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ABSTRACT: — The purpose of this paper is to summarize the results of my research on the enzymic steps of cholesterol biosynthesis (Dempsey, 1962; 1964; 1965; Dempsey, Seaton, and Trockman, 1963; Dempsey, Seaton, Sanford, and Trockman, 1964a; Dempsey, Seaton, Schroepfer, and Trockman, 1964b.[2] These data were obtained by use of an enzyme system isolated from rat liver homogenates. The findings have elucidated the pathways of and cofactor requirements for cholesterol synthesis from naturally occurring sterol precursors.

Methodology

The enzyme system was obtained by differential centrifugation of rat liver homogenates prepared at 0-4° in 0.1 M phosphate buffer, pH 7.4. For some experiments, the enzyme system was treated with Sephadex G-25 to remove loosely bound cofactors of low molecular weight. Substrates were synthetic or biosynthetic carbon-14 or tritium-labeled sterols, purified and characterized by physical and biochemical techniques. Some of the methods of assay for enzyme activity were, silicic acid column chromatography to separate and identify sterol intermediates (Frantz, 1963); gas–liquid chromatography to separate and identify the methyl ether derivatives of sterols (Clayton, 1962); the dibromide technique to measure cholesterol biosynthesis (Seaton, 1963); ultraviolet spectral analysis and epiperoxide derivative formation to detect $\Delta^{5,7}$–intermediate sterols (Dempsey, et al., 1964b; Dempsey, 1965).

Summary of Results

The Intermediary Role of $\Delta^{5,7}$–Cholestadien–3β–ol in Cholesterol Biosynthesis. The data presented in Table I show that a liver enzyme system capable of converting Δ^{7}–cholestenol and $\Delta^{5,7}$–cholestadienol to cholesterol is present in cell particles sedimenting at 105,000 × g and that the enzyme system is activated by material present in the 105,000 × g supernatant fraction (designated A in Table 1). In the absence of the high–speed supernatant fraction the enzyme system is slightly active. The high–speed supernatant fraction, individually, is also inactive. By use of a preparation containing a combination of the cell particles and the supernatant fraction, results were obtained showing that cholesterol biosynthesis from Δ^{7}–cholestenol is by way of $\Delta^{5,7}$–cholestadienol (steps 7 and 8, Fig. 1) as an intermediate (Dempsey, et al., 1963; 1964b). Oxygen was found to be required for conversion of Δ^{7}–cholestenol to $\Delta^{5,7}$–cholestadienol (step 7, Fig. 1), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) for conversion of $\Delta^{5,7}$–cholestadienol to cholesterol (step 8, Fig. 1).

Pathways of Enzymic Synthesis and Conversion to Cholesterol of $\Delta^{5,7,24}$– Cholestatrien–3β–ol and Other Naturally–Occurring Sterols. Results obtained using tritium-labeled $\Delta^{7,24}$–cholestadienol, isolated from rat liver, and $\Delta^{8,24}$–cholestadienol, isolated from yeast, as substrates for the rat liver enzyme system showed that $\Delta^{5,7,24}$–cholestatrienol is formed from $\Delta^{7,24}$–cholestadienol by an enzymic reaction requiring oxygen (step 2, Fig. 1). The $\Delta^{5,7,24}$–trienol is converted to cholesterol by enzymic reduction of the Δ^{7}– and Δ^{24}–bonds, reactions requiring NADPH (steps 3 and 4 or 10 and 8, Fig. 1). Enzymic synthesis of cholesterol from Δ^{7},^{24}–cholestadienol can also occur by a pathway involving Δ^{7}–cholestenol and $\Delta^{5,7}$–cholestadienol as intermediate and requireing oxygen and NADPH as cofactors (steps 9, 7, and 8, Fig. 1). The isomerization of $\Delta^{8,24}$–cholestadienol to $\Delta^{7,24}$–cholestadienol by the enzyme system (step 1, Fig. 1) does not require oxygen or added cofactors. In the presence of oxygen, enzymic synthesis of $\Delta^{5,7,24}$–cholestatrienol from $\Delta^{8,24}$–cholestadienol (steps 1 and 2, Fig. 1) occurs. Some of these results were obtained using MER–29, an inhibitor of Δ^{24}– and Δ^{7}–reductase activity, and AY–9944, an inhibitor of Δ^{7}–reductase activity.

These data offer evidence that enzymic conversion to cholesterol of naturally occurring sterols containing 27 carbon atoms can take place by apparently irreversible pathways – one in which the Δ^{24}–bond is preserved until

TABLE I. *Enzymic Cholesterol Synthesis by Liver Cell Fractions*
Incubations of Δ5,7–cholestadienol–4–^{14}C (9.2×10^4 c.p.m. per μmole) or Δ7–cholestenol–4–^{14}C (9.4×10^4 c.p.m. per μmole) with rat liver cell fractions (prepared in 0.1 M phosphate buffer, pH 7.4) were carried out for 1 hour at 37° in the presence of 0.5 mM NADPH and under oxygen. Cholesterol–4–^{14}C synthesis was assayed by the dibromide technique (Seaton, 1963).

Cell Fractions	Conversion to Cholesterol μM/mg Particle Protein	
	$\Delta^{5,7}$–Cholestadienol	Δ^{7}–Cholestenol
105,000 × g Sediment	0.4	0.1
105,000 × g Sediment + A	1.7	1.4
30,000 × g Sediment* + A	2.2	1.5
30,000 × g Supernatant* + A	1.8	0.3

A = The slower sedimenting portion (upper half) of the 105,000 × g supernatant fraction.
* Obtained by refractionation of the 105,000 × g sediment.

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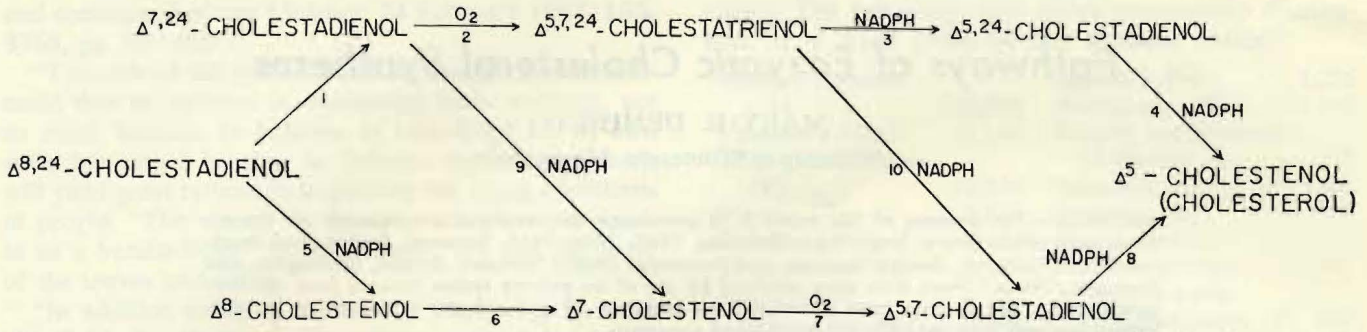

FIG. 1. Pathways of enzymic cholesterol synthesis involving naturally occurring 3,8-hydroxy sterols containing 27 carbon atoms. This scheme depicts the known cofactors and catalytic steps consistent with the results of this paper and previous findings (see references by Dempsey and Dempsey et al.). Reaction steps 4, 5, 9, and 10 are catalyzed by a $_{\Delta}{}^{24}$–reductase and inhibited by MER-29; steps 3 and 8 by a $_{\Delta}{}^{7}$-reductase and inhibited by MER-29 and AY-9944; steps 2 and 7 by a $_{\Delta}{}^{5}$–dehydrogenase; and steps 1 and 6 by a $_{\Delta}{}^{7-8}$–isomerase.

the final reduction yielding cholesterol (step 4, Fig. 1) and by others in which the $_{\Delta}{}^{24}$–bond is reduced prior to (step 5, Fig. 1) or concurrent with (steps 9 and 10, Fig. 1) reactions occurring in ring B. These results also show that $_{\Delta}{}^{5,7}$–intermediate sterols are essential components of all biosynthetic pathways leading to cholesterol.

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Pollution

The increasing problems of pollution of soil, water, and food products were investigated in two papers in this issue. The first, by Arthur F. Novak and M. R. Ramachandra Rao of Louisiana State University, is a report of endrin monitoring in the lower Mississippi River (reprinted from *Science*). The second, but only in terms of listing, is a report of pesticide residues in soil by Russell S. Adams, Jr., of the University of Minnesota, St. Paul.

In order to keep readers of the *Journal* informed of current research in the problems of pollution that concern the Northwest, investigators of such problems are urged to send brief descriptions of their research in progress or completed reports of the research to the Editor, Mrs. Sylvia W. Rosen, 2204 North Lexington Ave., St. Paul, Minn. 55113.