β -3-methyl ether	VII	35.10	1.06		

* See text for solvents.

† Chromatographed on Merck alumina PF_{254/366} (Type T); Merck Silica gel HF_{254/366} (Type 60) was used at all other times.

The results are shown in Table 1.

Virtually complete metabolism of the two substrates took place with the formation of a range of C₁₉- and C₁₈-steroids and their conjugates. The major metabolites, estimated by scanning the radioactivity on thin-layer plates, appeared to be the 20-reduced products of both substrates, accounting for approximately 79% and 93% respectively of the initial tritium and ¹⁴C radioactivity. These steroids were not investigated further and are not included in Table 1 but raise the question of their own participation in the formation of C₁₉- and C₁₈-steroids.

Of the metabolites characterized, oestrone sulphate was again (Pierrepont *et al.*, 1973) the principal product, followed by oestrone and epitestosterone. The total 'desmolase' activities for the two substrates, 17 α -hydroxypregnenolone and 17 α -hydroxyprogesterone, were very much greater than those achieved using pregnenolone or progesterone and support the findings of a previous report on 11-deoxycortisol (Pierrepont & co-authors, 1974) that 17 α -hydroxylated C₂₁-steroids can act as substrates for placental C₁₇-C₂₀ lyase.

Davies, Ryan & Petro (1970) failed to find oestrone after incubating sheep placenta alone with tritiated pregnenolone but when the tissue was co-incubated with maternal or fetal adrenals, a 0.1% and 0.17% conversion was achieved. This low yield may be accounted for by the poor ability of the fetal adrenal to form C₁₉-steroids from 17-deoxy-C₂₁-precursors (Davies *et al.*, 1970; Anderson, Pierrepont, Jones, Griffiths & Turnbull, 1970), the order of which is similar to that achieved in the placenta (Pierrepont *et al.*, 1973).

The implications from these results may be that there is less evidence for the existence of a feto-placental unit in the sheep as defined for women at mid-pregnancy (Diczfalusy, 1969), unless some of the 17 α -hydroxylated steroids arise in the fetus. The placenta, on the other hand, may be the site of all the steroid reactions from cholesterol to oestrogen synthesis with controlling factors originating in the fetus.

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