The Removal of the 32-Carbon Atom as Formic Acid in Cholesterol Biosynthesis

By K. ALEXANDER and M. AKHTAR*
(*Department of Physiology and Biochemistry, The University, Southampton*)
R. B. BOAR and J. F. MCGHIE
(*Department of Chemistry, Chelsea College of Science and Technology, London, S.W.3*)
and D. H. R. BARTON
(*Imperial College, London, S.W.7*)

Summary It is shown that the removal of the 32-carbon atom in cholesterol biosynthesis occurs at the oxidation state of an aldehyde resulting in the release of formic acid.

IN the conversion of lanosterol into cholesterol the methyl groups at C-4,4′ and 14 are removed. From the very early studies[1] on sterol biosynthesis it has been assumed that the methyl groups are eliminated as carbon dioxide subsequent to their oxidation into the corresponding carboxylic acid derivatives. That such a mechanism operates in the case of C-4,4′ demethylation is suggested by the isolation of 4α-carboxylic acid derivatives,[2–5] which have been shown to be intermediates in cholesterol biosynthesis. Furthermore using [4α-^{14}C]methylcholest-7-en-3β-ol we have shown (unpublished work) that the labelled carbon atom is removed exclusively as carbon dioxide and no significant amount of radioactivity is found in either formic acid or formaldehyde. We now show that unlike the C-4 steps the

C-14 alkyl group in cholesterol biosynthesis is removed as formic acid.

The crucial substrate [32-^{3}H]lanost-7-ene-32,3β-diol, required for the biosynthetic experiments, was prepared by the reduction[6] of 32-oxo-lanost-7-ene-3β-ol[7] with sodium borotritide. The [32-^{3}H]diol (**1**) (22·4 μM, 5·6 × 10^6 d.p.m./100 μg) was incubated with unwashed microsomes[8] (10 ml) supplemented with NAD (0·4 mM), in air for 2 h at 37 °C. After being acidified with phosphoric acid the reaction mixture was freeze dried and the distillate was shown to contain 76% of the radioactivity incubated. The aqueous solution was neutralized with sodium hydroxide and evaporated to dryness and the residue contained 43% of the original radioactivity. After the addition of carrier and recrystallization all this radioactivity was shown to be associated with the sodium formate. A part of this material (199,000 d.p.m./mmol) when converted into *p*-bromophenacylformate[9] retained over 85% of the radioactivity (170,000 d.p.m./mmol). In a control experiment performed with boiled enzyme less than 0·5% of the original radioactivity was in the distillate and less than 0·1% was associated with sodium formate.

Next, experiments were performed to establish the relationship between the release of formic acid and the formation of the product of C-14 demethylation: 4,4-dimethylcholesta-7,14-dien-3β-ol(**4**). Either [3α-^{3}H]lanost-7-ene-32,3β-diol[6](**1**) (26·9 μM, 1·0 × 10^6 d.p.m./100 μg) or [32-^{3}H] lanost-7-ene-32,3β-diol(**1**) (26.9 μM, 1·0 × 10^6d.p.m. /100 μg) were incubated† with washed microsomes supplemented with NAD (0·4 mM) and NADPH generator‡ (0·5 mM NADP) under aerobic conditions. The [32-^{3}H] diol gave 47% of its radioactivity with formic acid (Table) whereas 32% of the radioactivity from the [3α-^{3}H]diol was found to be present in the 4,4-dimethylcholesta-7,14-dien-3β-ol (**4**) band on t.l.c. That over 90% of the radioactivity chromatographically coincident with the diene (**4**) band was associated with 4,4-dimethylcholesta-7,14-dien-3β-ol (**4**) was shown in a similar experiment by the method described previously.[6] This suggests that the release of formic acid may be intimately linked to the formation of 4,4-dimethylcholesta-7,14-dien-3β-ol (**4**). Further evidence for this was provided when it was shown (Table) that factors influencing the formation of formic acid also affected the formation of the immediate product of demethylation in a similar way.

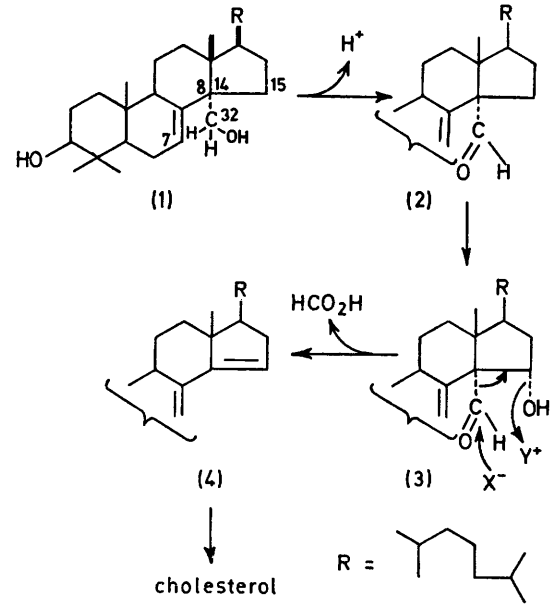
TABLE

Release of formic acid and formation of 4,4-*dimethylcholesta*-7,14-*dien*-3β-*ol* (**4**) *using* [3α-^{3}H] *or* [32-^{3}H]*lanost*-7-*ene*-32,3β-*diol* (**1**) *incubated*† *with washed microsomes supplemented with* NAD (0·4 mM) *and* NADPH *generator*‡ (0·5 mM NADP).

Conditions	Incubation time (h)	% of [32-^{3}H] radioactivity in formic acid	% of [3α-^{3}H] radioactivity associated with diene (**4**)
aerobic	0·5	47·6	32·2
aerobic	1·0[a]	46·5	17·7
partially anaerobic	1·0[a]	14·6	7·5

[a] The relatively low recovery of 4,4-dimethylcholesta-7,14-dien-3β-ol (**4**) in this experiment was due to the further metabolism of the diene (**4**) during the longer incubation period.

The examination of the cofactor requirements revealed that in the presence of oxygen and NADPH generator‡ (1·0 mM NADP) alone the [32-^{3}H]diol (**1**) (22·4 μM) was quantitatively metabolized giving 43·5% of its radioactivity with water and 56·5% with formic acid. That the removal of the 32-carbon atom from the diol (**1**) occurs through the intermediacy of the aldehyde (**2**) was suggested when the aerobic incubation† of the [3α-^{3}H]diol (**1**) (22·4 μM) with washed microsomes in the presence of a limited quantity of NADPH generator‡ (0·1 mM NADP) resulted in the 20%



SCHEME

accumulation of the aldehyde (**2**), isolated by t.l.c. No significant formation of the aldehyde (**2**) from the [3α-^{3}H]diol(**1**) (22·4 μM) was noted when the latter was incubated† under anaerobic conditions in the presence of either NAD (0·4 mM) or NADP (1·0 mM), all the radioactivity being recovered as unmetabolized [3α-^{3}H]diol (**1**). This experiment suggests that the conversion of the diol (**1**) to the aldehyde (**2**) does not occur through the conventional alcohol dehydrogenase type of reaction.

In conclusion it is shown that the removal of the 32-carbon atom in cholesterol biosynthesis occurs at the oxidation state of an aldehyde resulting in the release of formic acid.

† Incubation performed in the presence of a trap of 4,4-dimethylcholesta-7,14-dien-3β-ol (**4**) (0·4 mM).

‡ The NADPH generator contained glucose-6-phosphate (4 μmol) and glucose-6-phosphate dehydrogenase (0·6 units) per μmol of NADP.

The overall reaction (Scheme) requires the presence of NADPH and oxygen and appears to be mechanistically related to the process involved in the removal of the 19-methyl group in the formation of oestrogens from androgens.[10,11]

(*Received*, 1*st February* 1971; *Com.* 147.)

[1] J. A. Olson, jun., M. Lindberg, and K. Bloch, *J. Biol. Chem.*, 1957, **226**, 941.
[2] W. L. Miller and J. L. Gaylor, *J. Biol. Chem.*, 1970, **245**, 5369.
[3] W. L. Miller and J. L. Gaylor, *J. Biol. Chem.*, 1970, **245**, 5375.
[4] G. M. Hornby and G. S. Boyd, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 1452.
[5] D. P. Bloxham and M. Akhtar, *Biochem. J.*, 1971, **123**, 275.
[6] K. Alexander, M. Akhtar, R. B. Boar, J. F. McGhie, and D. H. R. Barton, Chem. Comm., 1971, 1479.
[7] T. J. Bentley, J. F. McGhie, and D. H. R. Barton, 1965, 2497.
[8] D. P. Bloxham, D. C. Wilton, and M. Akhtar, *Biochem. J.*, 1971, **125**, 625.
[9] O. Gabriel, *Analyt. Biochem.*, 1965, **10**, 143.
[10] M. Akhtar and S. J. M. Skinner, *Biochem. J.*, 1968, **109**, 318.
[11] S. J. M. Skinner and M. Akhtar, *Biochem. J.*, 1969, **114**, 75.