

INHIBITION OF THE CONVERSION OF 7-DEHYDROCHOLESTEROL
TO CHOLESTEROL BY AY-9944

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We have reported recently on the effect of trans-1,4-bis(2-chloro-benzylaminomethyl)cyclohexane dihydrochloride (AY-9944) (Humber, 1963) on hepatic cholesterogenesis in rat liver homogenates (Dvornik et al., 1963). In vitro, AY-9944 (a) prevented the incorporation of 2-C¹⁴-mevalonate into cholesterol but not into squalene or lanosterol, (b) did not affect the conversion of 26,27-C¹⁴-24-dehydrocholesterol to cholesterol and (c) inhibited the metabolism of 7-dehydrocholesterol (cf. Kandutsch, 1962). We considered it important to amplify the latter finding by investigating the effect of AY-9944 on the conversion of 4-C¹⁴-7-dehydrocholesterol to cholesterol by rat liver homogenates (cf. Schroepfer and Frantz, 1961). Experimental: 4-C¹⁴-7-Dehydrocholesterol was prepared from 4-C¹⁴-cholesterol according to Kulkarni, Blondin and Nes (1963) and had a specific radioactivity of 0.57 μ c per μ mole.

Livers taken from hooded rats were homogenized and incubated according to Bucher and McGarrah (1956) in the presence of cofactors as described by Popjak, Cornforth and Clifford (1960). Enzyme action was arrested by addition of KOH, carrier cholesterol added, the suspension heated and the nonsaponifiable lipids extracted with petroleum ether. After bromination (Fieser, 1953), 5,6-dibromocholestan-3 β -ol (DBC) was isolated, crystallized and counted. Ratios of specific radioactivity were verified by debromination to cholesterol (Schwenk and Werthessen, 1952) and recounting. The results are presented in Table I.

TABLE I

EFFECT OF AY-9944 ON THE CONVERSION OF 7-DEHYDROCHOLESTEROL
TO CHOLESTEROL BY RAT LIVER HOMOGENATES

Each flask contained: 4 ml. of homogenate, 0.65 μ mole of 4-C^{14} -7-dehydrocholesterol (0.37 μ c), 2 μ mole of NADP, 5 μ mole of ATP and 4 μ mole of glucose-6-phosphate. Incubation at 37° for 1 hour.

	Nonsap.	DBC ^(a)	Cholesterol
Control	103,367 ^(b)	79,625	86,640
AY-9944, 1×10^{-6} M (c)	106,710	16,004 (-80%) ^(d)	17,185 (-80%)
AY-9944, 3×10^{-7} M	98,788	74,918 (-6%)	79,440 (-8%)

(a) Recrystallized 5 times from ethyl acetate/methanol.

(b) Counts/min. per incubation.

(c) Final concentration.

(d) Per cent inhibition.

Summary: Based on incorporation studies we have shown recently that in vitro, AY-9944 inhibited hepatic cholesterogenesis at a stage between lanosterol and cholesterol without affecting the enzyme(s) involved in the reduction of the Δ^{24} -bond. The finding that AY-9944 prevents the conversion of 4-C^{14} -7-dehydrocholesterol to cholesterol by rat liver homogenates is in agreement with our earlier observation that the compound inhibited the metabolism of 7-dehydrocholesterol in rat liver homogenates. The evidence thus demonstrates that AY-9944 interferes with the synthesis of cholesterol in rat liver homogenates by inhibiting the enzymatic transformation of 7-dehydrocholesterol to cholesterol. This is in accordance with the finding of 7-dehydrocholesterol in livers (Dvornik et al., 1963) and serum (Chappel et al., 1963) of rats treated orally with AY-9944.

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