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Formation of dehydroepiandrosterone sulphate by previable human foetuses*

The steroid pattern of cord blood is characterized by a high concentration of dehydroepiandrosterone sulphate and 16α-hydroxydehydroepiandrosterone sulphate (refs. 2–4). These compounds are the principal precursors of the large amounts of oestrogens formed by the placenta[5,6]. To elucidate the pathway of dehydroepiandrosterone sulphate formation in the foeto-placental unit, we perfused mid-term placentas *in situ* with labelled 17α-hydroxypregnenolone. The perfused placentas were not capable of removing the steroid side-chain[7]. This suggested that the side-chain cleavage occurs in the foetal rather than placental compartment. The following study was therefore undertaken.

Two male foetuses (18 and 20 weeks) were obtained following hysterotomy and perfused for 60 min with 25 μC of [7α-^{3}H]17α-hydroxypregnenolone (batch 51-224-5a, with a specific activity of 0.4 μC/μg, New England Nuclear Corp., Boston, Mass.). The purification of this compound was described previously[7]. Upon completion of the perfusions, the perfusates and various foetal organs were extracted separately, using previously described methods[8,9]. The distribution of ether-soluble (unconjugated) and water-soluble (conjugated) radioactive material recovered from the various sources is shown in Table I. In the two cases, 85 and 92.6% of the perfused radioactivity was recovered. The bulk of the radioactive material recovered from all sources except the adrenals was in an unconjugated form.

TABLE I

DISTRIBUTION OF THE ETHER-SOLUBLE (UNCONJUGATED) AND WATER-SOLUBLE (CONJUGATED) RADIOACTIVE MATERIAL RECOVERED FROM THE VARIOUS TISSUES AND PERFUSATES FOLLOWING THE PERFUSION OF TWO PREVIABLE HUMAN FOETUSES WITH 25 μC OF ^{3}H-LABELLED 17α-HYDROXYPREGNENOLONE

Figures are expressed as percentage of administered material.

	Expt. A		*Expt. B*	
	Ether	*Water*	*Ether*	*Water*
Adrenals	0.2	0.8	0.3	0.3
Liver	5.2	0.8	14.0	5.0
Residual foetal tissues	19.0	2.0	25.0	3.0
Perfusate	53.0	4.0	41.0	4.0
Total	85.0		92.6	

The following trivial names are used: pregnenolone: 3β-hydroxypregn-5-en-20-one; pregnenolone sulphate: 20-oxopregn-5-en-3β-yl sulphate; 17α-hydroxypregnenolone: 3β,17α-dihydroxypregn-5-en-20-one; 17α-hydroxypregnenolone sulphate: 17α-hydroxypregn-5-en-20-one-3β-yl sulphate; dehydroepiandrosterone: 3β-hydroxyandrost-5-en-17-one; dehydroepiandrosterone sulphate: 17-oxoandrost-5-en-3β-yl sulphate; 16α-hydroxydehydroepiandrosterone sulphate: 16α-hydroxyandrost-5-en-17-one-3β-yl sulphate.

The following solvent systems were used: System A: isooctane–ethyl acetate–*n*-butanol–methanol–1 M NH_4OH (20:40:8:20:30); System B: isooctane–chloroform–*n*-butanol–methanol–0.3 M pyridinium sulphate–pyridine (40:20:8:20:19:1); System C: butanol–butyl ether–ammonium hydroxide–water (5:5:1:9); System D: ligroin–benzene–methanol–water (66:33:80:20).

* A preliminary report was presented at the 6th Pan American Congress of Endocrinology, Mexico, October, 1965 (ref. 1).

The conjugated material obtained from the various sources was subjected to column partition chromatography in System A (ref. 10). The dehydroepiandrosterone sulphate-like radioactive material was then subjected to paper partition chromatography with authentic dehydroepiandrosterone sulphate in System C, eluted and solvolysed[11]. The liberated material was subjected to paper partition chromatography with carrier dehydroepiandrosterone in System D, after which the identity of this material (with dehydroepiandrosterone) was established by reverse isotope dilution (Table II).

Chromatography of the dehydroepiandrosterone sulphate-like material in System B revealed the presence of another peak with the partition characteristics of 17α-hydroxypregnenolone sulphate. This material was chromatographed with authentic 17α-hydroxypregnenolone sulphate in System C. The 17α-hydroxypregnenolone sulphate-like radioactive material was then solvolysed, the liberated steroid and carrier chromatographed in System D, mixed with additional carrier and crystallized to constant specific activity (Table II). Neither dehydroepiandrosterone sulphate nor 17α-hydroxypregnenolone sulphate could be isolated from the liver.

TABLE II

CRYSTALLIZATION TO CONSTANT SPECIFIC ACTIVITY (disint./min per mg) OF DEHYDROEPIANDROSTERONE AND OF 17α-HYDROXYPREGNENOLONE ISOLATED IN THE FORM OF DEHYDROEPIANDROSTERONE SULPHATE AND 17α-HYDROXYPREGNENOLONE-3-SULPHATE, RESPECTIVELY, FROM PREVIABLE FOETUSES PERFUSED WITH ^{3}H-LABELLED 17α-HYDROXYPREGNENOLONE

Solvents used: a, methanol; b, methanol–ether; c, ethanol; d, ligroin.

Source	*Dehydroepiandrosterone*			*17α-Hydroxypregnenolone*		
	Solvent	*Crystals*	*Mother liquor*	*Solvent*	*Crystals*	*Mother liquor*
Adrenals	a	575	740	a	315	300
	c	590	610	d	295*	
	d	585*				
Residual foetal tissues	a	0.86**	0.93	a	610	3070
	c	0.85	0.95	c	415	740
	a	0.82	0.81	a	400	430
Perfusate	a	3690	4050			
	b	3630				
	c	3750	3710			

* Crystallized as the acetate.

** Crystallized to constant isotopic ratio following the addition of authentic [4-^{14}C]dehydroepiandrosterone.

These data establish the capacity of the mid-term human foetus to remove the side-chain of 17α-hydroxypregnenolone by converting this compound into dehydroepiandrosterone sulphate. The isolation of dehydroepiandrosterone sulphate from the perfusate indicates that the dehydroepiandrosterone sulphate formed is secreted by the foetus into the umbilical circulation. It appears from the data that the adrenal is an important site of side-chain cleavage for C_{21} steroids with a 3β-HO-Δ^5 structure. This seems to be in contrast to the fate of C_{21} steroids with an α,β-unsaturated 3-oxo group[12]. Whether other foetal tissues (*e.g.* the testicles) are also capable of removing the steroid side-chain *in vivo* remains to be studied.

Since pregnenolone sulphate is present in the umbilical circulation in substantial quantities[13–15] and was shown to be hydrolysed by mid-term placentas perfused

in situ[16] and, furthermore, since pregnenolone is converted by perfused previable foetuses into 17α-hydroxypregnenolone[17], the reactions leading to the formation of dehydroepiandrosterone sulphate in the human foetus at midpregnancy can be summarized as shown in Fig. 1. It remains to be established whether sulphurylation of 17α-hydroxypregnenolone precedes the removal of the side-chain.

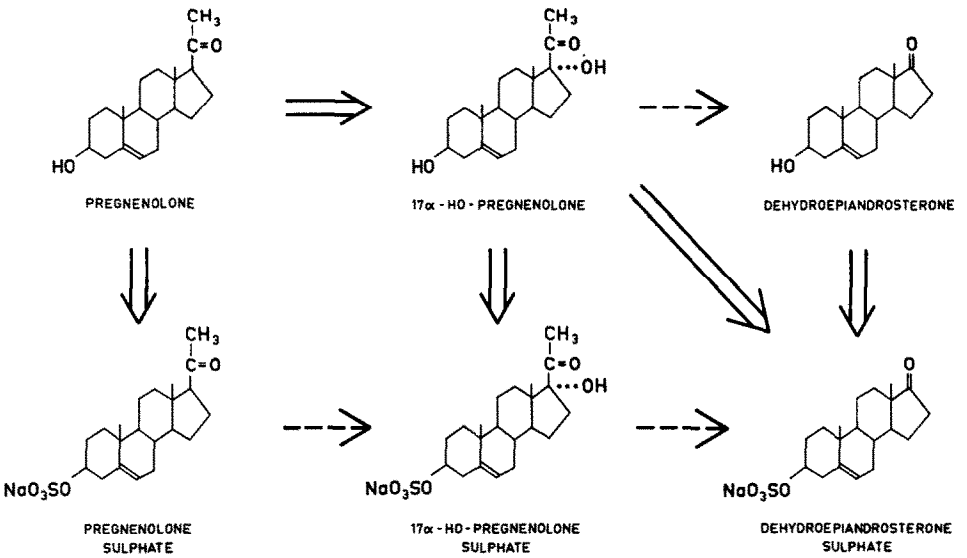

Fig. 1. Concept of the formation of dehydroepiandrosterone sulphate by the previable human foetus. Solid arrows: established pathways. Dotted arrows: postulated but not yet proven reactions.

It is of interest that cholesterol was shown to be converted into pregnenolone by the human placenta[18] but not by the foetus[17]. It seems possible therefore that the cleavage of the cholesterol side-chain takes place in the placental and that of the steroid side-chain in the foetal compartment.

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