The Origin of the 14α-Hydrogen in Cholesterol Biosynthesis

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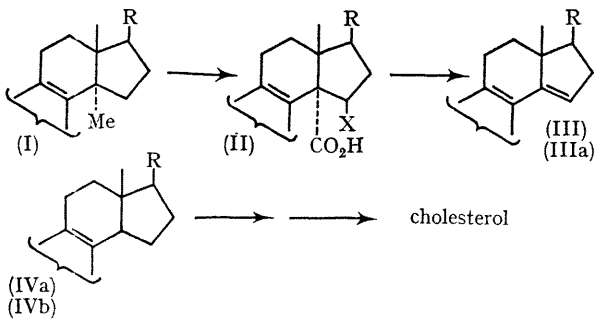
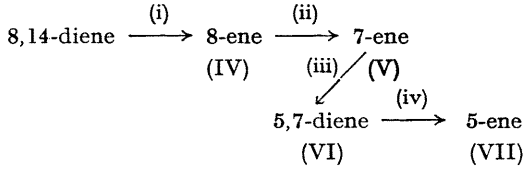
THE biosynthesis of cholesterol either from squalene[1,2] or from specifically labelled mevalonic acid[2-4] involves the labilization of one of the C-15 hydrogen atoms. It has been suggested that the involvement of a C-15 hydrogen atom in the biosynthesis is intimately linked with the removal of the 14α-methyl group.[2,5] We have found that both cholesta-8,14-dien-3β-ol (IIIa) and 4,4-dimethylcholesta-8,14-dien-3β-ol (IIIb) are efficiently converted into cholesterol by rat liver homogenate.[2,5] We have now studied the mechanism of action of the enzyme involved in the conversion (IIIa)→(IVa), and have shown that the C-14 hydrogen atom is derived from the 4-position of pyridine nucleotide and the C-15 hydrogen atom from a proton source.

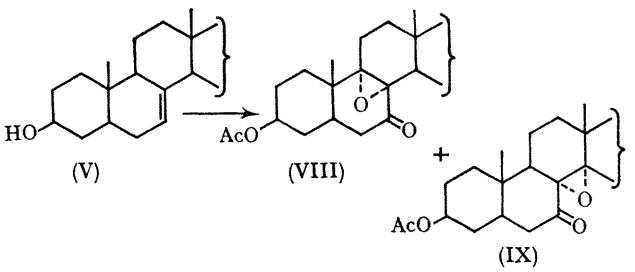


The aerobic incubation of cholesta-8,14-dien-3β-ol (IIIa) with a 10,000 *g* supernatant of rat liver homogenate resulted in the formation of cholesterol through the sequence shown in Scheme 2.[5]



(i), NADPH, anaerobic; (ii), anaerobic; (iii) O_2; (iv), NAPDH

In the absence of oxygen the above biosynthetic path stops at the stage of cholest-7-en-3β-ol (V).[5] When cholesta-8,14-diene-3β-ol (IIIa) was incubated anaerobically with a 105,000 *g* microsomal fraction of rat liver homogenate in the presence of [4-$^3H_2$]NADPH (6·6 × 10^6 c.p.m.), 1·66 × 10^5 c.p.m. were incorporated into cholest-7-en-3β-ol (V). The latter compound (V; 4·6 × 10^4 c.p.m./mmole) on oxidation[6] gave 3β-acetoxy-8,9-epoxycholest-7-one (VIII) (4·6 × 10^4 c.p.m./mmole) and cholest-3β-acetoxy-8,14-epoxycholest-7-one (IX) (5·0 × 10^2 c.p.m./mmole). The complete retention of radioactivity in the 8,9-epoxide (VIII) and its complete removal in the 8,14-epoxide (IX) shows that the labelled hydrogen atom in the biosynthetic cholest-7-en-3β-ol (V) was located at C-14.



In an alternate incubation cholesta-8,14-dien-3β-ol (IIIa) was incubated anaerobically with a 105,000 *g* microsomal fraction in the presence of tritiated water. The biosynthetic cholest-7-en-3β-ol (V; 465,000 c.p.m./mmole) on oxidation[6] gave the epoxides (VIII) (229,000 c.p.m./mmole) and (IX) (484,000 c.p.m./mmole). The loss of 50% of the radioactivity observed in the 8,9-epoxide (VIII) showed that this amount of radioactivity was present at C-9 and the complete retention of radioactivity in the 8,14-epoxide (IX) showed that C-7 contained no activity. That the remaining radioactivity is present at C-15 is suggested by the following degradation. Biosynthetic cholest-7-en-3β-ol (V) (465,000 c.p.m./mmole) was converted into cholesta-7,14-dien-3β-ol benzoate[9] (461,000 c.p.m./mmole). This was then diluted with non-radioactive material to give a specific activity of 48,400 c.p.m./mmole. The latter compound on acid isomerisation to cholesta-8,14-dien-3β-ol benzoate[9] (3280 c.p.m./mmole) lost 92% of the counts. In this isomerisation hydrogen atoms at positions 7, 9, and 15 are likely to be involved. Since it has already been shown that C-9 contains 50% of the radioactivity and C-7 contains no radioactivity we deduce that the remaining 50% of the radioactivity was at C-15. The radioactivity at C-15 in cholest-7-en-3β-ol (V) biosynthesized in the presence of

tritiated water must have been introduced during the reduction of the 14–15 double bond and the activity at C-9 during the isomerisation step (Reaction 2, Scheme 2).[7]

The cumulative evidence presented above shows that the enzyme 8,14-cholestadiene-reductase catalyses the reduction of the 14–15 double bond in the diene of the type (III) such that the C-14 hydrogen atom originates from the 4-position of NADPH and the C-15 hydrogen from a proton source. These results strongly support an earlier prediction[8] regarding the mechanism of reduction of C=C in biological systems. It has been shown that 4,4-dimethylcholesta-8,14-dien-3β-ol (IIIb) is efficiently converted into cholesterol.[2] If this conversion reflects the true intermediary nature of this compound in cholesterol biosynthesis, then it follows that the 14α-hydrogen atom of cholesterol is derived from the 4-position of NADPH.

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