The Biosynthesis of Cholesta-8,14-dien-3 β -ol by Chang Human Liver Cells

By D. C. WILTON

Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 5NH, U.K.

(Received 5 October 1971)

It is well established that many mammalian cells in culture are able to biosynthesize sterols *de novo* from acetate and mevalonate (Rothblat & Kritchevsky, 1968). I have been investigating cholesterol biosynthesis in Chang human liver cells and have observed that a number of radioactive sterol intermediates may be isolated when the cells are grown in the presence of [U-¹⁴C]acetate. One particular radioactive sterol peak accounted for about 10% of the total radioactivity in the sterol fraction, and this I have now identified as cholesta-8,14-dien-3 β -ol (I).

Exponentially growing Chang liver cells were incubated for 3 days with Eagle's medium supplemented with 10% (v/v) of calf serum in the presence of 20 μ Ci of [U-¹⁴C]acetate. The cells were harvested by trypsinization and saponified for 5 min at 60°C with an equal volume of 10% (w/v) potassium hydroxide in methanol. The neutral steroids were

extracted as described by Wilton, Munday, Skinner & Akhtar (1968), and the non-saponifiable extract was spotted on analytical-layer silica gel GF₂₅₄ plates that were developed in light petroleum (b.p. 60–80°C)–acetone (4:1, v/v). The plates were scanned with a Berthold radiochromatogram scanner, and the radioactive sterol band was eluted with methanol and rechromatographed on analytical-layer silica gel GF₂₅₄–10% (w/w) AgNO₃ plates along with various sterol standards (see Fig. 1). The plates were developed twice in chloroform–diethyl ether–acetic acid (970:23:5, by vol.) and then scanned for radioactivity (see Fig. 1). The radioactive peak corresponding to cholesta-8,14-dien-3 β -ol (I) was eluted with methanol, mixed with 100 mg of non-radioactive cholesta-8,14-dien-3 β -ol and recrystallized three times from methanol, during which process all the radioactivity was retained (see Table 1). Because

Fig. 1. Thin-layer radioscan of sterols biosynthesized from [U-¹⁴C]acetate and separated on silica gel–10% (w/w) AgNO₃. A, 4,4'-Dimethylcholesta-8,14-dien-3 β -ol; B, cholesterol; C, cholest-7-en-3 β -ol; D, cholesta-5,24-dien-3 β -ol; E, cholesta-8,14-dien-3 β -ol; F, cholesta-5,7-dien-3 β -ol.

Table 1. Recrystallization of unknown [U-¹⁴C]sterol and also synthetic [³H]cholesta-7,14-dien-3 β -ol with cholesta-8,14-dien-3 β -ol (I) Cholesta-8,14-dien-3 β -ol (I)

Source of radioactivity	Unknown [U- ¹⁴ C]sterol	[³ H]Cholesta-7,14-dien-3 β -ol
Amount of compound (I) used	100 mg	50 mg
Total radioactivity added	15000 c.p.m.	110000 c.p.m.
Theoretical specific radioactivity	150 c.p.m./mg	2200 c.p.m./mg
1st crystallization	100 c.p.m./mg	1530 c.p.m./mg
2nd crystallization	110 c.p.m./mg	1050 c.p.m./mg
3rd crystallization	125 c.p.m./mg	695 c.p.m./mg

cholesta-8,14-dien-3 β -ol (I) cannot be separated from cholesta-7,14-dien-3 β -ol (II) by using the chromatography systems described above, a portion of authentic tritiated compound (II), synthesized from cholesta-5,7-dien-3 β -ol (Knight, Klein & Szczepanik, 1966) in the presence of a trace of tritiated water (1 Ci/ml), was mixed with 50 mg of non-radioactive compound (I) and recrystallized three times from methanol. In this case the specific radioactivity fell with each crystallization, thus establishing that the 7,14-diene (II) does not crystallize with the 8,14-diene (I), and hence the unknown radioactive sterol is probably cholesta-8,14-dien-3 β -ol (I).

Another incubation mixture was worked up as described above to give a radioactive peak corresponding to cholesta-8,14-dien-3 β -ol (I), and this peak was subjected to mass-spectral analysis in an AEI MS30 combined g.l.c.-mass spectrometer. Ions at m/e 384 (M^+), 369 ($M^+ - CH_3$), 351 ($M^+ - CH_3 - H_2O$) and 271 ($M^+ - \text{side chain}$) were obtained, and these are consistent with structure (I).

The identification of cholesta-8,14-dien-3 β -ol (I)

as a major sterol intermediate biosynthesized by Chang human liver cells during growth further emphasizes the importance of 8,14-dienes in cholesterol biosynthesis (Canonica *et al.* 1968; Watkinson, Wilton, Munday & Akhtar, 1971). It is also noteworthy that these cells are able to remove the 4-methyl groups, originally present in lanosterol, before reduction of the Δ^{14-15} -double bond.

I thank Mrs S. Gahr for excellent technical assistance and Dr M. Akhtar for many helpful discussions.

- Canonica, L., Fiecchi, A., Kienle, M. G., Scala, A., Galli, G., Paoletti, E. G. & Paoletti, R. (1968). *J. Am. chem. Soc.* **90**, 6532.
 Knight, J. C., Klein, P. D. & Szczepanik, P. A. (1966). *J. biol. Chem.* **241**, 1502.
 Rothblat, G. H. & Kritchevsky, D. (1968). *Expl molec. Path.* **8**, 314.
 Watkinson, I. A., Wilton, D. C., Munday, K. A. & Akhtar, M. (1971). *Biochem. J.* **121**, 131.
 Wilton, D. C., Munday, K. A., Skinner, S. J. M. & Akhtar, M. (1968). *Biochem. J.* **106**, 803.