The Pathway for the Removal of C-32 in Cholesterol Biosynthesis

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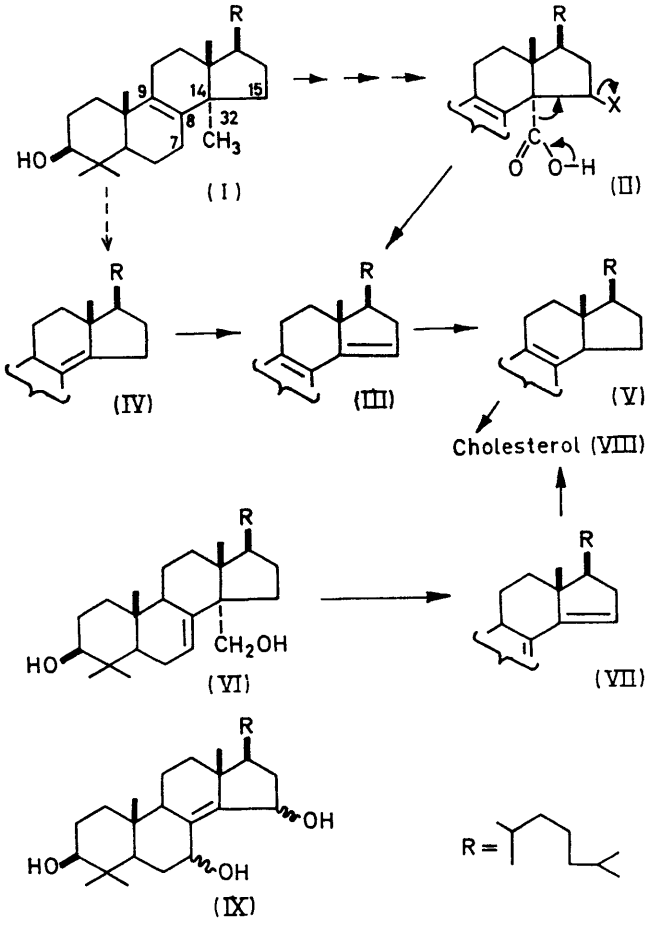
Summary It is shown that a $\Delta^{8(14)}$-sterol is not involved in cholesterol biosynthesis; the removal of C-32 in sterols containing a double bond at either $\Delta^{7(8)}$ or $\Delta^{8(9)}$ is attended exclusively with the formation of 7,14-diene and 8,14-diene sterols, respectively.

THE loss of the 15-hydrogen atom [1–5] in cholesterol biosynthesis suggested that the sequence (I) → (II) → (III) may be involved[1–3,5] in the removal of the 14-methyl group (C-32). In support of this hypothesis the obligatory participation of the 8,14-diene (III)[1,3,6–8] in the biosynthesis

has been established.[7,9] However, some additional observations indicated an intermediary role also for a $\Delta^{8(14)}$-sterol (IV)[10–15] and necessitated the consideration of an alternative sequence (I) → (IV) → (III) for the 14-demethylation. In this report we show that contrary to a previous claim[11] the $\Delta^{8(14)}$-sterol (IV) is not an intermediate in the metabolism of lanost-7-ene-3β,32-diol (VI), nor in that of dihydrolanosterol (I).



Samples of [3α-^{3}H]dihydrolanosterol (100 μg) were incubated [7,16] in the presence of a trap of 4,4-dimethylcholesta-8,14-dien-3β-ol (0·5 mg), one of the experiments in addition contained 4,4-dimethylcholest-8(14)-en-3β-ol (0·5 mg). In both cases about 20% of the radioactivity incubated was found to be associated with a material chromatographically identical with the 8,14-diene (III). If the $\Delta^{8(14)}$-sterol (IV) was the direct precursor of the 8,14-diene (III) then the presence of the unlabelled (IV) during biosynthesis would be expected to diminish the incorporation of radioactivity from dihydrolanosterol (I) into the 8,14-diene (III) through pool dilution. This was not the case. In a related experiment attempts to trap the $\Delta^{8(14)}$-sterol (IV) from [3α-^{3}H]dihydrolanosterol were unsuccessful (see also ref. 17).

The postulated mechanism (I) → (IV) → (III) would require dihydrolanosterol (I) and the $\Delta^{8(14)}$-sterol (IV) to be equally efficient precursors of the 8,14-diene(III). However, incubation of [3α-^{3}H]-4,4-dimethylcholest-8(14)-en-3β-ol (100 μg) in the presence of the 8,14-diene (III) (0·5 mg) as a trap revealed that only 1% of the radioactivity incubated was chromatographically identical with (III) compared to the 20% trapped from the [3α-^{3}H]dihydrolanosterol substrate in a parallel experiment.

The failure to demonstrate a convincing intermediary role for the $\Delta^{8(14)}$-sterol (IV) led to a re-examination of the previous report[11] claiming that (IV) was at least as good a precursor of cholesterol as dihydrolanosterol. Under our conditions, it was found that (IV) was only 1/6 as good a precursor of cholesterol as was dihydrolanosterol.

Consequently therefore, we investigated the report[11] that the $\Delta^{8(14)}$-sterol (IV) was a product of the metabolism of lanost-7-ene-3β,32-diol (VI). A sample of lanost-7-ene-32-carbonitrile-3β-ol acetate synthesised *via* the nitrite photolysis method,[18,19] on hydrolysis and oxidation followed by reduction with sodium borotritiide afforded [3α-^{3}H]-lanost-7-ene-32-carbonitrile-3β-ol. This was converted into the 32-aldehyde[19] with lithium aluminium hydride, which was reduced with sodium borohydride in methanol to give after t.l.c. [3α-^{3}H]lanost-7-ene-3β,32-diol. [3α-^{3}H]Lanost-7-ene-3β,32-diol (20 μg) was incubated in the presence of the $\Delta^{8(14)}$-sterol (IV) (2 mg). Isolation of the 4,4-dimethylmonoene alcohol region by t.l.c. and co-crystallisation with carrier 4,4-dimethylcholest-8(14)-en-3β-ol revealed that after 5 crystallisations only 0·12% of the radioactivity incubated was associated with the $\Delta^{8(14)}$-sterol (IV). In a parallel experiment [3α-^{3}H]lanost-7-ene-3β,32-diol (22 μg) was incubated in the presence of 4,4-dimethylcholesta-7,14-dien-3β-ol (VII) (2 mg). Isolation of the 4,4-dimethyl diene alcohol region by t.l.c. followed by acetylation and co-crystallisation with 4,4-dimethylcholesta-7,14-dien-3β-ol acetate[7] revealed that 13% of the radioactivity incubated was associated with the 7,14-diene (VII). This was unambiguously confirmed by 93% retention of radioactivity when 4,4-dimethylcholesta-7,14-diene-3β-ol acetate (67,500 c.p.m./mmole) was converted into 4,4-dimethylcholest-8(14)-ene-3β,7ζ,15ζ-triol[20] (IX) and crystallised to constant specific activity (1st cryst. = 63,000 c.p.m./mmole, 2nd cryst. = 61,800 c.p.m./mmole, 3rd cryst. = 62,300 c.p.m./mmole). In a control experiment it was shown that conversion of non-radioactive 4,4-dimethylcholesta-7,14-dien-3β-ol acetate in the presence of a trace amount of [3α-^{3}H]-4,4-dimethylcholesta-8,14-dien-3β-ol acetate afforded the triol (IX) which had lost at least 97% of the original radioactivity.

Contrary to a previous report[11] the present work has failed to show the formation of the $\Delta^{8(14)}$-sterol (IV) in cholesterol biosynthesis, although the trapping of the 7,14-diene (VII) from the diol (VI) has been demonstrated for the first time. The observation that the removal of the 32-carbon atom of the $\Delta^{8(9)}$-sterol (I) gives rise exclusively to the 8,14-diene (III), whereas a 7,14-diene intermediate (VII) is formed in high yield in the removal of the 32-carbon atom of the $\Delta^{7(8)}$-sterol (VI) rejects the intermediary role of a $\Delta^{8(14)}$-compound in a major pathway of cholesterol biosynthesis. If a $\Delta^{8(14)}$-sterol was an intermediate the same diene would be formed from both the $\Delta^{8(9)}$- and $\Delta^{7(8)}$-precursors. The mechanism for the removal of the

32-carbon atom is therefore best represented by the sequence (I) → (II) → (III)

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[1] M Akhtar I A Watkinson, A D Rahimtula, D. C. Wilton, and K A Munday, *Chem Comm*, 1968, 1406
[2] M Akhtar I A Watkinson, A D Rahimtula, D C Wilton, and K. A Munday, *European J Biochem*, 1969, **9**, 107
[3] M Akhtar I A Watkinson, A D Rahimtula, D. C. Wilton, and K A Munday, *Biochem J*, 1969, **111**, 757
[4] G F Gibbons, L J Goad, and T W Goodwin, *Chem Comm*, 1968, 1458
[5] L Canonica, A Fiecchi, M. G. Kienle, A Scala, G Galli, E Grossi Paoletti, and R Paoletti, *J Amer Chem Soc*, 1968, **90**, 3597
[6] L Canonica A Fiecchi, M G. Kienle, A Scala, G. Galli, E Grossi Paoletti, and R Paoletti, *J Amer Chem Soc*, 1968, **90**, 6532
[7] I A Watkinson, D C Wilton, K A Munday, and M Akhtar, *Biochem J*, 1971, **121**, 131
[8] B N Lutsky and G J Schroepfer *Biochem Biophys Res Comm*, 1968, **33**, 492
[9] A Fiecchi L Canonica, A Scala, F Cattabeni, E Grossi Paoletti, and R Paoletti, *Life Sci*, 1969, **8**, 629
[10] Lee Wen Hui and G J Schroepfer *Biochem Biophys Res Comm*, 1968, **32**, 635
[11] J Fried, A Dudowitz, and J W Brown, *Biochem Biophys Res Comm*, 1968, **32**, 568
[12] B N Lutsky and G J Schroepfer, *Biochem Biophys Res Comm*, 1969, **35**, 288
[13] L H Zalkow G A Cabat, G L Chetty, M Ghosal, and G Keen, *Tetrahedron Letters*, 1968, 5727
[14] S Rakhit M Dhar, N Anand, and M L Dhar, *J. Sci Ind Res, India*, 1959, **18B**, 422
[15] Lee Wen Hui, B N Lutsky, and G J Schroepfer, *J Biol Chem*, 1969, **244**, 5440
[16] D C Wilton, K A Munday, S J M Skinner, and M Akhtar, *Biochem J*, 1968, **106** 803
[17] R Paoletti G Galli, E Grossi Paoletti A Fiecchi, and A Scala, *Lipids*, 1970, **6**, 134
[18] D H R Barton, J M Beaton, L E Geller, and M M Pechet, *J Amer Chem Soc*, 1961, **83**, 4076
[19] T J Bentley, J F McGhie, and D H R Barton, *Tetrahedron Letters* 1965, 2497
[20] R B Woodward, A A Patchett, D H R Barton, D A J Ives and R B Kelly, *J Chem Soc*, 1957, 1131