Investigations into the Metabolism *in vitro* of C₁₉ Steroids by the Sheep Placenta

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Combined foetal and maternal placental tissues were removed from the uteri of two sheep after hysterectomy on days 122 and 130 of gestation respectively.

The tissues were finely chopped and 4g of each was incubated in 24 ml of Krebs-Ringer bicarbonate-buffered glucose solution (Cohen, 1957) at 39.5°C for 2 h with equimolar amounts of the following isotopically labelled substrates: (a) 122-day tissues, 2 μ Ci of [4-¹⁴C]testosterone (29.2 mCi/mmol) and 20 μ Ci of [7 α -³H]androstenedione (2.92 mCi/mmol); (b) 130-day tissue, 20 μ Ci of 17 α -hydroxy[1,2-³H]-androst-4-en-3-one (epitestosterone) (2.92 mCi/mmol) and 14.0 μ g of non-radioactive androstenedione. Incubations were stopped by the addition of 60 ml of acetone and refrigerated at -15°C. Non-radioactive carrier steroids, listed in Table 1, were added before homogenization and extraction by the method described by Pierrepoint, Galley, Griffiths & Grant (1967). Fractionation, isolation and proof of radiochemical purity of the carrier steroids was achieved as described previously (Pierrepoint *et al.* 1967; Pierrepoint, Anderson, Griffiths & Turnbull, 1969).

Steroids were chromatographed on thin layers of Merck silica gel HF_{253/366}, unless otherwise stated, with the following solvent systems: I, chloroform-acetone (37:3, v/v); II, cyclohexane-ethyl acetate (1:1, v/v); III, cyclohexane-ethyl acetate (3:1, v/v); IV, benzene-chloroform (1:1, v/v); V, cyclohexane-ethyl acetate-ethanol (9:9:2, by vol.); VI, benzene-ethyl acetate (9:1, v/v). Table 1 provides evidence of the radiochemical purity of the steroids isolated and the particular solvent systems employed in their separation: percentages of initial radioactivity associated with the steroids investigated are in parentheses.

This study of steroid metabolism by the sheep placenta provides information on both oestrogen formation and the interconversion of the C₁₉ steroid substrates incubated.

The isolation of radioactive epitestosterone is, in particular, noteworthy. The only previous report of its synthesis by placental tissue followed an investigation of steroid metabolism by bovine

foetal cotyledons at term (Pierrepoint *et al.* 1969), although, with regard to the species, sheep whole blood has shown the ability to form this steroid from androstenedione (Lindner, 1961, 1965). Its presence as a metabolite from incubation studies of tissues from other animals has also been reported (see Blaquier, Dorfman & Forchielli, 1967). These authors indicated that, from their own work, testosterone was the favoured substrate for the formation of epitestosterone. This is in contradistinction to the results reported here, where only a 3.86% formation from testosterone was achieved as compared with 16.5% from androstenedione. The equilibrium of the reactions testosterone $\rightleftharpoons$ androstenedione $\rightleftharpoons$ epitestosterone obviously favours the latter in the sheep placenta. Dorfman & Shipley (1956) reported that epitestosterone was practically devoid of androgenic activity and consequently no biological importance has been assigned to it. It is particularly pertinent therefore to report that the premature expulsion of the sheep foetus has been induced by the infusion of epitestosterone at the rate of 1 mg/day for 6 days into the intact lamb *in utero*. Further to this, doubly labelled epitestosterone has been isolated from the myometrium of the pregnant sheep after the short-term perfusion of the placenta *in situ* with [4-¹⁴C]-androstenedione and dehydro[7 α -³H]epiandrosterone (G. Rossier, C. G. Pierrepoint, A. B. M. Anderson, K. Griffiths & A. C. Turnbull, unpublished work).

Oestrogens were formed from each of the radioactive precursors, with oestrone predominating. Oestriol from either incubation was found not to be radioactive. Incubations of androstenedione with sheep placental preparations have previously been reported with a similar formation of oestrogens (Ainsworth & Ryan, 1966), although these authors were not able to conclude whether neutral or phenolic 17 α -hydroxy steroid dehydrogenase enzymes had been active. They did not look for epitestosterone. The present information suggests direct aromatization of the three substrates to the corresponding oestrogens, with the consequent greater formation of oestradiol-17 α from epitestosterone.

Table 1. *Details of the steroids investigated, the derivatives formed and the solvent systems employed in their purification*

Specific radioactivities are given as d.p.m./nmol and the percentages of the initial radioactivity associated with the isolated steroids are given in parentheses.

Compound isolated and derivative formed	Solvent systems	Incubation (a)		Incubation (b)	
		From [4- ¹⁴ C]-testosterone	From [7 α - ³ H]-androstenedione	From [1,2- ³ H]-epitestosterone	
Testosterone	I and II*	1981.2	127.8	3.82	
Androstenedione	II	1816.2 (52.7)	127.9 (0.65)	3.88 (0.02)	
Testosterone acetate	III*	1996.4	126.2	4.14	
Androstenedione	I and II	158.2	2420.5	359.7	
Testosterone	II	161.6 (4.33)	2438.8 (12.4)	354.3 (1.54)	
Testosterone acetate	II	152.7	2343.0	348.0	
Epitestosterone	I and II*	142.5	3282.5	6548.0	
Androstenedione	II	137.2 (3.86)	3106.5 (16.5)	6604.0 (27.9)	
Epitestosterone acetate	III*	144.2	3253.4	6197.6	
Oestrone	I and II	158.1	1464.5	776.7	
Oestrone acetate	III	149.2 (4.47)	1323.8 (7.65)	844.6 (7.48)	
Oestrone 3-methyl ether	III	153.4	1401.3	813.9	
Oestradiol-17 β	I and II	18.29	33.59	41.84	
Oestradiol-17 β diacetate	IV	18.05 (0.53)	33.47 (0.18)	41.30 (0.38)	
Oestradiol-17 β 3-methyl ether	VI	18.62	34.02	40.62	
Oestradiol-17 α	I and II	5.56	54.12	97.3	
Oestradiol-17 α diacetate	IV	4.87 (0.14)	57.59 (0.30)	100.9 (0.90)	
Oestradiol-17 α 3-methyl ether	VI	4.58	56.10	99.2	
Oestriol	V	0 (0)	0 (0)	0 (0)	

* Run on Merck alumina HF_{254/366}.

This sequence of biosynthetic events, the formation of epitestosterone and its further transformation into oestradiol-17 α , may have great significance in the control of myometrial function in the sheep. Certainly oestradiol-17 α is quantitatively the most important oestrogen in pregnant-sheep urine (Fèvre & Rombaut, 1966), it reaching a maximum excretion value at term.

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