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A STUDY OF STEROL AND STEROID SYNTHESIS FROM SODIUM ACETATE BY HUMAN FOETAL LIVER PREPARATIONS

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SUMMARY

Liver preparations from three midgestation human foetuses were incubated with carbon-14 labelled sodium acetate in the presence as well as in the absence of cofactors and attempts were made to isolate cholesterol, pregnenolone and dehydroepiandrosterone in a radiochemically homogeneous form. Tritium labelled internal standards were used to assess procedural losses.

Relatively large quantities of carbon-14 labelled sodium acetate (corresponding to 0·95–3·1% of the material incubated) were converted to radiochemically homogeneous cholesterol in each experiment, whereas all carbon-14 labelled material was dissociated from crystalline pregnenolone and dehydroepiandrosterone.

It is concluded that liver preparations of midgestation human foetuses convert considerably large quantities of sodium acetate to cholesterol, but none to steroids.

IN PREVIOUS investigations radiochemically homogeneous cholesterol, pregnenolone and dehydroepiandrosterone were isolated from the liver of midgestation human foetuses perfused with carbon-14 labelled sodium acetate [1, 2]. These findings were confirmed in subsequent studies, in which tritium labelled internal standards were used to assess procedural losses [3, 4]. In one of the foetuses perfused in the study reported by Archer *et al.* [4], the amount of labelled pregnenolone sulphate and dehydroepiandrosterone sulphate isolated from the liver greatly exceeded the quantity of these two compounds isolated from the perfusates or adrenals. These findings prompted us to investigate whether or not labelled sodium acetate is converted to cholesterol, pregnenolone and dehydroepiandrosterone by liver preparations obtained from midgestation human foetuses.

EXPERIMENTAL

Clinical material. Three livers were obtained from midgestation foetuses following laparotomy in connection with the interruption of gestation. Permission for interruption of pregnancy was granted by the Swedish National Board of Health and Welfare upon request of the patients, under the statute of 1938, as amended in 1953 and 1964. The period of gestation was between the 16th and 20th week.

Conditions of incubation. The foetal livers were removed immediately after the termination of gestation, minced and incubated for 5 h at 37°C. In Experiment No. 1 incubation was carried out without the addition of cofactors. In Exp. No. 2 and 3 half of the minced liver was incubated without cofactors in Krebs–Ringer

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bicarbonate buffer (pH 7·4) in an atmosphere of 95% O_2 + 5% CO_2 (Exps. 2A and 3A), whereas the other half was incubated with cofactors in a Krebs–Ringer phosphate buffer (pH 7·4) in an atmosphere of air (Exps. 2B and 3B). The amounts of cofactors added per 25 ml incubation volume were as follows: 5 μmol ATP (Calbiochem), 5 μmol NADP (Sigma), 5 μmol DPN (Sigma), 30 μmol glucose-6-phosphate (Sigma), 20 Kornberg units glucose-6-phosphate dehydrogenase (Sigma) and 8 mmol nicotinamide.

Radioactive precursur. [1,2-^{14}C]-Sodium acetate (NES 553, Lot No. 620–173, S.A.: 55·4 mCi/mmol) was used without purification.

The amount added to each incubation vial was 1·0 mCi.

Internal standards. [7α-^{3}H]-*Cholesterol* (NET-030, Lot. No. 460–170, S.A.: 15 Ci/mmol) was purified with added carrier by crystallization to constant specific activity. The purity was 99·4%. [7α-^{3}H]-*Pregnenolone* (NET-039, Lot. No. 460-180, S.A.: 25 Ci/mmol) was purified by paper chromatography in ligroin, methanol, water (5:4:1) and crystallized with authentic carrier to constant S.A. The purity was 99·8%, [7α-^{3}H]-*Dehydroepiandrosterone* (NET-033, Lot No. 184-150, S.A.: 25·1 Ci/mmol) was purified with added carrier by crystallization to constant S.A. The purity was 98·5%.

Measurement of radioactivity. This was carried out by liquid scintillation spectrometry, using a Nuclear Chicago Mark II system. Correction for quenching was made by external standardisation. The results are expressed in DPM.

Extraction and separation of cholesterol and steroids. Upon completion of the incubations internal standards were added, the tissue was homogenized and extracted with 5 volumes of ethanol–acetone (3:2 v/v) mixture. This was followed by extraction with 1 vol. of acetone, 3 × 1 vol. of ether and 3 × 1 vol. of 80% (v/v) of ethanol. The extracts were combined and evaporated to almost dryness under reduced pressure at a temperature not exceeding 40°C. The residue was dissolved in 70% (v/v) methanol and the insoluble material precipitated at −17°C was removed[5]. The supernatant was evaporated and subjected to a 24-transfer countercurrent distribution between n-hexane and 90% (v/v) methanol[1]. The contents of tubes 13–24 (sterol fraction) were pooled and from this fraction cholesterol was isolated, using the method described previously[1]. The contents of tubes 0–12, containing the steroid fraction, were subjected to ether-water partition to separate the conjugated and unconjugated steroids[5]. The conjugated fraction was subjected to enzymic hydrolysis and subsequent solvolysis [2] and extracted with ethyl acetate and ether. The extracts containing the unconjugated and conjugated materials were combined and subjected to paper partition chromatography in the ligroin, methanol, water (5:4:1 by vol.) system. In this system pregnenolone was separated from dehydroepiandrosterone. The isolated fractions were rechromatographed in the same system and crystallized to constant specific activity.

RESULTS

Representative examples of crystallization to constant specific activity of cholesterol, pregnenolone and dehydroepiandrosterone are presented in Table 1.

It should be noted that the pregnenolone and dehydroepiandrosterone crystallizations indicated in Table 1 represent the total steroid pooled from four experiments; the two compounds were crystallized separately in each experiment and these crystals were then used for the formation of derivatives. In two instances

Table 1. Specific activities following repeated crystallizations of cholesterol, pregnenolone and dehydroepiandrosterone isolated following the incubation of ^{14}C sodium acetate with liver preparations from midgestation human foetuses. Tritium labelled internal standards were added to assess methodological losses. Figures are expressed in DPM/mg steroid

	Cholesterol†				Pregnenolone‡				Dehydroepiandrosterone‡			
	^3H		^{14}C		^3H		^{14}C		^3H		^{14}C	
	X	ML	X	ML	X	ML	X	ML	X	ML	X	ML
S.M.	443		8290		779[f]	868	2	13	5020[f]	4840	7	84
1.	405[a]	448	6470	9930	737[g]	801	4	5	4780[g]	5050	6	9
2.	360[b]	369	7900	6060								
3.	369[c]	362	7100	6630	729[h]	735	2	2	4780[h]	4840	2	2
4.	358[d]	304	6000	6900	740[i]	731	0	1	4720[i]	4820	0	0
5.	324[e]	349	5450	6870								
6.	308[f]	324	5310	5600								
7.	298[a]	z	5430	z								
8.	294[a)(v)]	320	5430	5600								
9.	328[e)(w)]	z	5430	z								
10.	296[d)(y)]	z	5350	z								
11.	290[e)(y)]	z	5260	z								
12.	294[d)(y)]	z	5260	z								

†Results obtained in Exp. No. 3B.

‡Material pooled from 4 experiments.

X: crystals, ML: mother liquor, S.M.: starting material.

Solvents: (a) methanol, (b) acetone–water, (c) ethanol–water, (d) methanol–ethylacetate, (e) methanol–acetone, (f) methanol–water, (g) petroleum ether–ethanol, (h) hexane, (i) heptane.

(v) crystallized as acetate, (w) crystallized as cholesterol dibromide, (y) crystallized as cholesterol acetate dibromide, (z) insufficient material for weighing.

(pregnenolone from Exp. No. 1 and dehydroepiandrosterone from Exp. No. 2B) the initial crystallizations seemed to indicate that carbon-14 labelled material may remain associated with these compounds. Therefore these two extracts were not combined for derivative formation with the four other ones. However, after crystallizations in the form of the unconjugated compound followed by 5 crystallizations of their acetates, radiochemical homogeneity could not be achieved with respect to the carbon-14 labelled material. By this time only some 15 DPM/mg carbon-14 labelled material was associated with the crystals.

The amount of radiochemically homogeneous cholesterol formed in the five experiments is indicated in Table 2.

It appears from the data of Table 2 that the yield of conversion of labelled sodium acetate to cholesterol varied between 0·95 and 3·1%.

DISCUSSION

The data reported in this communication indicate that human foetal liver preparations are capable of converting relatively large quantities of labelled sodium acetate to cholesterol, but cannot convert the cholesterol formed to pregnenolone or dehydroepiandrosterone. The conversion to cholesterol was higher in the presence than in the absence of cofactors; however, no steroids were formed from labelled acetate regardless of the presence of cofactors. Our data confirm those reported recently by Givner and Jaffe[6], who also found an extensive conversion of labelled sodium acetate to cholesterol by human foetal liver preparations. They have not studied the possible formation of steroids from acetate. Thus, on the basis of the present *in vitro* studies it may be concluded that the large quantities of labelled cholesterol isolated by us from the livers of midgestation foetuses perfused with labelled sodium acetate[1, 3] were partly, if not mainly formed there, whereas the labelled pregnenolone and dehydroepiandrosterone isolated from the same organ in the same experiments[2, 4] were only deposited in the liver, but were not synthesized there. Hence, although the foetal liver is capable of carrying out a large number of steroid reactions, such as various hydroxylations, dehydrogenations, reductions, conjugations and also aromatisation of neutral steroids[7], this organ is not able to remove the cholesterol side-

Table 2. Cholesterol isolated in a radiochemically homogeneous form following the incubation of 1·0 mCi of [^{14}C] sodium acetate with liver preparations from midgestation human foetuses. Figures were calculated from the mean of the last two sets of crystals and were corrected for methodological losses on the basis of the recovery of internal standards added

Experiment N	Tissue (g)	DPM ($\times 10^3$)	Conversion (per cent)
1	2·73	27·808	1·25
2A[a]	4·00	44·783	2·02
2B[b]	4·77	58·311	2·63
3A[a]	6·20	21·077	0·95
3B[b]	4·45	71·031	3·15

(a) without cofactors.
(b) with cofactors.

chain and convert cholesterol to pregnenolone, or dehydroepiandrosterone. On the other hand, foetal liver preparations seem to be capable of removing the steroid side-chain *in vitro*[8, 9]; this reaction has not been demonstrated in perfusion experiments, using pregnenolone[10], or 17α-hydroxypregnenolone [11] as precursors.

It is of some interest to note that cholesterol metabolism seems to be highly specialized in the different compartments of the foeto-placental unit. The liver can synthesize cholesterol, but is not able to convert this compound to steroids; the placenta is not able to synthesize cholesterol[1, 12] but it is capable of transforming cholesterol into C-21, C-19 and C-18 steroids[13] and finally, the foetal adrenals and testicles are capable of synthesizing cholesterol as well as metabolizing this compound to a variety of steroids [e.g. 14, 15].

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REFERENCES

1. Telegdy G., Weeks J. W., Lerner U., Stakemann G. and Diczfalusy E.: *Acta endocr. (Kbh)* **63** (1970a) 91.
2. Telegdy G., Weeks J. W., Archer D. F., Wiqvist N. and Diczfalusy E.: *Acta endocr. (Kbh)* **63** (1970b) 119.
3. Mathur R. S., Archer D. F., Wiqvist N. and Diczfalusy E.: *Acta endocr. (Kbh)* **65** (1970) 663.
4. Archer D. F., Mathur R. S., Wiqvist N. and Diczfalusy E.: *Acta endocr. (Kbh)* **66** (1971) 666.
5. Mikhail G., Wiqvist N. and Diczfalusy E.: *Acta endocr. (Kbh)* **42** (1963) 519.
6. Givner M. L. and Jaffe R. B.: *Steroids* **18** (1971) 1.
7. Diczfalusy E.: *Excerpta med. (Amst) Int. Congr. Ser.* **183** (1969) 65.
8. Jungmann R. A. and Schweppe J. S.: *J. clin. Endocr.* **27** (1967) 1151.
9. Jaffe R. B., Lamont K. G. and Perez-Palacios G.: *Excerpta med. (Amst) Int. Congr. Ser.* **157** (1968) 171.
10. Solomon S., Bird C. E., Ling W., Iwamiya M. and Young P. C. M.: *Rec. progr. Horm. Res.* **23** (1967) 297.
11. Pion R. J., Jaffe R. B., Wiqvist N. and Diczfalusy E.: *Biochim. biophys. Acta* **137** (1967) 584.
12. Van Leusden H. A., Siemerink M., Telegdy G. and Diczfalusy E.: *Acta endocr. (Kbh)* **66** (1971) 711.
13. Telegdy G., Weeks J. W., Wiqvist N. and Diczfalusy E.: *Acta endocr. (Kbh)* **63** (1970c) 105.
14. Jaffe R. B., Perez-Palacios G., Lamont K. G. and Givner M. L.: *J. Clin. Endocr.* **28** (1968) 1671.
15. Serra G. B., Perez-Palacios G. and Jaffe R. B.: *J. Clin. Endocr.* **30** (1970) 128.