# The Transfer of the Double Bond from 8(9) to 7(8) in Cholesterol Biosynthesis

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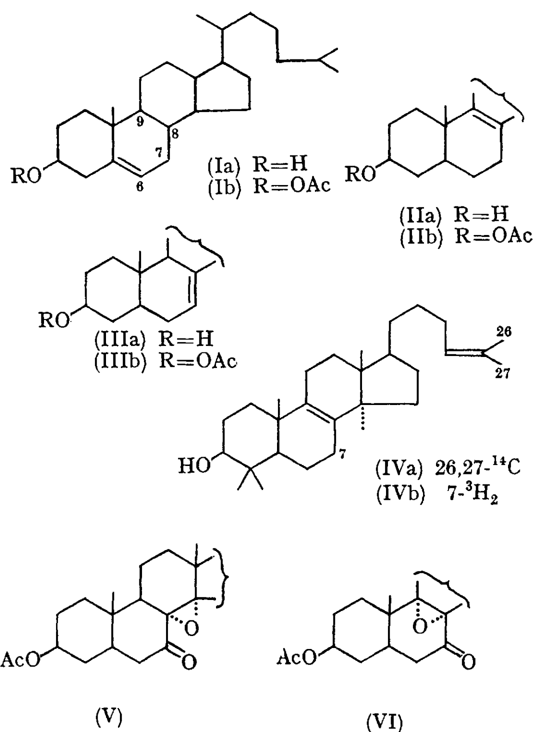
RECENT evidence from several laboratories suggests that the transfer of the double bond from 8(9) to 5(6) in cholesterol biosynthesis occurs through the combination of three reactions.[1-4] An initial migration of the double bond from 8(9) to 7(8) which is then followed by an oxidative dehydrogenation[2,3] to give a 5,7-diene system; and finally, a NADPH-linked reduction of the 7(8) bond to furnish the 5(6) double bond of cholesterol.[4] Here we report observations pertinent to the migration of the double bond from 8(9) (IIa) to 7(8) (IIIa). Our main objective was to differentiate between two main mechanisms:

(i) an intramolecular hydrogen transfer mechanism in which one of the C-7 hydrogens is transferred to C-9,

(ii) an "addition–elimination" mechanism in which one of the C-7 protons is lost and one from water is incorporated at C-9 during the biosynthesis. Evidence proves that the migration of the double bond from 8(9) to 7(8) occurs through an "addition–elimination" mechanism.

For our mechanistic studies we needed a $^{14}C$-labelled lanosterol and lanosterol labelled specifically with tritium at C-7. [26,27-$^{14}C$]Lanosterol was synthesised by the method described previously[5] and [7-$^{3}H_2$]lanosterol was prepared as follows: dry chloroform (10 ml.) was saturated with tritiated HCl gas for 10—15 min. Lanosteryl acetate dibromide (250 mg.) was then added and allowed to equilibrate at room temperature for 4 hr. The resulting mixture of tritiated $\Delta^8$- and $\Delta^7$-dibromide acetates was debrominated (Zn/benzene–methanol) and separated on a silica gel–silver nitrate plate. The [7-$^{3}H_2$]lanosteryl acetate thus prepared incorporates radioactivity at both the C-7 hydrogens.

When a mixture of [26,27-$^{14}C$]- and [7-$^{3}H_2$]-lanosterol (IVa and IVb) (T/C = 5·6) was incubated with a 10,000 g. supernatent of rat liver




homogenates under aerobic conditions,[4] the biosynthetic cholesterol (Ia; T/C = 1·9) which was isolated as the dibromide had lost 66% of the tritium activity. That the remaining activity

was still located at C-7 of cholesterol was shown by the conversion of the biosynthetic cholesteryl acetate (Ib) into 3β-acetoxycholest-5-en-7-one which resulted in the removal of 82% of the remaining activity (T/C = 0·34). This experiment therefore proves that in the migration of the double bond from 8(9) to 7(8) during biosynthesis, one of the hydrogens at C-7 is lost and the other remains undisturbed. (The loss of 66% of the activity instead of 50% shows the uneven incorporation of label at C-7α and C-7β during the chemical synthesis.)†

In order to locate the origin of the C-9 hydrogen during the biosynthesis, non-radioactive cholest-8(9)-enol (IIa) was incubated for 2 hr. with rat liver microsomes[6] under anaerobic conditions in the presence of tritiated water (counts/min./mg. atom of hydrogen = $3·4 \times 10^8$). This resulted in the incorporation of $10^5$ counts/min. in the biosynthetic cholest-7-enol.

After the addition of carrier, biosynthetic 3β-acetoxycholest-7-ene (IIIb; counts/min./mmole = $6 \times 10^4$) was oxidised with chromium trioxide–acetic acid[7] to give a mixture of 3β-acetoxy-8,9-epoxycholestan-7-one (VI; counts/min./mmole = 1150) and 3β-acetoxy-8,14-epoxycholestan-7-one (V; counts/min./mmole = $5·8 \times 10^4$). The complete retention of activity in (V) and its complete removal in (VI) unambiguously proves that the C-9 hydrogen of the biosynthetic cholest-7-enol (IIIa) contained all the radioactivity.

We therefore conclude that the biological transfer of the 8(9)-double bond to 7(8) occurs through an "addition–elimination" mechanism.

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† The distribution of radioactivity between the two hydrogen atoms at C-7 of lanosterol may vary from experiment to experiment, because of the difficulty involved in maintaining a known concentration of hydrogen chloride in the reaction mixture. Also one sample of labelled lanosterol prepared by the equilibration method contained 15—20% of the activity at positions other than C-7.

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