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

1. R. C. Gould, *Amer. J. Med.* **11**, 209 (1951); E. P. M. Bhattathiry and M. D. Siperstein, *J. Clin. Invest.* **42**, 1613 (1963).
2. Abbreviations: NADP, nicotinamide adenine dinucleotide phosphate; CoA, coenzyme A.
3. M. D. Siperstein and V. M. Fagan, *Advan. Enzyme Regulation* **2**, 249 (1964).
4. M. D. Siperstein, personal communication.
5. M. Siddiqi and V. W. Rodwell, *Biochem. Biophys. Res. Commun.* **8**, 110 (1962).
6. G. M. Fimognari, thesis (1964).
7. D. K. Bloomfield, *Proc. Natl. Acad. Sci. U.S.* **50**, 117 (1963).
8. A. Pihl, *Acta Chem. Scand.* **34**, 206 (1955).
9. N. B. Myant and H. A. Eder, *J. Lipid Res.* **2**, 363 (1961).
10. N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.* **221**, 1 (1956).
11. F. Lynen and M. Grassl, *J. Physiol. Chem.* **313**, 291 (1959).
12. I. F. Durr and H. Rudney, *J. Biol. Chem.* **235**, 2572 (1960).
13. Supported by grants from the PHS (GM-06468) and from the University of California.

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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)

the C(1) hydroxyl group axial and the other substituent groups equatorial) is not altered by the refinement; nor was it expected to be altered. There are, however, significant changes in details of the skeletal structure. Furthermore, the structure determination is completed by the precise location of the hydrogen atoms. Because of the extraordinary biochemical importance of α-D-glucose, we wish to present the atomic coordinates and the most important derived structural parameters without detailed comment at this time.

Our determination is based on intensity data of 1619 Bragg reflections from a crystal specimen (*2*) weighing 31.7 mg. The raw data from our automatic neutron diffractometer (*3*) were reduced by standard procedures (*4*) to a set of values $|F_o(hkl)|^2$ and $w(hkl)$, where $|F_o(hkl)|^2$ is the observed value of the square of the magnitude of the structure factor for reflection *hkl*, and $w(hkl)$ is the weight of the observation. Absorption corrections were applied. In the least-squares refinement (*5*), the sum

$$S = \sum_{h,k,l} w(hkl)\ [|F_o(hkl)|^2 - |F_c(hkl)|^2]^2$$

was minimized by adjustment of an overall scale factor on the calculated structure-factor magnitudes $|F_c(hkl)|$ and of three coordinates and six anisotropic thermal parameters for each atom. The discrepancy index

$$R = \sum_{h,k,l} ||F_o(hkl)|^x - |F_c(hkl)|^x| / \sum |F_o(hkl)|^x$$

is 0.060 when the exponent x is 1 and 0.059 when x is 2. A better indicator of the quality of the fit of the model to the data is the value 1.10 for the standard deviation of fit (*6*), which is close to the unit value expected at convergence for the correct model when the observational errors are normally distributed and correctly estimated.

The spatial configuration in molecule and crystal is specified completely by the atomic coordinates (Table 1) of the asymmetric unit molecule (*7*), in conjunction with the space-group symmetry and cell parameters determined by McDonald and Beevers (orthorhombic, $P2_12_12_1$, $a = 10.36 \pm 0.02$ Å, $b = 14.84 \pm 0.02$ Å, $c = 4.97 \pm 0.02$ Å, 4 molecules per cell).

All bond lengths and a selection of bond angles are shown in Fig. 1 (*8*). The six conformation angles (*9*) about the pyranose ring bonds are: C(1) → C(2), 54.1°; C(2) → C(3), −51.3°; C(3) → C(4), 53.3°; C(4) → C(5), −57.5°; C(5) → O(5), 62.2°; O(5) → C(1), −60.9°. The computed standard errors of these molecular parameters, including contributions from errors in the unit-cell parameters (*10*), are as follows: C−C and C−O bonds, about 0.003 Å (except 0.006 Å for C(1)−O(1)); C−H and O−H bonds, 0.004 Å to 0.006 Å; bond angles involving only carbon and oxygen atoms, 0.1° to 0.2°; C−O−H angles, 0.2° to 0.4°; conformation angles, 0.2° to 0.3°. The thermal parameters have not yet been interpreted, and no corrections for thermal motion have been applied to any bond lengths or angles.

The hydrogen-bonding pattern established by McDonald and Beevers (*1*) is specified further by the precise location of the hydrogen atoms. The distances between the oxygen atoms and the angles at the hydrogen atoms in the five different hydrogen bonds are as follows: hydrogen bond O(1)−H(8) . . . O(5), 2.847 Å, 161.0°; O(2)−H(9) . . . O(6), 2.776 Å, 170.1°; O(3)−H(10) . . . O(2), 2.707 Å, 164.8°; O(4)−H(11) . . . O(4), 2.773 Å, 167.7°; O(6)−H(12) . . . O(3), 2.711 Å, 169.7°. The standard errors are 0.004 Å to 0.009 Å for the distances and 0.3° to 0.4° for the angles.

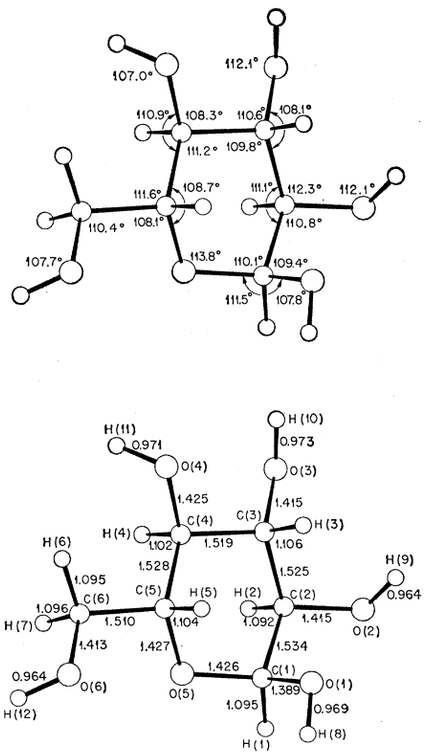

Fig. 1. Bond angles (top) and bond lengths (bottom) in the α-D-glucose molecule. The molecule is represented to scale as it is found in the crystal.

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References and Notes

1. T. R. R. McDonald and C. A. Beevers, *Acta Cryst.* **5**, 654 (1952).
2. We thank Professor A. VanHook of the College of the Holy Cross for growing crystals for us.
3. W. R. Busing, H. G. Smith, S. W. Peterson, H. A. Levy, *J. Phys. (Paris)* **25**, 495 (1964).
4. See for example, G. M. Brown and H. A. Levy, *ibid.*, p. 497.
5. We used various modified versions of "OR FLS, A Fortran Crystallographic Least-Squares Program," W. R. Busing, K. Martin, H. A. Levy, Report No. TM-305, Oak Ridge National Laboratory, 1962.
6. This quantity is given by the expression $[S/(n - p)]^{1/2}$, where n is the number of observations and p is the number of parameters adjusted.
7. Our coordinates when referred to the right-handed axis system of the crystal describe the molecule in correct absolute configuration. The McDonald and Beevers coordinates describe the optical antipode. The absolute configuration is known from chemical work relating α-D-glucose to D-tartaric acid, the absolute configuration of which was established by application of the x-ray anomalous scattering effect by A. F. Peerdemann, A. J. van Bommel, J. M. Bijvoet, *Koninkl. Ned. Akad. Wetenschap. Proc. Ser. B* **54**, 16 (1951).
8. The drawings, except for the lettering, were executed automatically through use of an *X–Y* plotter program of our co-worker Dr. C. K. Johnson (see Abstracts of Annual Meeting of American Crystallographic Association, Bozeman, Montana, 1964, p. 22).
9. For definition see G. M. Brown and H. A. Levy, *Science* **141**, 921 (1963).
10. Standard errors computed without the contributions from the errors of the unit-cell parameters are generally smaller by 30 to 50 percent. These are the errors which are relevant in considering parameter shifts between our structure and that of McDonald and Beevers.

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Histoplasma Capsulatum from the Liver of a Bat in Colombia

Abstract. *Of 135 bats (eight species), one of the species* Glossophaga sorcina sorcina, *a nectar-feeding bat from Girardot, Colombia, harbored* Histoplasma capsulatum. *The microorganism was cultured from the liver. This is the second report of recovery of this fungus from bat tissue.*

Histoplasma capsulatum has been isolated from the tissues of various mammals (*1*); Shacklette *et al.* (*2*) were the first to isolate it from the