MECHANISM OF ACTION OF MER-29, AN INHIBITOR OF CHOLESTEROL BIOSYNTHESIS *

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Received December 28, 1959

MacKenzie and Blohm (1959) have shown that administration of MER-29 (1-[p-(β-diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol) to rats leads to a marked depression of the rate of cholesterol biosynthesis and to a decrease in serum and tissue levels of cholesterol. Their studies, confirmed in this laboratory, showed that the incorporation of C^{14}-acetate radioactivity into the total nonsaponifiable lipid fraction and into the digitonin-precipitable fraction of the liver in rats receiving MER-29 was comparable to that observed in control rats. However, when the cholesterol liberated from the digitonide was purified through the dibromide, it was found that actually very little of the incorporated radioactivity was in cholesterol itself, most of the radioactivity remaining in the supernatant solution.

The present studies show that there is an accumulation of 24-dehydrocholesterol (desmosterol) in the liver and in the serum of rats receiving MER-29 in the diet. This sterol has been postulated as an intermediate in cholesterol biosynthesis and it has recently been shown by Stokes and coworkers (1958) that labeled 24-dehydrocholesterol injected into rats is rapidly converted to cholesterol. Our tracer studies in rats fed MER-29 are compatible with a precursor-product relationship between 24-dehydro-

*Presented at Conference on MER-29, Princeton, New Jersey, December 16, 1959.

cholesterol and cholesterol. It appears that the principal site of action of MER-29 is at the very last step in cholesterol biosynthesis, that is, in the reduction of the side chain double bond of 24-dehydrocholesterol.

The presence of significant amounts of an unusual sterol in the livers of animals treated with MER-29 (0.1% in the diet for 14 days) was first indicated by the fact that the digitonin-precipitable sterol fraction yielded very little or no insoluble dibromide even though the total sterol level was not markedly depressed. Addition of sterol from the livers of drug-treated animals to an equal amount of sterol from normal rat livers reduced the yield of insoluble dibromide from the latter, suggesting the presence of a new component that interfered with cholesterol dibromide precipitation. Initial efforts to separate pure cholesterol from the mixture by fractional crystallization and by silicic acid chromatography were unsuccessful. After recrystallization from methanol the sterol mixture melted at 128-132°; $[\alpha]_D^{20}$, -39° (standard cholesterol: M.P. 148°, $[\alpha]_D^{20}$, -40°). The infrared spectrum of the mixture was very similar to that of cholesterol but differed in that it showed a shoulder at 820 cm^{-1}, a doubling of the peak at 950 cm^{-1} and less of a shoulder at 1360 cm^{-1}. In these respects it resembled the spectrum of 24-dehydrocholesterol as published by Stokes and coworkers (1958). On catalytic hydrogenation this sterol mixture took up 1.5 moles of hydrogen per mole of sterol, calculated on the molecular weight of cholesterol.

Resolution of the sterol mixture was accomplished by preparing the p-phenylazobenzoyl esters and chromatographing them on silicic acid-Celite as described by Idler and Baumann (1952). This yielded two major components which were eluted and saponified. The component eluted first was identified as cholesterol (mixed melting point, optical rotation and infrared spectrum). The second component, representing approximately 50% of the mixture, was identified as 24-dehydrocholesterol by comparison with an authentic sample that was generously provided by Dr. U. H. M. Fagerlund. The melting point of the

free sterol (119-120°) was not depressed on mixing it with authentic 24-dehydrocholesterol. Its infrared spectrum was identical with that of 24-dehydrocholesterol. Acetate and benzoate esters were prepared and these were identical in melting point and infrared spectrum with the respective derivatives of 24-dehydrocholesterol. The new sterol was brominated in chloroform solution and the recrystallized product contained 43.5% bromine (theoretical value for tetrabromocholestanol, 45.4%). Finally, a rat treated with MER-29 was given 35 μc of 1-C^{14}-acetate intraperitoneally and 4 hours later the liver sterols were isolated and fractionated as described above. The specific radioactivity of the 24-dehydrocholesterol was 44 times the specific radioactivity of the cholesterol, ruling out the possibility that the 24-dehydrocholesterol is derived from cholesterol.

We therefore conclude that the major block in cholesterol biosynthesis caused by MER-29 is in the conversion of 24-dehydrocholesterol to cholesterol. The accumulation of 24-dehydrocholesterol in the treated animals and the ratio of the specific radioactivities of the sterols synthesized in vivo provides important additional evidence that 24-dehydrocholesterol is in fact an intermediate in cholesterol biosynthesis. The new sterol is present in significant amounts not only in the liver but also in the serum of drug-treated animals and patients (Steinberg, Avigan and Feigelson,1959). For this reason use of MER-29 in patients should be undertaken with caution until more information becomes available concerning the biological properties of 24-dehydrocholesterol.

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