Cholesterol Biosynthesis: Mevalonate Synthesis Inhibited by Bile Salts

Abstract. *Certain bile salts inhibit biosynthesis of mevalonate from acetate-2-C^{14} in normal rat liver homogenates. The physiological control of cholesterol biosynthesis is discussed.*

Dietary cholesterol inhibits synthesis of cholesterol *de novo* from acetate in human liver and rat liver preparations (*1*). The chief site of this "feedback" regulation is the mevalonate—NADP oxidoreductase reaction (acylating CoA) (*2*) which results in the formation of mevalonate (*3*). Control of synthesis of cholesterol therefore depends on control of mevalonate synthesis. Since cholesterol synthesis has not been inhibited by addition of cholesterol to liver slices or homogenates of normal rat liver (*4*), the actual regulatory molecule apparently is not cholesterol itself but some compound more or less directly derived from cholesterol. We now offer evidence that bile salts may be the cholesterol metabolites responsible for control of cholesterol synthesis.

During attempts to increase the solubility of mevalonate oxidoreductase from rat liver by treatment with bile salts we observed marked inhibition of mevalonate synthesis from acetate in treated preparations. This prompted us to consider whether bile salts might play a role as physiological regulators of mevalonate, and hence of cholesterol biosynthesis. Addition of bile salts to rat liver homogenates indeed strongly inhibits mevalonate synthesis from acetate (Table 1). Furthermore, these compounds inhibit at concentrations ($2 \times 10^{-4} M$) which may approximate physiological concentrations.

The mechanism by which bile salts are inhibitory may be the disruption of the particulate mevalonate oxidoreductase of liver. However, the failure of another detergent, Triton X-100, to inhibit is an argument against this view. A second possibility is that bile salts may inhibit by simple substrate-analog competition. Studies with a soluble but otherwise analogous mevalonate oxidoreductase from bacteria (*5*) provide indirect evidence in support of this interpretation. In two bacterial systems tested, mevalonate oxidoreductase assayed in the direction of mevalonate oxidation is competitively inhibited by cholate or deoxycholate (*6*).

These results suggest that bile salts may be the cholesterol metabolites responsible for physiological regulation of cholesterol synthesis. A similar suggestion, based on nutritional studies, was made by Bloomfield (*7*). Consistent with this view are observations that cholesterol synthesis from acetate is depressed by administration not only of cholesterol but also of bile salts (*8*), and that it is increased in animals with a bile fistula which removes bile salts from the body (*9*).

Table 1. Inhibition by bile salts of biosynthesis of mevalonate from acetate-2-C^{14}.*

Addition	Final concn. (m*M*)	Inhibition (%)
Cholesterol	2.5	0–15
Cholate	2.5	95
Cholate	0.25	38
Deoxycholate	2.5	98
Deoxycholate	0.25	97
Deoxycholate	0.12	23
Taurocholate	2.5	88
Taurocholate	0.25	11
Taurodeoxycholate	2.5	97
Taurodeoxycholate	0.25	89
Taurine	5.0	4
Triton X-100	40 μg/ml	0

* The homogenate (*10*) of livers from Long-Evans rats fed on a stock diet was centrifuged at 5000*g*. In control tubes, the supernatant (a portion containing 50 mg of protein) was incubated (2 hours at 37°C) with 260 μmole of potassium phosphate buffer (*p*H 7.0), 12.5 μmole of glucose-1-phosphate, 1.1 μmole of NADP, 6.0 μmole of acetate-2-C^{14} (specific activity, 4.3×10^5 count/min per μmole), and 10 μmole of carrier mevalonate. Experimental tubes contained also the additions listed above. The reaction was stopped by heating at 80°C for 3 minutes. Precipitated protein was removed by centrifugation, washed with 1.0 ml of water, and discarded. The supernatant and washing water were treated (*11*) to convert mevalonate to mevalonalactone which was isolated by chromatography on a column of Celite (*12*). The isolated material was homogeneous on paper chromatography in a mixture of ethanol, 15*N* NH_3, and H_2O (8:1:1) and migrated in the manner of an authentic sample. Radioactivity was determined with a Packard Tri-Carb scintillation counter. The results represent selected values from several experiments. In control tubes, incorporations ranged from 5000 to 20,000 count/min in several trials.

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References and Notes

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2. Abbreviations: NADP, nicotinamide adenine dinucleotide phosphate; CoA, coenzyme A.
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α-D-Glucose: Precise Determination of Crystal and Molecular Structure by Neutron-Diffraction Analysis

Abstract. *This analysis provides the first precise crystal and molecular structural parameters for α-D-glucose. The C—C, C—H, and O—H bond lengths deviate only slightly from their means of 1.523 Å, 1.098 Å, and 0.968 Å. The C(1)—O(1) bond length, 1.389 Å, is significantly shorter than the mean value, 1.420 Å, of the other C—O bonds. The valence angle of the ring oxygen is 113.8 degrees.*

From three-dimensional neutron-diffraction data, we have determined precise crystal and molecular structural parameters for α-D-glucose ($C_6H_{12}O_6$), by least-squares refinement starting with the approximate parameters of McDonald and Beevers (*1*). The general description of the carbon-oxygen skeleton of glucose (chair conformation, with

Table 1. Fractional coordinates *x*, *y*, and *z* of atoms in the asymmetric unit of the α-D-glucose unit cell. Each coordinate is followed by its least-squares standard error in parentheses.

Atom	$10^4 x$	$10^4 y$	$10^4 z$
C(1)	3329(1)	6007(1)	−762(3)
C(2)	4184(1)	6746(1)	455(3)
C(3)	5611(1)	6514(1)	154(3)
C(4)	5860(1)	5569(1)	1200(3)
C(5)	4956(1)	4886(1)	−123(3)
C(6)	5141(1)	3953(1)	1026(4)
O(1)	3470(2)	6007(1)	−3541(4)
O(2)	3880(1)	7598(1)	−657(5)
O(3)	6334(1)	7154(1)	1634(5)
O(4)	7176(1)	5348(1)	688(4)
O(5)	3654(1)	5154(1)	369(4)
O(6)	4458(2)	3311(1)	−518(5)
H(1)	2327(2)	6124(2)	−154(9)
H(2)	3958(3)	6776(2)	2599(7)
H(3)	5888(3)	6543(2)	−1995(7)
H(4)	5677(3)	5565(2)	3385(6)
H(5)	5136(3)	4865(2)	−2312(7)
H(6)	6172(3)	3792(2)	1032(12)
H(7)	4804(5)	3947(3)	3116(9)
H(8)	2753(4)	5674(3)	−4299(9)
H(9)	4485(3)	7776(2)	−2031(9)
H(10)	7247(2)	7137(2)	1178(8)
H(11)	7517(3)	5082(3)	2326(8)
H(12)	4127(3)	2861(2)	699(10)