Sterol 27-hydroxylase: high levels of activity in vascular endothelium

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Abstract Sterol 27-hydroxylase activity in bovine aortic endothelial (BAE) cells in culture has been compared with that in HepG2 cells and in Chinese hamster ovary (CHO) cells using identical culture conditions. The total enzyme activity of BAE cells (3.0 nmol/72 h per mg cell protein) was comparable with that of HepG2 cells (4.0 nmol/72 h per mg protein) and both values were significantly greater than that in CHO cells (0.002 nmol/72 h per mg protein). The enzyme was identified in the mitochondria extracted from BAE cells by Western blotting using an antibody of proven specificity, and its metabolites 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid were identified by mass spectrum analysis. The presence of the enzyme in endothelium provides a mechanism for preventing accumulation of intracellular cholesterol by initiating a pathway of bile acid synthesis different from that initiated by 7α-hydroxylation of cholesterol in the liver.—**Reiss, A. B., K. O. Martin, N. B. Javitt, D. W. Martin, E. A. Grossi, and A. C. Galloway.** Sterol 27-hydroxylase: high levels of activity in vascular endothelium. *J. Lipid Res.* 1994. **35:** 1026–1030.

Supplementary key words bovine aortic endothelial cells • HepG2 cells • CHO cells • 27-hydroxycholesterol • 3β-hydroxy-5-cholestenoic acid

Sterol 27-hydroxylase is a mitochondrial P450 enzyme that stereospecifically catalyzes the hydroxylation of one of the two terminal methyl groups of both endogenously occurring and synthetic C_{27} sterols (1). Because its biological role was thought to be confined only to the oxidation of 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol, intermediates in bile acid synthesis that are formed in liver microsomes, these substrates are usually used to estimate its activity. However, the presence of the enzyme in kidney (2) and in cultured fibroblasts (3) and the finding that an mRNA for its synthesis is present in many tissues (4) implies a broader biological role.

Because of our interest in an independently regulated metabolic pathway of bile acid synthesis beginning with the oxidation of cholesterol to 27-hydroxycholesterol (5), a compound with many potent biological properties that relate to both cholesterol synthesis and smooth muscle cell proliferation (6), we have been evaluating the level of 27-hydroxylase activity in different tissues. To our surprise, we found that sterol 27-hydroxylase activity in bovine aortic endothelial cells (BAE) is comparable with that present in liver-derived HepG2 cells.

METHODS

Cell culture

Bovine aortic endothelial (BAE) cells (7) obtained from a slaughterhouse (gift from Dr. D. Rifkin) were plated at low density (2×10^5 cells/cm^2) in 100-mm dishes and were grown to confluence in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 50 units/ml penicillin, and 50 units/ml streptomycin at 37°C in a 5% CO_2 atmosphere. The confluent monolayer was rinsed once with Hank's balanced salt solution (HBSS, Gibco, Grand Island, NY), and 4 ml DMEM containing 10% delipidated FBS and either 20 μM cholesterol dissolved in 2-hydroxypropyl-β-cyclodextrin (8) or an equivalent amount of vehicle alone was added to each dish. HepG2 cells and Chinese hamster ovary cells (CHO) were cultured under identical conditions and for the same length of time. The media and cells were harvested at 24-h intervals. The medium obtained from each dish was analyzed for metabolites. Cells were pooled for immunoblot analysis.

Mitochondrial preparation

The method is as described (9) with some minor modifications. Briefly, 12 100-mm dishes of BAE cells and

Abbreviations: BAE, bovine aortic endothelial cells; CHO, Chinese hamster ovary cells; DMEM, Dulbecco's modified Eagle's medium; HBSS, Hank's balanced salt solution; GLC-MS, gas-liquid chromatography-mass spectrometry; TLC, thin-layer chromatography; FBS, fetal bovine serum.

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12 100-mm dishes of HepG2 cells were grown to confluence in DMEM containing 10% FBS. All cells were harvested by scraping with a rubber policeman; then cells of the same type were pooled and washed with HBSS. Cells were then rinsed twice with NKM buffer (0.13 M NaCl, 5 mM KCl, 1 mM $MgCl_2$), resuspended in 6 ml of Tris buffer (0.01 M Tris, pH 6.7, 0.01 M KCl, 0.15 mM $MgCl_2$, 0.25 M sucrose), and broken using a Parr bomb.

The resulting homogenates were centrifuged at 1200 *g* for 3 min. The supernates were collected and centrifuged at 5000 *g* for 10 min to precipitate mitochondria. The mitochondrial pellets were resuspended in 3 ml of a solution of 0.25 M sucrose, 0.01 M Tris acetate, pH 6.7, and 0.15 mM $MgCl_2$, and the suspension was centrifuged at 1100 *g* for 2 min. The final supernate was spun at 500 *g* for 10 min. The final pellet was resuspended in 1.0 ml of a solution of 0.25 M sucrose and 0.01 M Tris acetate, pH 7.0. Protease inhibitors (phenylmethylsulfonyl fluoride, trasylol, and leupeptin) were added to all buffers at 1:1000 dilution and all centrifugations were done at 4°C. The mitochondrial protein concentration was measured using the BCA Protein Assay Reagent (Pierce, Rockford, IL).

Western blotting

Protein samples were subjected to electrophoresis on a 10% SDS-polyacrylamide gel and were transferred electrophoretically onto a nitrocellulose membrane. The immunoreactive protein was detected using a BCIP/NBT Phosphatase Substrate System (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The first antibody, with known specificity (10), is a rabbit polyclonal antipeptide antibody raised against residues 15–28 of the 27-hydroxylase protein. It was a gift from Dr. D. W. Russell.

GLC–MS analysis

Sample preparation. A 1-ml aliquot of harvested medium was combined with internal standards (500 ng each) of deuterated 27-hydroxycholesterol (11), 3β-hydroxy-5-cholestenoic acid (prepared by Jones oxidation of the 3-monoacetate of the deuterated 27-hydroxysterol), and [^{13}C]3β-hydroxy-5-cholenoic acid (12) and was allowed to equilibrate for 30 min at room temperature. After acidification and extraction into ethyl acetate, the dried residue was saponified. In some studies solvolysis (13) was also done prior to extraction. The dried extract was applied to a Silica gel G thin-layer chromatography (TLC) plate together with authentic standards in parallel lanes; after development (chloroform–acetone 97:3) the standards were visualized by spraying with phosphomolybdic acid and the appropriate areas of the plate were removed for elution of 27-hydroxycholesterol and the C_{27} and C_{24} acids. The diacetate of 27-hydroxycholesterol and the methyl ester acetates of the C_{27} and C_{24} acids were then prepared using dimethoxypropane–HCl for methylation and pyridine–acetic anhydride for acetylation. It was found that complete methylation of the C_{27} acid with dimethoxypropane–HCl took longer than that of the C_{24} acid. Therefore methylation was allowed to proceed at room temperature overnight (approximately 18 h). Formation of a 3-methoxy derivative by this prolonged methylation procedure was not detected.

Isotope ratio mass spectrometry

Using a Hewlett-Packard GLC-MS (Model #5890-5970) and a fused silica column (CP-sil 19 CB, 0.25 mm i.d., 25 m length; Chrompack, Raritan, NJ), the appropriate TLC fractions were injected in the splitless mode with temperatures that began either at 260°C or at 275°C and rose by 1°C/min. The column head pressure was 5 psi.

To quantify 27-hydroxycholesterol the detector was programmed in the simultaneous ion monitoring mode for *m/z* 426 [mol ion diacetate = 486 – 60 (acetate)] and *m/z* 430, and the amount of endogenous 27-hydroxycholesterol was calculated from the respective areas. For the C_{27} acid the ion pair that was used was *m/z* 412 [mol ion methyl ester acetate = 472 – 60 (acetate)] and *m/z* 418, and for the C_{24} acid *mz*/370 [mol ion methyl ester acetate = 430 – 60 (acetate)] and *m/z* 373.

RESULTS

The complete spectra of 27-hydroxycholesterol and of 3β-hydroxy-5-cholestenoic acid isolated from the sterol-

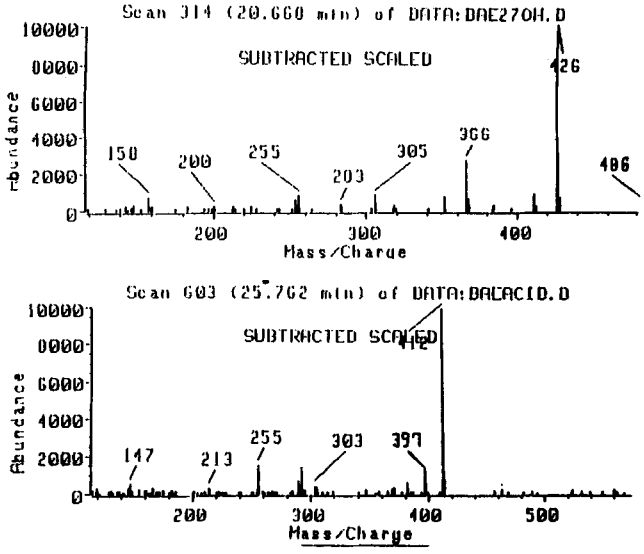

Fig. 1. Mass spectra of metabolic products of sterol 27-hydroxylase activity identified in the medium of BAE cells. The upper panel illustrates the spectrum obtained for 27-hydroxycholesterol as the diacetate (20.6 min): mol ion = *m/z* 486 [402 + (42 × 2)]; *m/z* 426 (486 – 60 acetate); *m/z* 366 [486 – (60 × 2)]. The lower panel illustrates the spectrum obtained for 3β-hydroxy-5-cholestenoic acid as the methyl ester acetate (25.7 min). The molecular ion *m/z* 472 was not obtained, but *m/z* 412 [472 – 60 (acetate)], *m/z* 397 [412 – 15 (methyl)], and *m/z* 255 (steroid nucleus) are prominent characteristic peaks (ref. 16).

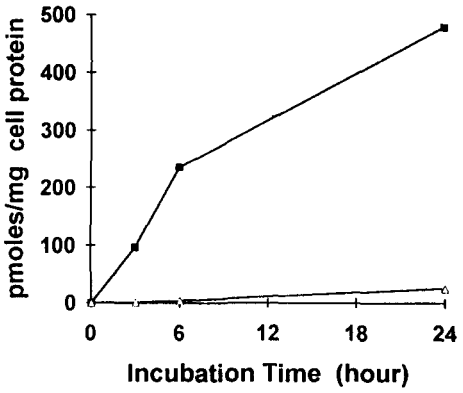

Fig. 2. Synthesis of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid by BAE cells during the initial 24-h period. The initial oxidation product of mitochondrial sterol 27-hydroxylase is the C_{27} alcohol (squares), which is further oxidized to the C_{27} acid (open triangles).

free medium that was in contact with BAE cells for 72 h are shown in **Fig. 1.** Both the spectra and the retention times are identical to those of authentic standards of the diacetate and methyl ester acetate derivatives, respectively. After the identity of these compounds was established by complete spectrum analysis (Fig. 1), an isotope ratio program was used to compare their rates of synthesis in sterol-free and cholesterol-supplemented medium.

Fig. 2 indicates the rates of synthesis of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid by BAE cells during the initial 24-h period. Initially, the synthesis rate of 27-hydroxycholesterol is much greater than its metabolite, 3β-hydroxy-5-cholestenoic acid. Throughout the 72-h period of study, the acid metabolite continued to accumulate in the medium, indicating continuing sterol-27-hydroxylase activity (**Table 1**). However, in medium supplemented with 20 μM cholesterol, which induced a significantly higher rate of production of 27-hydroxycholesterol, the amount of this metabolite in the medium became constant after 24 h.

By 72 h the total amount of metabolite in 5.0 ml of medium was 6200 pmol [(1029 + 211) × 5], which represented 6.2% of the cholesterol added to the medium (6.2 × 100 ÷ 20 × 5).

The activity of sterol 27-hydroxylase in BAE cells was compared with that in HepG2 and CHO cells using the cholesterol-supplemented medium. As shown in **Table 2,** the amount of 27-hydroxycholesterol in the medium collected from BAE cells at 72 h was greater than that from HepG2 cells. The amount present in medium from CHO cells was below our limit of detection (10 ng per assay). No 27-hydroxycholesterol could be detected in medium that was incubated in dishes for 72 h but without a monolayer of cells, further supporting the knowledge that it does not arise by auto-oxidation (14).

The medium from CHO cells always contained a small amount of 3β-hydroxy-5-cholestenoic acid, but much less than that found in the medium from HepG2 or BAE cells.

Because HepG2 cells synthesize 3β-hydroxy-5-cholenoic acid from 27-hydroxycholesterol (15), the medium from all the cell lines was analyzed before and after solvolysis for both this derivative and 3β-hydroxy-5-cholestenoic acid. No increase in the yield of 3β-hydroxy-5-cholestenoic acid was obtained after solvolysis of media derived from HepG2 or the other cell lines. Medium from HepG2 cells was found to contain 3β-hydroxy-5-cholenoic acid, which increased in amount after solvolysis.

As all the metabolic products are derived from the sterol 27-hydroxylase activity of the cells, the total amounts produced by HepG2 and BAE cells are comparable and are much greater than that from CHO cells.

Addition of 5β-cholestane-3α,7α,12α-triol to the medium inhibited the synthesis of both 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid (**Table 3**). The total amount of metabolites derived from cholesterol decreased from 1178 to 275 pmol/mg cell protein, a 77% decrease. As the molar ratio of the triol with respect to cholesterol in the medium was 0.25, the decrease was greater than expected assuming equal access of both substrates to the catalytic site. GLC–MS analysis of the medium indicated that the triol had been metabolized to the C_{27} tetrol (5β-cholestane-3α,7α,12α-27 tetrol, data not shown).

The presence of the enzyme in mitochondria harvested from BAE cells was detected by using an antibody of known specificity. (**Fig. 3**). The doublet identified in BAE cells (lane # 4) corresponds to that found previously with transfected cells (10) and represents the precursor form of

TABLE 1. Synthesis of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid by BAE cells: time course and effect of cholesterol added to the medium

	27-Hydroxycholesterol			3β-Hydroxy-5-cholestenoic Acid		
Culture Medium	24 h	48 h	72 h	24 h	48 h	72 h
	pmol/ml medium			*pmol/ml medium*		
Delipidated FBS[a] (n = 4)[b]	89 ± 12[c]	98 ± 11	103 ± 9	11.9 ± 1.3	32.0 ± 1.9	78.6 ± 11
+ 20 nmol/ml Cholesterol	1089 ± 111	1064 ± 161	1029 ± 99	42.5 ± 6.1	112 ± 12.4	211 ± 48.3

[a] Delipidated fetal bovine serum.
[b] Number of dishes.
[c] Mean ± SD.

TABLE 2. Comparison of sterol 27-hydroxylase activity in bovine aortic endothelial (BAE), HepG2, and Chinese hamster ovary (CHO) cells

Cells[a]	Metabolites Derived from Sterol 27-Hydroxylase[b]			
	27OH-Chol.	3βOH-5-Cholest.a.	3βOH-5-Cholen.a.	Total
	pmol/mg cell protein			
BAE (n = 6)[c]	2555 ± 348	474 ± 118	not detected	3029
HEPG2 (n = 6)	1622 ± 291	471 ± 126	1940 ± 270[d]	4033
CHO (n = 3)	not detected	2 ± 1	not detected	2

[a] All cells were maintained for 72 h in DMEM enriched with 10% delipidated FBS containing 20 μM cholesterol.
[b] 27OH-Chol., 27-hydroxycholesterol; 3βOH-Cholest.a., 3β-hydroxy-5-cholestenoic acid; 3βOH-Cholen.a., 3β-hydroxy-5-cholenoic acid.
[c] Number of dishes.
[d] Mean value of two dishes after solvolysis.

sterol 27-hydroxylase with attached signal sequence of about 30 amino acids (upper band) together with the mature form of the enzyme, both of which are immunoreactive.

DISCUSSION

The finding that the mRNA for a sterol 27-hydroxylase is present in many tissues (4) has changed the perspective in regard to its biological role. It is known that in the liver the enzyme initiates side-chain oxidation of the 5β-cholestane intermediates in bile acid synthesis (1), and kinetic studies have shown that this pathway of side-chain oxidation accounts for approximately 95% of total bile acid synthesis (16).

Studies of mitochondrial enzyme isolated from liver showed that the turnover rate of 5β-cholesten-3α,7α,12α-triol as a substrate is much higher than that of added cholesterol (1), further supporting the concept that the 5β-cholestane intermediates are the predominant, if not the exclusive, substrate for the enzyme in the liver. Currently it is not possible to obtain sufficient endothelial cells to study the properties of the mitochondrial enzyme in this tissue. However, the finding that the addition of 5β-cholestane-3α,7α,12α-triol to the medium greatly decreases the synthesis of metabolites derived from cholesterol implies that the endothelial enzyme has properties that are similar to the enzyme isolated from liver.

The data that we obtained with BAE cells using cholesterol as a substrate are comparable with those obtained using 5β-cholestane-3α,7α,12α-triol as a substrate in transfected COS cells. Initially, the C_{27}-tetrol increases were followed by an increase in the C_{27} acid, a typical precursor–product relationship. With the transfected COS cells, however, the proportion of the C_{27} acid metabolite was much greater. We attribute this difference to the co-transfection of the COS cells with adrenodoxin reductase, known to enhance mitochondrial P450 oxidations (4).

The recognition that sterol 27-hydroxylase is distributed in many tissues and our finding that its activity in aortic endothelial cells is comparable with that in HepG2 cells make it more likely that this metabolic pathway, beginning in nonhepatic tissues, can make a significant contribution to total bile acid synthesis. In a recent review in this journal, it was speculated that the 27-hydroxycholesterol pathway may account for as much as 50% of bile acid synthesis in humans (17).

The rate-limiting step(s) in bile acid synthesis via initial 27-hydroxylation of cholesterol have not been identified and will probably be difficult to ascertain. Traditionally, the rate-limiting step has often been found to be the initial step. If one compares the value for the activity of the mitochondrial 27-hydroxylase in rat liver of 113 pmol/min per mg of protein (18), which is greater than we observed for mitochondria obtained from hamster liver (unpublished data), with the value of 2398 pmol/min per mg protein for 27-hydroxycholesterol 7α-hydroxylase of hamster liver (5) then it would appear that the initial step is rate-limiting. However, sterol 27-hydroxylase is found in many

TABLE 3. Effect of 5β-cholestane-3α,7α,12α-triol on 27-hydroxycholesterol and 3β-hydroxycholestenoic acid synthesis by bovine aortic endothelial cells

Culture Medium	27OH-Cholesterol	3βOH-5-Cholestenoic Acid	Total
	pmol/mg cell protein		
Delipidated fetal bovine serum			
+ 20 nmol/ml cholesterol	920 ± 124 (3)	258 ± 25 (3)	1178
+ 20 nmol/ml cholesterol + 5 nmol/ml 5β-cholestane-triol	243 ± 21 (3)	33 ± 1.0 (3)	275

Values given as mean ± SD; number of dishes in parentheses.

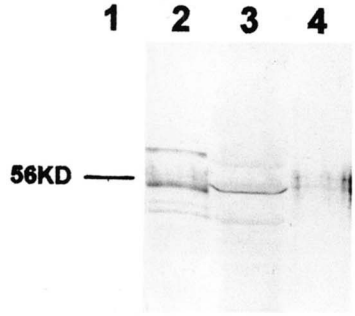

Fig. 3. Western blot analysis of mitochondrial proteins from BAE cells and HepG2 cells. Lane 1: molecular weight standard; lane 2: partially purified sterol 27-hydroxylase protein (gift from Dr. N. G. Avadhani); lane 3: 50 μg of mitochondrial protein isolated from HepG2 cells; lane 4: 15 μg of mitochondrial protein isolated from BAE cells. Immunodetection was carried out using a 1:2000 dilution of a rabbit polyclonal anti-peptide antibody specific for the mitochondrial sterol 27-hydroxylase.

tissues and we do not know either the total mass of tissue in which it is expressed or the activities in each tissue. Therefore, comparison of relative activities in liver are of limited value.

In addition, as shown in these studies, two products are formed in nonhepatic tissues, 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid, both of which normally circulate in plasma (19, 20). Although the C_{27} acid is metabolized in vivo mostly to chenodeoxycholic acid (21, 22), the 7α-hydroxylase enzyme catalyzing this pathway has not been ascertained. Thus, though uncertainty as to the rate-limiting steps exists, this pathway merits full evaluation since potentially it could be used for enhancing reverse cholesterol transport and for increasing the proportion of chenodeoxycholic acid in the bile acid pool.

The authors are deeply grateful to Dr. David W. Russell for providing the cDNA for both the rabbit and the human sterol 27-hydroxylase and for his interest and many helpful suggestions. We thank Mrs. Suzanne Javitt for her editorial assistance in preparing this manuscript. This work was supported by Grant RO1-DK32995 from the National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health.

Manuscript received 18 August 1993, in revised form 9 December 1993, and in re-revised form 24 January 1994.

REFERENCES

1. Atsuta, Y., and K. Okuda. 1981. On the stereospecificity of cholestanetriol 26-monooxygenase. *J. Biol. Chem.* **256:** 9144–9146.
2. Postlind, H., and K. Wikvall. 1989. Evidence for the formation of 26-hydroxycholesterol by cytochrome P-450 in pig kidney mitochondria. *Biochem. Biophys. Res. Commun.* **159:** 1135–1140.
3. Skrede, S., I. Björkhem, E. A. Kvittingen, M. S. Buchmann, S. O. Lie, C. East, and S. Grundy. 1986. Demonstration of 26-hydroxylation of C_{27}-steroids in human skin fibroblasts and deficiency of this activity in CTX. *J. Clin. Invest.* **78:** 729–735.
4. Andersson, S., D. L. Davis, H. Dahlbäck, H. Jörnvall, and D. W. Russell. 1989. Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile acid biosynthetic enzyme. *J. Biol. Chem.* **264:** 8222–8229.
5. Martin, K., K. Budai, and N. B. Javitt. 1993. Cholesterol and 27-hydroxycholesterol 7α-hydroxylation: evidence for two different enzymes. *J. Lipid Res.* **34:** 581–588.
6. Javitt, N. B. 1990. 26-Hydroxycholesterol: synthesis, metabolism, and biologic activities. *J. Lipid Res.* **31:** 1527–1533.
7. Gospodarowicz, D., J. Moran, D. Braun, and C. Birdwell. 1976. Clonal growth of bovine vascular endothelial cells: fibroblast growth factor as a survival agent. *Proc. Natl. Acad. Sci. USA.* **73:** 4120–4124.
8. DeCaprio, J., J. Yun, and N. B. Javitt. 1992. Bile acid and sterol solubilization in 2-hydroxypropyl-β-cyclodextrin. *J. Lipid Res.* **33:** 441–443.
9. Attardi, G., and E. Ching. 1979. Biogenesis of mitochondrial proteins in Hela cells. *Methods Enzymol.* **56:** 66–79.
10. Cali, J. J., C. Hsieh, U. Francke, and D. W. Russell. 1991. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J. Biol. Chem.* **266:** 7779–7783.
11. Javitt, N. B., E. Kok, J. Lloyd, A. Benscath, and F. H. Field. 1982. Cholest-5-ene-3β,26-diol: synthesis and biomedical use of a deuterated compound. *Biomed. Mass Spectrom.* **9:** 61–63.
12. Kok, E., S. Burstein, N. B. Javitt, M. Gut, and C. Y. Byon. 1981. Bile acid synthesis. Metabolism of 3β-hydroxy-5-cholenoic acid in the hamster. *J. Biol. Chem.* **256:** 6155–6159.
13. Cohen, B. I., K. Budai, and N. B. Javitt. 1981. Solvolysis of chenodeoxycholic acid sulfates. *Steroids.* **37:** 621–626.
14. Kudo, K., G. T. Emmons, E. W. Casserly, D. P. Via, L. C. Smith, J. S. Pyrek, and G. J. Schroepfer, Jr. 1989. Inhibitors of sterol synthesis. Chromatography of acetate derivatives of oxygenated sterols. *J. Lipid Res.* **30:** 1097–1111.
15. Javitt, N. B., and K. Budai. 1989. Cholesterol and bile acid synthesis in HepG2 cells. *Biochem. J.* **262:** 989–992.
16. Duane, W. C., P. A. Pooler, and J. N. Hamilton. 1988. Bile acid synthesis in man: in vivo activity of the 25-hydroxylase pathway. *J. Clin. Invest.* **82:** 82–85.
17. Björkhem, I. 1992. Mechanism of degradation of the steroid side chain in the formation of bile acids. *J. Lipid Res.* **33:** 455–471.
18. Petrack, B., and B. J. Latario. 1993. Synthesis of 27-hydroxycholesterol in rat liver mitochondria: HPLC assay and marked activation by exogenous cholesterol. *J. Lipid Res.* **34:** 643–649.
19. Javitt, N. B., E. Kok, S. Burstein, B. Cohen, and J. Kutscher. 1981. 26-Hydroxycholesterol. Identification and quantitation in human serum. *J. Biol. Chem.* **256:** 12644–12646.
20. Axelson, M., B. Mörk, and J. Sjövall. 1988. Occurrence of 3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid, and 7α-hydroxy-3-oxo-4-cholestenoic acid as normal constituents in human blood. *J. Lipid Res.* **29:** 629–641.
21. Ayaki, Y., E. Kok, and N. B. Javitt. 1989. Cholic acid synthesis from 26-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid in the rabbit. *J. Biol. Chem.* **264:** 3818–3821.
22. Ayaki, Y., E. Kok, and N. B. Javitt. 1990. Metabolism of 3β-hydroxy-5-cholestenoic acid in hamsters. *Experientia.* **46:** 267–269.