The Removal of the 14-Methyl Group in Cholesterol Biosynthesis

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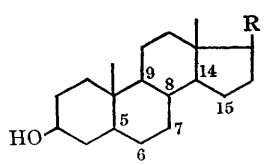
ONE of the key reactions in the biological conversion of lanosterol into cholesterol is the removal of the 14-methyl group. The work of Bloch and his co-workers[1] has shown that the methyl group is lost as carbon dioxide subsequent to its oxidation to a carboxylic acid. We show that cholesta-8,14-dien-3β-ol (IV) is converted into cholesterol (I) with a 10,000 g. supernatant of rat liver homogenate. We suggest that this observation has important implications regarding the mechanism of the removal of the 14-methyl group in steroid biosynthesis.

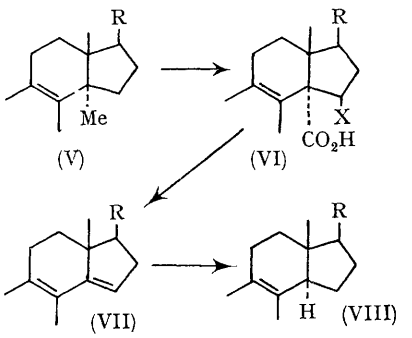
We have investigated the biosynthesis of cholesterol from squalene in the presence of tritiated

(I; 5,6-ene)
(II; 7,8-ene)
(III; 8,9-ene)
(IV; 8,14-diene)



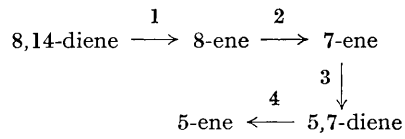
water.[2] Chemical degradation of the biosynthetic cholesterol strongly indicated that the removal of

the 14-methyl group in cholesterol biosynthesis may be facilitated by the oxidation of the 15-position.[3] One possible sequence of reactions for the decarboxylation is shown in Scheme 1. In order to



SCHEME 1

test the main features of Scheme 1, we have synthesised a number of $\Delta^{8,9}$-15 functionalised steroids and have studied their metabolisms with the rat liver enzymes. This Scheme would predict that cholesterol-synthesising tissues must contain an enzyme system capable of reducing the 14,15-double bond in the 8,14-diene of the type (VII).† We show that cholesta-8,14-dien-3β-ol (IV) is converted into cholesterol (I), and we suggest the following sequence:



1,NADPH, anaerobic; 2, anaerobic; 3, O_2; 4, NADPH

SCHEME 2

Cholesta-5,7-dien-3β-ol acetate was rearranged[4] in the presence of tritiated water to yield, after hydrolysis, cholesta-8,14-dien-3β-ol (IV). This diene (IV) should, in principle, be radioactive at positions 5, 6, 7, and 15. When a sample of the tritiated diene [(IV); (500 μg.), containing $4{\cdot}3 \times 10^5$ counts/min.] was incubated[5] with a 10,000 g. supernatant of rat liver homogenate under aerobic conditions about 30% of the original radioactivity was incorporated into cholesterol, which was isolated as its dibromide derivative. In an identical incubation carried out in the absence of oxygen less than 1% of the activity was incorporated into cholesterol.

The enzyme believed to participate in the reduction of the 14,15-double bond of the diene (IV) is present in the 106,000 g. microsomal pellet.[5]

When non-radioactive diene (IV) was incubated under anaerobic conditions with a 10,000 g. supernatant in the presence of tritiated water (the medium had 8×10^7 counts/min./1 mg. atom of hydrogen) about 9×10^4 counts/min. were incorporated in a fraction which crystallised with cholest-7-en-3β-ol. In this experiment no activity was found associated with cholesterol. This observation is in accordance with the requirements of Scheme 2 because the further conversion of cholest-7-en-3β-ol (II) into cholesterol involves an oxygen-dependent step[6] (step 3, Scheme 2).

When non-radioactive diene (IV) was incubated under anaerobic conditions with a 106,000 g. microsomal pellet in the presence of [4-$^3H_2$]-NADPH (3×10^6 counts/min./mg.) about $1{\cdot}5 \times 10^5$ counts/min. were incorporated in a fraction which crystallised with cholest-7-en-3β-ol. Since step 2 of Scheme 2 does not depend on the participation of pyridine nucleotides,[7] therefore NADPH must be required in an early stage of the reaction. It would be interesting to see if the reduction of the double bond in the diene (IV) occurs by a mechanism similar to the one already established for the conversion of cholesta-5,7-dien-3β-ol into cholesterol.[5]

Therefore under aerobic conditions the diene (IV) is converted into cholesterol and the evidence is consistent with the involvement of Scheme 2 for this conversion.

(*Received, August 5th*, 1968; *Com.* 1074.)

† Since this diene is expected to be the immediate product of decarboxylation of lanosterol, the physiological substrate for the enzyme should be a 4,4′-dimethylcholesta-8,14-dien-3β-ol system.

[1] J. A. Olson, M. Lindberg, and K. Bloch, *J. Biol. Chem.*, 1957, **226**, 941.
[2] M. Akhtar, D. C. Wilton, and K. A. Munday, *Biochem. J.*, 1966, **101**, 23C.
[3] D. C. Wilton, Ph.D. Thesis, University of Southampton, 1967, page 83; For recent evidence see L. Canonica, A. Fiecchi, M. G. Kienle, A. Scala, G. Galli, E. G. Paoletti, and R. Paoletti, *J. Amer. Chem. Soc.*, 1968, **90**, 3597; M. Akhtar, I. A. Watkinson, A. D. Rahimtula, D. C. Wilton, and K. A. Munday, *Biochem. J.*, in the press.
[4] L. F. Fieser and G. Ourisson, *J. Amer. Chem. Soc.*, 1953, **75**, 4404.
[5] D. C. Wilton, K. A. Munday, S. J. M. Skinner, and M. Akhtar, *Biochem. J.*, 1968, **106**, 803.
[6] M. Akhtar and A. D. Rahimtula, *Chem. Comm.*, 1968, 259.
[7] M. Akhtar and S. Marsh, *Biochem. J.*, 1967, **102**, 462.