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LACK OF SQUALENE AND LANOSTEROL SYNTHESIS BY THE PERFUSED HUMAN PLACENTA AT TERM

H. A. VAN LEUSDEN, CH. THOMAS, G. TELEGDY and E. DICZFALUSY

University Department of Obstetrics and Gynaecology, St. Radboud Ziekenhuis, Nijmegen, The Netherlands, and Reproductive Endocrinology Research Unit, Swedish Medical Research Council, Karolinska sjukhuset, Stockholm, Sweden

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SUMMARY

Three human term placentas were perfused *in vitro* with 1·6–2·4 mCi of carbon-14 labelled sodium acetate for 90–120 min and attempts were made to isolate from the placentas and perfusates labelled squalene, lanosterol, cholesterol, pregnenolone and progesterone. All carbon-14 labelled material was dissociated from the squalene, cholesterol, pregnenolone and progesterone isolated from the three placentas and three perfusates. The same was found with regard to lanosterol isolated from three perfusates and one placenta. Minute amounts of radiochemically homogeneous lanosterol were isolated from the second placenta, whereas the evidence was inconclusive in the case of minute amounts of lanosterol-like material recovered from the third placenta.

It is concluded that little, if any, squalene, lanosterol and cholesterol are synthesized by the human placenta perfused at term.

IN PREVIOUS papers, evidence has been presented indicating that mid-gestation human foetuses perfused with labelled sodium acetate synthesize squalene, lanosterol and cholesterol[1, 2]. On the other hand, little, if any, squalene, lanosterol and cholesterol were formed by perfused mid-gestation placentas[1, 3]. However, human term placental preparations were repeatedly shown to convert *in vitro* both acetate and mevalonate to squalene, lanosterol and cholesterol [4–7]. Therefore, it was of interest to find out whether or not perfused term placentas are capable of synthesizing sterols and steroids from acetate.

EXPERIMENTAL

Clinical material. Three placentas were obtained immediately after delivery from apparently healthy women.

Conditions of perfusion. These were the same as those described previously [8]. The placental tissue perfused in the three experiments was 200, 380 and 400 g, respectively. The amount of [^{14}C] sodium acetate (CFA 229 Batch 19, SA 56·0 mCi/mmol) in these three experiments was 1·6, 2·4 and 2·4 mCi, respectively, and the perfusion time was 90, 120 and 120 min.

Abbreviations and trivial names. Cholesterol: 5-cholesten-3β-ol; DPM: disintegrations per min; lanosterol: 8,24-lanostadien-3β-ol; mevalonic acid: 3,5-dihydroxy-3-methylbutan-1-oic acid; squalene: 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosanehexane; t.l.c.: thin-layer chromatography; pregnenolone:3β-hydroxy-5-pregnen-20-one; progesterone: 4-pregnene-3,20-dione.

Internal standard. [7α-^{3}H]-Cholesterol as internal standard was the same as

used previously [9]. After the perfusion but before extraction,225,000 d.p.m. were added to each extract.

Measurement of radioactivity.-A Nuclear Chicago Scintillation Spectrometer (MI) with external standardization was used.

Thin-layer chromatography. t.l.c. was carried out on Silica Gel G. The following systems were used at room temperature:

1. Benzene–ethanol (5 : 1 v/v)
2. Benzene–ethyl acetate (3 : 1 v/v)
3. Benzene–ethyl acetate (8 : 2 v/v)
4. Chloroform–acetate (8 : 2 v/v).

Extraction procedure. This was the same as described previously [1].

Separation of squalene, lanosterol, cholesterol, pregnenolone and progesterone. Following saponification with NaOH in methanol for 1 h at 60°C, the pH was adjusted to 7·4. The material was evaporated and the residue partitioned between n-hexane and 90% methanol in a countercurrent fashion using 5 upper phase transfers.

From the n-hexane fraction, squalene, lanosterol and cholesterol were separated using two subsequent t.l.c.'s in systems Nos. 1 and 2. To the 90% methanol fraction, carrier pregnenolone and progesterone were added, the material was evaporated and partitioned between toluene and 1 N NaOH. Pregnenolone was separated from progesterone by t.l.c. in system No. 3. Pregnenolone was purified subsequently by re-chromatography in system No. 3 and progesterone in system No. 4.

Isolation and identification of squalene. To the squalene-like material, 200–300 mg authentic carrier was added; squalene hexahydrochloride was prepared and re-crystallized from hot ethyl acetate.

Isolation and identification of lanosterol, cholesterol, pregnenolone and progesterone. Authentic carriers were added and the various fractions obtained from the placentas and perfusates were subjected to a series of re-crystallizations using different solvent systems.

Determination of the mass. Squalene hexahydrochloride was estimated gravimetrically. Lanosterol was assayed spectrophotometrically by a modified Liebermann–Burchard reaction by incubating for 40 min in the presence of 2 ml glacial acetic acid and 4 ml of a freshly prepared mixture of acetic anhydride-sulphuric acid (20 : 1 v/v). The colour was read after 40 min at 456 nm. Cholesterol was determined by the Liebermann–Burchard reaction.

Pregnenolone was assayed by the Pettenkofer reaction and progesterone by U.V. absorptiometry at 240 nm.

RESULTS

The results are presented in Tables 1–3.

The data presented in Table 1 indicate that all radioactive material was dissociated from the crystalline squalene hexahydrochloride.

The crystallizations of lanosterol are indicated in Table 2.

The data of Table 2 indicate that all radioactive material was dissociated from the lanosterol isolated from the three perfusates. It can also be seen from the data that in two of the three placentas analyzed some carbon-14 labelled material remained associated with the crystals. Evidence of radio-chemical homogeneity

Table 1. Crystallization from ethyl acetate of placental squalene-like radioactive material following the preparation of squalene hexahydrochloride. Human term placentas were perfused with sodium acetate, and tritium labelled cholesterol was added after perfusion before extraction. Values are expressed as d.p.m./mg

	Exp. No. 1				Exp. No. 2				Exp. No. 3			
	Placenta		Perfusate		Placenta		Perfusate		Placenta		Perfusate	
	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
Starting material*	520	5370	0	0	5000	192200	230	3290	0	7500	410	6810
Crystals 1	0	0			0	0	0	0	0	0	0	0
Mother liquor 1	2400	4700			0	214000	200	3700	0	6800	4700	7050

*Starting material indicates the total number of d.p.m. which was diluted with 200–300 mg authentic squalene prior to hexahydrochloride formation.

Table 2. Crystallization of lanosterol. In experiments 1, 2 and 3, human term placentas were perfused with 1·6, 2·4 and 2·4 mCi of [^{14}C]-sodium acetate, respectively; 225·000 d.p.m. [7α-^3H] cholesterol was added after perfusion before extraction and further analysis

	Exp. No. 1				Exp. No. 2				Exp. No. 3			
	Placenta		Perfusate		Placenta		Perfusate		Placenta		Perfusate	
	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C	^3H	^{14}C
Starting material[a]	400	18800	0	0	1100	59300	0	1800	200	15600	0	70900
Crystals[b] 1	0	2060			30	240	0	130	110	1580	0	4520
2	0	1590			0	160	0	150	0	1310	0	4400
3	0	1650			0	110	0	100	0	1390	0	3140
4	0	1350			0	110	0	90		1170	0	1050
5	0	1120			0	100	0	0		990	0	610
6	0	960								840	0	370
7	0	750								570	0	270
8	0	800								490	0	0
	0	0			0	120[c]				460[c]		
Mother liquors 1	50	2710			370	3700	300	1180	90	6390	320	26400
2	0	5150			0	1810	0	910	0	5440	280	14200
3	0	2740			0	520	100	420	0	5900	200	13200
4	0	3010			0	200	100	380		3450	0	4490
5	0	1600			0	100	60	190		1390	0	3170
6	0	1960								2430	0	2340
7	0	1110								900	0	1670
8	0	780								830	0	1270

[a]Indicates the total amount of d.p.m. which was diluted with 10070 μg (placentas) or 10770 μg (perfusates) of authentic lanosterol prior to crystallization.

[b]1. acetone 2. methanol 3. ethanol 4. acetone 5. methanol 6. ethanol 7. acetone 8. methanol. Expressed as d.p.m./mg.

[c]Specific activity upon acetylation and t.l.c. in cyclohexane/chloroform/acetic acid (7:2:1 by vol). expressed as d.p.m./mg lanosterol.

Table 3. Crystallization of cholesterol isolated from the placentas and perfusates

		Exp. No. 1				Exp. No. 2				Exp. No. 3			
		Placenta		Perfusate		Placenta		Perfusate		Placenta		Perfusate	
		^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Starting material[a]		15100	56400	69500	1000	6600	66300	10300	55800	10700	33900	24600	240800
Crystals[b]	1	240	450	1160	0	130	690	250	660	240	190	550	1080
	2	240	200	1210	0	120	400	240	240	240	90	660	380
	3	250	90			130	130	250	90	240	40	650	120
	4	260	0			130	0	260	0	270	0	550	0
Mother liquors	1	400	10840	1290	80	160	9400	240	8920	430	5150	500	63900
	2	320	10000	1150	0	100	8860	280	5840	250	1500	490	6380
	3	320	3330			130	4400	300	5040	270	660	590	4310
	4	260	1130			160	2940	250	390	250	600	580	550

[a]Total d.p.m. which was diluted with 25100 μg (placentas) or 25000 μg (perfusates) of standard cholesterol prior to crystallization.

[b]1. acetone 2. methanol 3. ethanol 4. acetone. Expressed as d.p.m./mg.

was obtained in experiment 2, whereas the evidence was inconclusive in experiment 3.

The crystallization data of the isolated cholesterol are presented in Table 3.

It can be seen from the data of Table 3 that evidence of radiochemical homogeneity was obtained in each case with regard to the tritium label, whereas all carbon-14 labelled material was dissociated from the crystals. Furthermore, all carbon-14 labelled material was dissociated from crystalline pregnenolone and progesterone in all sources studied.

DISCUSSION

In a previous study we have reported that little, if any, squalene and lanosterol were formed by the human placenta at mid-gestation[1]. However, we could isolate a minute quantity of radiochemically homogeneous lanosterol from the extract of one out of the four placentas perfused with labelled acetate. Since, the conversion of sodium acetate and mevalonate to squalene, lanosterol and cholesterol by human term placental preparations was repeatedly demonstrated *in vitro* [4–7], it appeared possible that during the last trimester of gestation the human placenta acquires a limited ability to synthesize squalene, lanosterol and cholesterol. The data presented in this paper seem to rule out the above possibility. No squalene or cholesterol formation could be demonstrated in any of the experiments. However, some evidence was obtained suggesting the formation of minute quantities of lanosterol in one study.

It is easy to see, however, from the figures of Table 2 that this conversion (if true) would be insignificant from the quantitative point of view. The significance of this finding is further reduced by the fact that the presence of both squalene and cholesterol was excluded in the same placenta.

Finally, one may ask whether squalene, lanosterol and cholesterol were possibly formed by the perfused placentas in a transient fashion, and that they escaped detection? Such a possibility appears to be unlikely; the perfusates were collected throughout the experiment and the entire perfusate and placental tissue were processed. Neither the placentas nor the perfusates contained any labelled squalene, cholesterol or characteristic metabolites formed from cholesterol by the placenta, such as pregnenolone or progesterone.

Another problem to be solved is the seeming discrepancy between the results of the previous *in vitro* and the present *in vivo* studies. Although no satisfactory explanation can be offered for this discrepancy for the time being, it should be remembered that the amounts of labelled squalene, lanosterol and cholesterol reported to be formed in previous *in vitro* incubations were very small indeed. Hence, in view of our previous[1] and present findings, it can be concluded that neither the mid-gestation nor the human term placenta has the ability to synthesize significant quantities of squalene, lanosterol and cholesterol from perfused acetate.

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