review

25R,26-Hydroxycholesterol revisited: synthesis, metabolism, and biologic roles

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Abstract **The *CYP27* gene is expressed in arterial endothelium, macrophages, and other tissues. The gene product generates sterol intermediates that function as ligands for nuclear receptors prior to their transport to the liver for metabolism, mostly to bile acids. Most attention has been given to 27-hydroxycholesterol as a ligand for LXR activated receptors and to chenodeoxycholic acid as a ligand for farnesoid X activated receptors (FXRs). Expression of the pathway in macrophages is essential for normal reverse cholesterol transport. Thus, ABC transporter activity is upregulated, which enhances cholesterol efflux. Absence of these mechanisms probably accounts for the accelerated atherosclerosis that occurs in cerebrotendinous xanthomatosis. Accumulation of 27-hydroxycholesterol in human atheroma is puzzling and may reflect low levels of oxysterol 7α-hydroxylase activity in human macrophages. The same enzyme determines the proportion of mono-, di-, and trihydroxy bile acids synthesized in the liver. Oxysterol 7α-hydroxylase deficiency is a molecular basis for cholestatic liver disease. Chenodeoxycholic acid, the major normal end product, downregulates expression of cholesterol 7α-hydroxylase via the FXR/short heterodimer protein nuclear receptor and thus limits total bile acid production. The challenge is to quantify in a physiologic setting the magnitude of the pathway in different tissues and to further evaluate the biologic roles of all the intermediates that may function as ligands for orphan nuclear receptors or via other regulatory mechanisms.**—Javitt, N. B. **25R,26-Hydroxycholesterol revisited: synthesis, metabolism, and biologic roles.** ***J. Lipid Res.*** **2002.** 43: **665–670.**

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When I last reviewed 27-hydroxycholesterol (known then as 26-hydroxycholesterol) (1), that it would become a "mainstream" player was not yet evident (2). Considering the complexity of the biological roles that 27-hydroxycholesterol subserves, the challenge will be to design studies that permit a valid assessment of when the level of expression of this metabolic pathway becomes critical to individual well-being. The widely variable phenotypic expressions in people born with a mutant CYP27 gene (3) emphasize the need to define the operative environmental conditions that lead to injury when the pathway is not expressed. Before tackling these issues, it is useful to review the history of the discovery of the pathway and what is known of the pathway as expressed in humans and other species.

As far as I have been able to discover, 25R,26-hydroxycholesterol was identified by Fredrickson (4) with the help of Scheer, Thompson, and Mosettig (5) at the National Institutes of Health. It represents a classic example of how cooperation between the biological sciences and physical sciences can bring to fruition observations that otherwise would have remained ill-defined.

Fredrickson incubated radioactive cholesterol with mouse liver mitochondria and obtained some products that were difficult to characterize. He was quite certain based on chromatographic criteria that one of the products was 25-hydroxycholesterol but another had slightly different properties. He suspected that it might be 26-hydroxycholesterol but obtaining complete characterization of the miniscule amount of radioactive metabolite was not possible. Enlisting the interest of a group of synthetic organic chemists led to a chemical synthesis of 25R,26-hydroxycholesterol (5), and thus to providing abundant crystalline material for definitive identification of the radioactive metabolite by reverse isotope dilution and other parameters.

Although no one disputes the correctness of the designation of the compound derived from kryptogenin or diosgenin as 25R,26-hydroxycholesterol based on the rules governing the priority of numbering carbon atoms attached to the pro-chiral C25 carbon (6), some ambiguity exists if the designation is shortened to either 26-hydroxycholesterol or 27-hydroxycholesterol. In medical parlance, the designation 26-hydroxycholesterol was changed to 27-hydroxycholesterol in 1991 (7, 8). With the designation of the gene as *CYP27* it is sensible to refer to the product as 27-hydroxycholesterol. However, Chemical Abstracts continues to index the sterol as 26-hydroxycholesterol when the stereospecificity is not known or as 25R,26- or 25S,26-hydroxycholesterol, respectively, when the stereospecificities are known.

Abbreviations: StAR, steroid acute response.

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Actually, the compound derived from natural sources contains both isomers after crystallization. The two enantiomorphs can be separated by chromatographic procedures (9) into mostly 25R,26-hydroxycholesterol with a small amount of 25S,26-hydroxycholesterol. Chemical synthesis, which essentially consists of attaching a stereospecific side chain to the steroid nucleus, can produce only the naturally occurring 25R-isomer (10).

Fredrickson and Ono (11) went on to show that 25R,26-hydroxycholesterol was metabolized to chenodeoxycholic acid in the rat, an exception to the then current paradigm (12). Danielsson (13) repeated these studies and confirmed the correctness of the precursor/product relationship for chenodeoxycholic acid but excluded 25-hydroxycholesterol as an endogenous metabolite, since its occurrence could be accounted for entirely by auto-oxidation.

Using the same approach to the synthesis of 25R,26-hydroxycholesterol as Scheer, Thompson, and Mosettig, Sidney Emerman, and I prepared a radioactive compound of much higher specific activity (14) and found that in the rat it was metabolized to both chenodeoxycholic and cholic acid, although the latter was present in much smaller amounts.

Thus, it was established in both rodents and humans (15) that bile acids could be derived from cholesterol beginning with the oxidation of the terminal carbon atom. However, the existence in both rats and mice of an alternate pathway of bile acid synthesis that yields muricholic rather than cholic acid (16) skewed the precursor/product relationships. These species differences in the genes that govern bile acid production continue to limit the usefulness of knockout mouse models for relating to the changes that occur to the phenotypic expressions as they occur in humans.

OVERVIEW OF THE CHOLESTEROL 27-HYDROXYLASE METABOLIC PATHWAY

In hepatocytes, mitochondrial cholesterol 27-hydroxylase initiates side-chain oxidation of the sterol intermediates generated initially by cholesterol 7α-hydroxylase expressed in the endoplasmic reticulum (**Fig. 1**). The possibility of a reverse sequence, initial mitochondrial 27-hydroxylation of cholesterol followed by 7α-hydroxylation was excluded because cholic acid was not identified as a metabolite of 3β-hydroxy-5-cholenoic acid in the rat (12, 17). Also, the purified 27-hydroxylase enzyme oxidized the 7α-hydroxylated sterol intermediates at rates more than 100-fold greater than that of cholesterol, implying that the latter was not a physiologic substrate (18).

We know now that cholesterol was always at a disadvantage to other substrates because it is the least water soluble. Therefore, it was never certain that cholesterol remained at concentrations sufficient to saturate the enzyme site, an essential requirement for zero order kinetics. With the advent of substituted β-cyclodextrins for the solubilization of cholesterol (19), much higher rates of turnover were obtained in assays of the activity of both

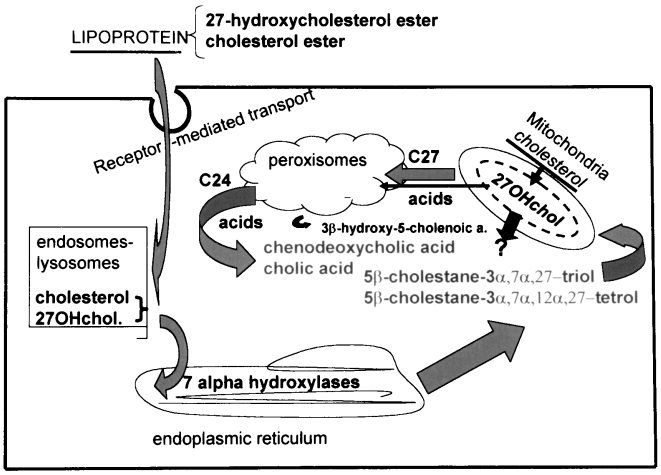

Fig. 1. Cholesterol 27-hydroxylase metabolic pathway. Two subcellular routes exist for the production of bile acids. 27-hydroxycholesterol, which is generated in mitochondria, is oxidized to the C27 acid and then transferred to peroxisomes for further side chain oxidation and shortening to the C24 acid (the acidic pathway) prior to the initiation of sterol ring transformation. Alternatively, 27-hydroxycholesterol is 7α-hydroxylated prior to further side chain oxidation by subcellular routes that are not defined (see text). Esters of 27-hydroxycholesterol circulating in plasma as part of LDL and HDL presumably follow a route parallel to that of esters of cholesterol. The free sterol is 7α-hydroxylated in the endoplasmic reticulum by oxysterol 7α-hydroxylase and is further transformed to 5β-cholestane-3α,7α,27-triol and 3α,7α,12α,27-tetrol prior to completion of side chain oxidation in mitochondria and peroxisomes to yield chenodeoxycholic and cholic acids.

cholesterol 7α-hydroxylase (20–22) and cholesterol 27-hydroxylase (23). Thus, the more recent data could support the view that both metabolic routes are normally expressed in hepatocytes.

However, in the last several years we have learned that steroid acute response (StAR) protein normally expressed in the adrenal gland but not the liver is essential for the normal rate of delivery of cholesterol to the inner mitochondrial membrane for 24*S*-hydroxylation (24). A similar role for StAR protein can be demonstrated in cell culture for 27-hydroxylation of cholesterol but not 7α-hydroxylated intermediates in bile acid synthesis. (25). Thus, because the liver normally lacks StaR protein it is possible that little if any 27-hydroxylation of cholesterol occurs in hepatocytes in vivo.

The subcellular routing that occurs following the generation of mitochondrial 27-hydroxycholesterol is less clear. Because the enzyme is multifunctional and also generates the C27 acid, 3β-hydroxy-5-cholestenoic acid (8), it might be assumed that all the generated 27-hydroxycholesterol undergoes successive oxidation to the C27 acid before leaving the mitochondria, referred to as the "acidic" pathway (26). The monohydroxy C27 bile acid is then metabolized to 3β-hydroxy-5-cholenoic acid, a precursor for chenodeoxycholic acid in peroxisomes. However, when the C27 bile acid is administered to rabbits, less than 5% is metabolized to cholic acid (27). By contrast, 27-hydroxycholesterol administered to rabbits (27)

and humans (15) yields approximately 90% and 40% cholic acid, respectively. Thus it seems likely that not all the 27-hydroxycholesterol that may occur in hepatic mitochondria is further oxidized to the C27 acid but that some undergoes 7α-hydroxylation prior to further side chain oxidation. This view is consistent with cell culture studies in which 27-hydroxycholesterol appears in the medium before the C27 acid (28), indicating that it is released from the enzyme site before complete oxidation.

Another subcellular route depicted in Fig. 1 derives from the finding that the 27-hydroxylase is widely expressed in tissues (7), including vascular endothelium (28) and macrophages (29). Most of the 27-hydroxycholesterol that is generated is transported in plasma esterified with fatty acids and together with esterified cholesterol is part of LDL and HDL (30). Following receptor mediated uptake and the generation of the free sterols by well-defined mechanisms, 7α-hydroxylation occurs in the endoplasmic reticulum. Further transformation to 5β-cholestane-3α,7α,27-triol and 3α,7α,12α-27-tetrol occurs prior to side chain oxidation in the mitochondria to form C27 bile acids. Further oxidation and shortening of the side chain occurs in peroxisomes to form chenodeoxycholic and cholic acids, respectively.

Recognition that a different 7α-hydroxylase (20) determines the production of mostly chenodeoxycholic acid from 27-hydroxycholesterol established the existence of two different but interdependent pathways (31). Thus, chenodeoxycholic acid is a potent ligand for farnesoid X activated receptors (FXRs), which modulates cholesterol 7α-hydroxylase activity (32), and therefore the amount of bile acid generated from cholesterol in the liver.

Initial interest in the biological effect of 27-hydroxycholesterol stemmed from the knowledge that it can downregulate cholesterol synthesis (33) at concentrations that could occur intracellularly. Recent studies of the mechanism for downregulation indicate that it is mediated by interruption of the transport of sterol regulatory binding protein by blocking sterol activating cleavage protein-mediated transport (34). Because 27-hydroxycholesterol is rapidly 7α-hydroxylated in the liver and the triol no longer downregulates cholesterol synthesis (35), it is difficult to know if 27-hydroxycholesterol has a physiologic role in regulating cholesterol synthesis in the liver. It is known that the activities of both HMG-CoA reductase and cholesterol 7α-hydroxylase are greater than normal in the livers of patients who do not generate 27-hydroxycholesterol because of a mutant *CYP27* gene. However, feeding chenodeoxycholic acid to these patients returns the activities toward normal, an event that presumably is not mediated by 27-hydroxycholesterol. The finding implies that the higher rate of cholesterol synthesis in the liver than that in most other tissues is related to bile acid production and that this interrelationship is governed by determinants that are independent of oxysterol regulation.

To fully evaluate the role of the cholesterol 27-hydroxylase metabolic pathway, quantitative data are needed with respect to total production of 27-hydroxycholesterol, the tissues of origin, the biologic roles of the intermediates that are generated in these tissues, and the biologic effects of the bile acid end products that are generated in the liver. How to achieve these goals remains an unsolved problem. Some in vivo kinetic data have been obtained (36) using techniques initially developed for determining steroid hormone production (37), but further validation is needed. In these limited studies in older adults (36), the cholesterol 27-hydroxylation pathway accounts for approximately 10% of the daily production of chenodeoxycholic and cholic acids.

Monohydroxy bile acid synthesis

Synthesis of the C27 bile acid, 3β-hydroxy-5-cholestenoic acid is unique to the cholesterol 27-hydroxylase metabolic pathway and it is probably the the sole source of the C24 bile acid, 3β-hydroxy-5-cholenoic acid, although one cannot exclude a very minor contribution from 24S-hydroxycholesterol (38). Both monohydroxy bile acids normally circulate in plasma in nanomolar amounts, but only the latter has been reported in amniotic fluid, meconium, bile, and urine (39–42). Presumably the C27 acid undergoes further oxidation to the C24 acid in peroxisomes, perhaps exclusively in the liver, since the C24 acid was not identified as a metabolite when 27-hydroxycholesterol was incubated with nonhepatic tissues (28).

Other metabolic fates are 7α-hydroxylation to 3β,7α-dihydroxy-5-cholenoic acid, which is further transformed to chenodeoxycholic acid (43, 44), or direct ring transformation to the monohydroxy bile acid 3α-hydroxy-5β-cholanoic acid (lithocholic acid). The latter bile acid, which is a primary bile acid in the newborn period (45), also derives later in life by bacterial 7α-dehydroxylation of chenodeoxycholic acid in the intestines. In urine monohydroxy, bile acids are found almost exclusively as ester sulfates (41).

The interrelationships of the two metabolic pathways in neonatal life are illustrated by the CYP7α knockout mouse (46). A transient neonatal cholestatic syndrome occurs because oxysterol 7α-hydroxylase activity is not normally expressed in neonatal mice. Absence of both oxysterol and cholesterol 7α-hydroxylase activities yields only monohydroxy bile acids. That these events in the mouse do not regularly occur in human neonates must be attributed to the expression of *CYP7B* in fetal life since in humans, in contrast to mice, the *CYP7A* gene is not expressed (47).

Bile acid synthesis in fetal and neonatal life

Relatively little data are available in humans to generate a complete picture of bile acid production during fetal and neonatal life. One needs to consider three sources: placental transfer, initial 7α-hydroxylation, and initial 27-hydroxylation.

Although bile acids normally circulate in maternal plasma mostly bound to albumin, exchange of the unbound species across the maternal/fetal capillary circulation can occur and probably accounts for the small amounts of deoxycholic acid found in sterile meconium at birth (48). However, the high proportion of chenodeoxycholic acid in fetal gallbladder bile in early pregnancy does not mimic the composition of maternal bile, and

therefore the accumulation of a fetal bile acid pool cannot be attributed entirely to placental exchange.

The knowledge that amniotic fluid in early pregnancy contains relatively large amounts of monohydroxy bile acids (39) indicates the existence of a metabolic pathway that begins with C27 hydroxylation. Expression of the *CYP27* gene during fetal life can also be inferred from the finding of monohydroxy bile acids in neonatal urine (41, 42). Oxysterol 7α-hydroxylase but not cholesterol 7α-hydroxylase is also normally expressed in fetal liver (49). Thus, one can conclude that the neonatal pool of bile acids is derived both via placental transfer and the cholesterol 27-hydroxlation metabolic pathway. Further evidence for this conclusion can also be derived from the statement by Setchell and coworkers that they cannot detect expression of CYP7α in normal neonates for at least the initial few months of life (47). When in the course of normal development the cholesterol 7α-hydroxylase pathway begins to contribute to bile acid production and then dominate total bile acid production is not known.

Since the cholesterol 27-hydroxylation metabolic pathway will generate only monohydroxy bile acids unless 7α-hydroxylation occurs, the status of expression of *CYP7B* becomes critical to the proportion of monohydroxy bile acids that are formed. Indeed, two instances of absent oxysterol 7α-hydroxylase activity have been associated with severe persistent cholestatic liver disease (47, 50). The severity of the cholestasis depends not only on oxysterol 7α-hydroxylase activity, but also on that of cholesterol 7α-hydroxylase, since the production of chenodeoxycholic and cholic acids via this latter pathway moderates the cholestatic effect of monohydroxy bile acids (51).

Postnatal bile acid synthesis

Because of the high extraction efficiency of hepatocytes for bile acids returning via the portal vein, the circulating pool of bile acids is confined for the most part to the enterohepatic circulation and supports three major functions. *A*) Bile acid dependent bile flow is essential for the excretion of endogenous metabolites such as bilirubin glucuronide and many xenobiotics, as well as to maintain in solution the relatively high concentration of cholesterol characteristic of human bile (52); *B*) Bile acids are essential for the absorption of adequate amounts of vitamin E and other fat-soluble vitamins as well as for the hydrolysis of fat and absorption of long-chain fatty acids; and *C*) Perhaps less essential but nevertheless important is the role of bile acids in regulating water reabsorption in the ascending colon (53).

Role of the cholesterol 7α-hydroxylase metabolic pathway

These biologic roles are maintained by an auto-regulatory process that focuses on the regulation of the activity of cholesterol 7α-hydroxylase. Thus, interruption of the enterohepatic circulation by ileal disease, bile fistula, or bile acid sequestrant administration, all of which threaten to reduce bile acid pool size, result in increased enzyme activity to maintain homeostasis. Artifical expansion of pool size by chenodeoxycholic acid administration is a potent mechanism for decreasing enzyme activity (54). This knowledge coupled with the newer information concerning FXR/short heterodimer protein (SHP) nuclear receptor regulation (55) and its relationship to chenodeoxycholic and deoxycholic acids (56) focuses on transcriptional regulation of the *CYP7A* gene as the dominant but not exclusive mechanism.

Regulation of bile acid pool size and synthesis by this mechanism is essentially independent of dietary cholesterol and depends mostly on the upregulation of cholesterol synthesis in the liver to satisfy the demand for increased bile acid synthesis. However, pharmacologic manipulation of the pathway can adapt it as a mechanism for lowering LDL cholesterol (LDL-C). Thus, suppressing HMG-CoA reductase activity in the liver by the administration of allosteric inhibitors makes it possible to enhance receptor-mediated LDL-C delivery to the enzyme as a source for bile acid production in lieu of de novo synthesis.

Roles of the cholesterol 27-hydroxylase metabolic pathway

Because adult *cyp7A* knockout mice appear phenotypically normal (46, 57), it could be argued that the cholesterol 27-hydroxylase metabolic pathway can replace all the essential functions, particularly the maintenance of bile acid dependent bile flow. However, until a human counterpart of the knockout cyp7A mouse is identified, one should reserve judgment with respect to the importance of this pathway in human physiology.

Because of species differences in bile acid synthesis (16), knocking out the *cyp*27 gene in the mouse does not result in the phenotypic expression of cerebrotendinous xanthomas characteristic of the human inherited disease.

The markedly accelerated atherosclerosis in some patients with a mutant *CYP27* gene can now be explained at least in part by the loss of 27-hydroxycholesterol/LXR-mediated cholesterol efflux from macrophages as part of reverse cholesterol transport (58). The major question that now requires resolution is how this inherited defect, which affects all cells normally expressing the gene, relates to acquired defects that could occur in selective tissues such as macrophages.

Reverse cholesterol transport

ABC A1 transporter activity. Since the recognition that the molecular basis of Tangier disease is a mutant ABC A1 transporter (59), progress has been very rapid (60, 61). 27-hydroxycholesterol generated in macrophages has been identified as an important endogenous ligand for up-regulating cholesterol efflux via this ABC transporter pathway (58).

Metabolism of cholesterol to 27-hydroxycholesterol in macrophages. The rate of conversion of cholesterol to 27-hydroxycholesterol followed by its further metabolism to other intermediates in the bile acid biosynthetic pathway represents a mechanism for returning cholesterol to the liver that is independent of its role of 27-hydroxycholesterol as a ligand for LXR-mediated cholesterol efflux via the ABC A1 transporter.

It is of particular interest, therefore, that the 27-hydroxycholesterol concentration in atheroma obtained from the

TABLE 1. Steroids unique to the cholesterol 27-hydroxylase metabolic pathway

A. Sterol intermediates
1. 27-hydroxycholesterol
2. 27-hydroxycholesterol-3 sulfate
3. 27-hydroxycholesterol-27 sulfate
4. 27-hydroxycholesterol disulfate
5. 7α,27-dihydroxycholesterol[+]
B. Acidic intermediates[++]
1. 3β-hydroxy-5-cholestenoic acid
2. 3β-hydroxy-5-cholestenoic acid-3 sulfate
3. 3β-hydroxy-5-cholenoic acid
4. 3β-hydroxy-5-cholenoic acid sulfate

[+] can be generated by initial 7α-hydroxylation followed immediately by side-chain oxidation. Potentially a common intermediate for the cholesterol 7α-hydroxylase and cholesterol 27-hydroxylase metabolic pathways.

[++] 7α-hydroxylated acidic species not shown. They can arise either by initial 7α- or 27-hydroxylation.

carotid and coronary arteries and the aorta is much greater than that in plasma (62). Since the major cellular components of atheroma are macrophages that express the *CYP27* gene (63), it is reasonable to think that they are the source of this hydroxysterol and account for the much greater ratio of 27-hydroxycholesterol/cholesterol in atheroma than in plasma. The concentration gradient between atheroma and plasma indicates a block in reverse cholesterol transport that is puzzling and may affect the natural history of atheroma. Thus, the local concentration of 27-hydroxycholesterol when expressed per weight of tissue is greater than that needed to initiate apoptosis in cell culture (64) and sufficient to inhibit smooth muscle cell migration (65). On the other hand we do not know what proportion of the 27-hydroxycholesterol may be esterified with fatty acid and/or sulfated and what the local biologic effects of these different naturally occurring derivatives are. It could be argued that the local activity of oxysterol 7α-hydroxylase should be equivalent to that of cholesterol 27-hydroxylase. The more water-soluble triol would then enter plasma. Currently, it is not possible to evaluate either the potentially beneficial or the potentially deleterious effects of 27-hydroxycholesterol in atheroma. A major challenge is to design critical studies in an experimental model that might begin to give insights on the biologic significance of 27-hydroxycholesterol and the other steroid intermediates that form locally.

Other ligands regulating gene expression. Thus far only the biologic role of 27-hydroxycholesterol as a ligand has been studied in detail. One study indicates that 3β-hydroxy-5-cholenstenoic acid also may function as a ligand (66). **Table 1** indicates the number of intermediates exclusive of fatty acid esters that can be generated by and are unique to the cholesterol 27-hydroxylase metabolic pathway. With the exception of 3β-hydroxy-5-cholestenoic acid, their possible role as ligands for nuclear receptors have not been evaluated.

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REFERENCES

1. Javitt, N. B. 1990. 26-Hydroxycholesterol: synthesis, metabolism, and biologic activities. *J. Lipid Res.* **31:** 1527–1533.
2. Cooper, A. D. 1997. Bile salt biosynthesis: an alternate synthetic pathway joins the mainstream. *Gastroenterology.* **113:** 2005–2008.
3. Verips, A., L. H. Hooseloot, G. C. H. Steenbergen, J. P. Theelen, R. A. Wevers, F. J. M. Gabriels, B. G. M. Endelen, and L. P. W. J. van den Heuvel. 2000. Clinical and molecular genetic characteristics of patients with cerebrotendinous xanthomatosis. *Brain.* **123:** 908–919.
4. Fredrickson, D. S. 1956. The conversion of cholesterol-4-C^{14} to acids and other products by liver mitochondria. *J. Biol. Chem.* **222:** 109–120.
5. Scheer, I., M. J. Thompson, and E. Mosettig. 1956. 5-Cholestene-3β,26-diol. *J. Amer. Chem. Soc.* **78:** 4733–4737.
6. Nomenclature Committee 1989. The nomenclature of steroids. *Eur. Journ. Biochem.* **186:** 429–458.
7. Andersson, S., D. L. Davis, H. Dahlback, H. Jornvall, 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.
8. Cali, J. J., and D. W. Russell. 1991. Characterization of human sterol 27-hydroxylase. A mitochondrial cytochrome P-450 that catalyzes multiple oxidation reaction in bile acid biosynthesis. *J. Biol. Chem.* **266:** 7774–7778.
9. Arunachalam, J. C. H., and E. Caspi. 1986. Facile high-performance liquid chromatographic resolution of (25R)- and (25S)-26-hydroxycholesterol -3,26-di-p-bromobenzoates. *J. Chromatogr.* **351:** 604–607.
10. D'Ambra, T. E., N. B. Javitt, and T. Warchol. 2000. Synthesis of (25R)-cholest-5-ene-3β,26-diol and its radiolabeled Analog. *Tetrahedron Lett.* **38:** 3801–3804.
11. Ono, M., and D. S. Fredrickson. 1956. The in vitro production of 25- and 26-hydroxycholesterol and their in vivo metaboism. *Biochim. Biophys. Acta.* **22:** 183–184.
12. Bergstrom, S. 1955. Formation and metabolism of bile acids Bile Acids and Steroids 20. *Record of Chemical Progress.* **16:** 63–83.
13. Danielsson, H. 1961. Formation and metabolism of 26-hydroxycholesterol. *Arkiv.Kemi.* **17:** 373–379.
14. Wachtel, N., S. Emerman, and N. B. Javitt. 1968. Metabolism of cholest-5-ene-3 beta, 26-diol in the rat and hamster. *J. Biol. Chem.* **243:** 5207–5212.
15. Anderson, K. E., E. Kok, and N. B. Javitt. 1972. Bile acid synthesis in man: metabolism of 7 -hydroxycholesterol- 14 C and 26-hydroxycholesterol- 3 H. *J. Clin. Invest.* **51:** 112–117.
16. Elliott, W. H., and P. M. Hyde. 1971. Metabolic Pathways of Bile Acid Synthesis. *Am. J. Med.* **51:** 439–452.
17. Bergstrom, S., H. Danielsson, and B. Samuelsson. 1960. Formation and metabolism of bile acids. *In* Lipid Metabolism. K. Bloch, editor. Wiley, New York. 291–336.
18. Okuda, K., O. Masumoto, and Y. Ohyama, 1988. Purification and characterization of 5β-cholestane-3α,7α,12α-triol 27-hydroxylase from female rat liver mitochondria. *J. Biol. Chem.* **263:** 18138–18142.
19. De Caprio, J., J. Yun, and N. B. Javitt. 1992. Bile acid and sterol solubilization in 2-hydroxypropyl-beta-cyclodextrin. *J. Lipid Res.* **33:** 441–443.
20. Martin, K. O., K. Budai, and N. B. Javitt. 1993. Cholesterol and 27-hydroxycholesterol 7 alpha-hydroxylation: evidence for two different enzymes. *J. Lipid Res.* **34:** 581–588.
21. Souidi, M., M. Paraquet, J. Ferezou, and C. Lutton. 1999. Modulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase activities by steroids and physiological conditions in hamster. *Life Sci.* **64:** 1585–1593.
22. Souidi, M., M. Parquet, and C. Lutton. 1998. Improved assay of hepatic microsomal cholesterol 7 alpha-hydroxylase activity by the use of hydroxypropyl-beta-cyclodextrin and an NADPH-regenerating system. *Clin. Chim. Acta.* **269:** 201–217.
23. 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.
24. Bose, H. S., T. Sugawara, J. F. Strauss, 3rd, and W. Miller. 1996. The

pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. *N. Engl. J. Med.* **335:** 1870–1878.
25. Sugawara, T., D. Lin, J. A. Holt, K. O. Martin, N. B. Javitt, W. L. Miller, and J. F. Strauss, III. 1995. Structure of the human steroidogenic acute regulatory protein (StAR) gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity. *Biochemistry.* **34:** 12506–12512.
26. Axelson, M., and J. Sjovall. 1990. Potential bile acid precursors in plasma–possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J. Steroid Biochem.* **36:** 631–640.
27. Ayaki, Y., E. Kok, and N. B. Javitt. 1989. Cholic acid synthesis from 26-hydroxycholesterol and 3-hydroxy-5-cholestenoic acid in the rabbit. *J. Biol. Chem.* **264:** 3818–3821.
28. Reiss, A. B., K. O. Martin, N. B. Javitt, D. W. Martin, E. A. Grossi, and A. C. Galloway. 1994. Sterol 27-hydroxylase: high levels of activity in vascular endothelium. *J. Lipid Res.* **35:** 1026–1030.
29. Bjorkhem, I., O. Andersson, U. Diczfalusy, B. Sevastik, R. J. Xiu, C. Duan, and E. Lund. 1994. Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proc. Natl. Acad. Sci. USA.* **91:** 8592–8596.
30. 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.
31. Javitt, N. B. 1994. Bile acid synthesis from cholesterol: regulatory and auxiliary pathways. *FASEB J.* **8:** 1308–1311.
32. Tu, H., A. Y. Okamoto, and B. Shan. 2000. FXR, a bile acid receptor and biological sensor. *Trends Cardiovasc. Med.* **10:** 30–35.
33. Esterman, A. L., H. Baum, N. B. Javitt, and G. J. Darlington. 1983. 26-hydroxycholesterol: regulation of hydroxymethylglutaryl-CoA reductase activity in Chinese hamster ovary cell culture. *J. Lipid Res.* **24:** 1304–1309.
34. Brown, M. S., and J. L. Goldstein. 1998. Sterol regulatory element binding proteins (SREBPs): controllers of lipid synthesis and cellular uptake. *Nutr. Rev.* **56:** S1–S3.
35. Martin, K. O., A. B. Reiss, R. Lathe, and N. B. Javitt. 1997. 7α-hydroxylation of 27-hydroxycholesterol: biologic role in the regulation of cholesterol synthesis. *J. Lipid Res.* **37:** 1053–1058.
36. Duane, W. C., and N. B. Javitt. 1999. 27-hydroxycholesterol: production rates in normal human subjects. *J. Lipid Res.* **40:** 1194–1199.
37. Gurpide, E. 1990. Experimental designs used to estimate rates of steroid production and metabolism in vivo and in vitro. *Ann. N. Y. Acad. Sci.* **595:** 165–172.
38. Bjorkhem, I., D. Lutjohann, U. Diczfalusy, L. Stahler, G. Ahborg, and J. Wahren. 1998. Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation. *J. Lipid Res.* **39:** 1594–1600.
39. Deleze, G., G. Paumgartner, G. Kartaganus, W. Giger, M. Reinhard, and D. Sideropoulos. 1978. Bile acid pattern in human amniotic fluid. *Eur. J. Clin. Invest.* **8:** 41–45.
40. Jonsson, G., A-C. Midtvedt, A. Norman, and T. Midtvedt. 1990. Intestinal microbial bile acid transformation in healthy infants. *J. Pediatr. Gastr. & Nutr.* **20:** 394–402.
41. Tohma, M. 1996. Determination of fetal bile acids and related steroidal compounds and their profile in neonatal biologic fluids. *Yakugaku Zasshi.* **116:** 753–775.
42. Tazawa, Y., M. Yamada, M. Nakagawa, T. Konno, and K. Tada. 1984. Direct measurement of urinary bile acids of infants by high-performance liquid chromatography connected with an enzyme immobilized column. *Tohoku J. Exper. Med.* **143:** 361–371.
43. Kulkarni, B., and N. B. Javitt. 1982. Chenodeoxycholic acid synthesis in the hamster: a metabolic pathway via 3 beta, 7 alpha-dihydroxy-5-cholen-24-oic acid. *Steroids.* **40:** 581–589.
44. Javitt, N. B., E. Kok, F. Carubbi, T. Blizzard, M. Gut, and C. Y. Byon. 1986. Bile acid synthesis. Metabolism of 3 beta-hydroxy-5-cholenoic acid to chenodeoxycholic acid. *J. Biol. Chem.* **261:** 12486–12489.
45. Finni, K., S. Simila, M. Koivisto, and K. Kouvalainen. 1983. Deoxycholic and sulpholithocholic acid concentrations in serum during infancy and childhood. *Acta. Paediatrica. Scandinavica.* **72:** 215–218.
46. Arnon, R., T. Yoshimura, A. Reiss, K. Budai, and R. Lefkowitch. 1998. Cholesterol 7-hydroxylase knockout mouse: a model for monohydroxy bile acid-related neonatal cholestasis. *Gastroenterology.* **115:** 1223–1228.
47. Setchell, K. D., M. Schwarz, N. C. O'Connell, E. G. Lund, D. L. Davis, R. Lathe, H. R. Thompson, R. W. Tyson, R. J. Sokol, and D. W. Russell. 1998. Identification of a new inborn error in bile acid synthesis neonatal liver disease. mutation of the oxysterol 7 alpha-hydroxylase gene causes severe neonatal liver disease. *J. Clin. Invest.* **102:** 1690–1703
48. Sharp, H. L., J. B. Carey, Jr., and W. Krivit. 1971. Primary and secondary bile acids in meconium. *Pediatr. Res.* **5:** 274–279.
49. Collins, J. A., R. P. Altman, K. O. Martin, and N. B. Javitt. 1994. Neonatal cholestatic syndromes: molecular basis for transient "physiologic cholestasis". *Hepatology.* **35:** 340A.
50. Javitt, N. B., E. Kok, M. Gut, I. Rajagoplan, and K. Budai. 1984. Neonatal cholestasis: Identification of a metabolic error in bile acid synthesis. *Pediatr. Res.* **18:** 200A.
51. Javitt, N. B., and S. Emerman. 1968. Effect of sodium taurolithocholate on bile flow and bile acid excretion. *J. Clin. Invest.* **47:** 1002–1014.
52. Javitt, N. B. 1976. Hepatic bile formation (first of two parts). *N. Engl. J. Med.* **295:** 1464–1469.
53. Hofmann, A. F. 1999. The continuing importance of bile acids in liver and intestinal disease. *Arch. Intern. Med.* **159:** 2647–2658.
54. Reihner, E., B. Angelin, I. Bjorkhem, and K. Einarsson. 1989. Bile acid synthesis in humans: regulation of hepatic microsomal cholesterol 7α-hydroxylase activity. *Gastroenterology.* **97:** 1498–1505.
55. Denson, L. A., E. Sturm, W. Echevarria, T. L. Zimmerman, M. Makishima, D. J. Mangelsdorf, and S. J. Karpen. 2001. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. *Gastroenterology.* **121:** 140–147.
56. Makishima, M., A. Y. Okamoto, J. J. Repa, H. Tu, R. M. Learned, A. Luk, M. V. Hull, K. D. Lustig, D. J. Mangelsdorf, and B. Shan. 1999. Identification of a nuclear receptor for bile acids. *Science.* **284:** 1362–1365.
57. Schwarz, M., E. G. Lund, K. D. Setchell, H. J. Kayden, J. E. Zerwekh, I. Bjorkhem, J. Herz, and D. W. Russell. 1996. Disruption of cholesterol 7alpha-hydroxylase gene in mice. II. Bile acid deficiency is overcome by induction of oxysterol 7alpha-hydroxylase. *J. Biol. Chem.* **271:** 18024–18031.
58. Fu, X., J. G. Menke, Y. Chen, G. Zhou, K. L. MacMaul, S. D. Wright, C. P. Sparrow, and E. G. Lund. 2001. 27-Hydroxycholesterol Is an endogenousl ligand for liver X receptor in cholesterol-loaded cells. *J. Biol. Chem.* **276:** 38378–38387.
59. Bodzioch, M., E. Orso, J. Klucken, T. Langmann, A. Bottcher, W. Diederich, W. Drobnik, S. Bariage, C. Buchler, M. Porsch-Ozurumez, W. E. Kaminski, H. W. Hahmann, K. Oette, G. Rothe, C. Aslanidis, K. J. Lackner, and G. Schmitz. 1999. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat. Genet.* **22:** 316–318.
60. Neufeld, E. B., A. T. Remaly, S. J. Demosky, J. A. Stonik, A. M. Cooney, M. Comly, N. K. Dwyer, M. Zhang, J. Blanchette-Mackie, S. Santamarina-Fojo, and H. B. Brewer, Jr. 2001. Cellular localization and trafficking of the human ABCA1 transporter. *J. Biol. Chem.* **276:** 27584–27590.
61. von Eckardstein, A., J. R. Nofer, and G. Assmann. 2001. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* **21:** 13–27.
62. Carpenter, K. L., S. E. Taylor, J. A. Ballentine, B. Fassell, B. Halliwell, M. J. Mitchinson. 1993. Lipids and oxidised lipids in human atheroma and normal aorta. *Biochim. Biophys. Acta.* **1167:** 121–130.
63. Babiker, A., O. Andersson, E. Lund, R. J. Xiu, S. Deeb, A. Reshef, E. Leitersdorf, U. Diczfalusy, and I. Bjorkhem. 1997. Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. *J. Biol. Chem.* **272:** 26253–26261.
64. Panini, S. R., and M. S. Sinensky. 2001. Mechanisms of oxysterol-induced apoptosis. *Curr. Opin. Lipidol.* **12:** 529–533.
65. Corsini, A., D. Verri, M. Rateri, P. Quaroto, R. Paoletti, and R. Fumagelli. 1995. Effects of 26-aminocholesterol, 27-hydroxycholesterol, and 25-hydroxycholesterol on proliferation and cholesterol homeostasis in arterial myocytes. *Arterioscler. Thromb. Vasc. Biol.* **15:** 420–428.
66. Song, C., and S. Liao. 2000. Cholestenoic acid is a naturally occurring ligand for liver X receptor alpha. *Endocrinology.* **141:** 4180–4184.