Oxysteroids are a new classification for sterol intermediates in cholesterol synthesis that undergo enzyme-catalyzed stereo-specific 25R,26-hydroxylation and thus bypass cholesterol as the expected end-product. Recently, they were identified in micromolar amounts in the plasma of patients with Smith-Lemli-Opitz syndrome (SLOS). An additional three oxysteroids, the 25,26-hydroxy derivatives of lanosterol, zymosterol, and desmosterol, respectively, were generated in vitro by CYP27A1-transfected bacteria. As there are 19 steps between cholesterol and lanosterol, the first post-squalene sterol, a potentially large class of oxysteroids exists. Limited studies of 25r,26–7-dehydrocholesterol indicate a traditional role as a ligand for nuclear receptors, but complete evaluation of oxysteroids for novel biologic activities is lacking. Currently, the lack of authentic oxysteroid standards limits both their detection in biologic fluids and evaluation of their biologic effects.

Although it has been recognized for many years that 7-dehydrocholesterol, a normal intermediate in cholesterol synthesis, is also the precursor of vitamin D, the idea that other intermediates might also be used to generate potent steroids has received little, if any, attention.

Because of the low activity of 7-dehydrocholesterol-7-reductase in Smith-Lemli-Opitz syndrome (SLOS) (Figure 1) (Table 1), a marked increase in the levels of intermediates in cholesterol synthesis in biologic fluids occurs. The opportunity to detect novel metabolites therefore became easier as, if they occur, their concentration in biologic fluids is also likely to be increased. Even so, certain additional precautions are necessary because of the possible light-sensitive nature of the metabolites.

The finding of micromolar amounts of the 25r,26-hydroxy metabolites of both 7-dehydrocholesterol and 8-dehydrocholesterol in the plasma of patients with SLOS confirmed the existence of alternate metabolic pathways. Their role as ligands for the Liver X-activated group of nuclear receptors (LXR) indicate they also have biologic activities [1].

The oxysteroid hormone concept extrapolates both from these findings and from the knowledge that there are 19 intermediate steps between the formation of lanosterol, the first post-squalene sterol intermediate, and cholesterol [2]. Of this large group, lanosterol, lathosterol, and desmosterol among others are normally present in human plasma [3]. A search for oxysteroid metabolites of these intermediates was not done perhaps because of the lack of known standards and the much longer retention times that require lengthy programs for their detection. The in vitro studies that indicate that lanosterol, zymosterol and desmosterol are all substrates for the mitochondrial p450 enzyme cholesterol 27-hydroxylase provide a rationale for seeking their presence under physiologic and particularly pathophysiologic conditions [4]. Thus, potentially, a variety of novel metabolites can be generated under physiologic and/or pathophysiologic conditions.

The unifying concept that permits the new classification as oxysteroids is based on the initial step in the pathway: stereo-specific hydroxylation of the terminal carbon atom of the side chain, referred to by biochemists as C25r,C26-hydroxylation and in the medical literature as C27 hydroxylation [5].

This review focuses on the properties of the enzyme that initiates the production of oxysteroids, the biologic activities on the known ligands, and the potential role that unrecognized ligands might have in modulating gene expression.

Biochemistry of mitochondrial p450 CYP27A1

Many parallels exist between CYP11A1, the steroid-generating side-chain cleavage enzyme (scc), and CYP27A1, the side-chain terminal methyl group hydroxylase enzyme. Both are multifunctional mitochondrial cytochrome p450 enzymes that require NADPH and a two-protein redox chain, ferredoxin and ferredoxin reductase [6]. To generate pregnenolone, two successive hydroxylations occur followed by cleavage between C20 and C22. For CYP27A1, two successive hydroxylations at the terminal CH3 produce an unstable gem-diol that quickly transforms to the aldehyde, followed by a third hydroxylation to generate a C27 acid. Thus a major difference between steroid hormone and oxysteroid hormone synthesis is that CYP11A1 generates only one product, pregnenolone, in contrast to CYP27A1, which generates both a neutral dihydroxy product and a C27 acid product. Of particular interest is the report that on a molar basis, 25r,26-hydroxycholesterol was less potent as a ligand for the nuclear receptor LXR than was the C27 acid derived from it [7]. Because lanosterol was also found to form both an acidic and a neutral product in vitro [4], a rich group of biologically active oxysteroids can occur in vivo. With the finding that desmosterol is a substrate, we know that the modified
stereochemistry caused by a double bond between C24 and C25 does not preclude terminal methyl-group oxidation.

The regulation of the proportions of dihydroxy and acidic metabolites derived from CYP27A1 activity is also of interest. Both metabolites normally circulate in human plasma [3], but their tissues of origin might be different. It has been proposed that the C_{27} acidic metabolite is uniquely derived from macrophages in the lung [8], in contrast to the origin of 25r,26-hydroxycholesterol from arterial endothelium [9] and macrophages [10]. Cell culture studies indicate that a rise in 25r,26-hydroxy-cholesterol in the medium precedes the increase in the C27 acid [9], but gives little insight into the events that occur in vivo that determine the proportions of the different oxysteroids.

**Biologic roles of mitochondrial CYP27A1**

In humans the CYP27A1 gene is expressed in most tissues. Because metabolic products of the gene are known to occur in fetal life [11], it can be assumed that expression begins early in development; however, studies indicating the onset of gene expression in different tissues are lacking and could provide insights into the biologic roles of the metabolites. Nevertheless, because expression is eventually present in most tissues, in contrast to the genes that initiate steroid hormone synthesis, it can be

![Figure 1. Oxysteroid hormone synthesis in Smith-Lemli-Opitz Syndrome (SLOS). The dark arrows indicate steps in the normal metabolic pathway for cholesterol synthesis. In SLOS low levels of 7-dehydrocholesterol-7-reductase reduce or prevent cholesterol synthesis and result in normal intermediates following an alternate metabolic pathway (open arrows) with an increased production of 25R,26-hydroxy-7-dehydrocholesterol.](image-url)

**Table 1. Altered oxysteroid ligand–nuclear receptor relationships in genetically determined diseases**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosome location</th>
<th>Birth frequency</th>
<th>Normal gene product</th>
<th>Pathophysiology</th>
<th>Phenotypic expressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrotendinous xanthomatosis</td>
<td>2; 2q35</td>
<td>Rare; +/− 300 patients reported</td>
<td>CYP 27A1 P450 enzyme</td>
<td>Lowered ATP-binding cassette transport activity</td>
<td>Atherosclerosis, neurologic syndromes</td>
</tr>
<tr>
<td>Tangier disease</td>
<td>9; 9q31.1</td>
<td>Rare; &lt;100 patients reported</td>
<td>ABCA1 transporter</td>
<td>Loss of ATP-binding transporter activity</td>
<td>Atherosclerosis, neurologic syndromes</td>
</tr>
</tbody>
</table>
assumed that the products of the enzyme have local effects, referred to conventionally as autocrine or paracrine rather than endocrine.

With the recognition that both 7-dehydrocholesterol and 8-dehydrocholesterol are substrates for CYP27A1, it was reasonable to determine whether other normal intermediates in cholesterol synthesis are also substrates for the enzyme. Only three, lanosterol, zymosterol and desmosterol, are readily available commercially. When tested in vitro using the human CYP27A1 cDNA expressed in Escherichia coli, it was found that they were all substrates for the enzyme [4]. Thus, it is possible that a new group of potent biologic steroid ligands might exist, all fitting the definition that they are normal intermediates in cholesterol synthesis that undergo enzyme-catalyzed stereo-specific 25r,26-hydroxylation as the initial step. To distinguish this group from the steroid hormones derived from cholesterol, the term ‘oxysteroids’ is appropriate, particularly as both groups appear to function as ligands for nuclear receptors, a traditional biologic role.

Although it is clear that the Steroid Acute Response (STAR) protein regulates the access of cholesterol to CYP11A1 in the adrenal gland [12], little is known about the regulation of access to CYP 27A1, also located on the inner mitochondrial membrane [13]. In doubly transfected cells it can be shown that STAR protein enhances the production of 27-hydroxycholesterol [14]. Thus, another normal restriction of intermediates in cholesterol synthesis that undergo 25r,26-hydroxylation might be their requirement for STAR protein, particularly in relation to cholesterol, which is normally present in much greater amount.

Another biologic role for CYP27A1 unrelated to oxy steroid production is the initiation of side-chain oxidation of 7α-hydroxylated C27 intermediates in bile acid synthesis; their generation in the liver commences after upregulation of CYP7A1 in postnatal life [15]. STAR protein is not normally found in liver and studies in cell culture [14] indicate that it does not regulate access of these intermediates to the enzyme.

Physiology and pathophysiology of oxysteroids
Although picomolar amounts of 25,26r-hydroxy-7-dehydrocholesterol were found in the plasma of three normal individuals [1], it is the presence of micromolar amounts in the plasma of patients with SLOS that focuses on a pathophysiologic role for oxysteroids in the disorder. It is reasonable to think that the high production rates represent a combination of high tissue levels of the precursor, 7-dehydrocholesterol, together with relatively low levels of cholesterol. This dual alteration in tissue levels also probably accounts for the finding of both steroid hormones and bile acids derived from intermediates in cholesterol synthesis in patients with SLOS [16,17]. Because similar circumstances exist in other disorders of cholesterol synthesis [18], it can be anticipated that complete evaluation of the metabolic alterations will indicate the occurrence of other oxysteroids.

Generation of oxysteroid intermediates, acting as ligands, might then alter gene expression in expected or perhaps unexpected ways. The specificity of 25r,26-hydroxy-7-dehydrocholesterol for LXRα and not LXRβ, in contrast to that of 25r,26-hydroxycholesterol [1], reaffirms the knowledge that subtle changes in steroid structure can have major effects on biologic activities.

The occurrence of these novel metabolites in vivo also raised a question with regard to the origin of 25r,26-hydroxycholesterol that is normally present in plasma [19]. It had been thought that 25r,26-hydroxycholesterol is derived solely from cholesterol by a stereo-specific hydroxylation catalyzed by the mitochondrial P450 enzyme, CYP27A1. Because children with SLOS have low plasma levels of cholesterol and plasma from normal individuals shows a correlation between cholesterol and 25r,26-hydroxycholesterol levels [20], one would have predicted that the plasma levels of 25r,26-hydroxycholesterol would be low rather than significantly increased in SLOS [21]. Although it remains to be established, the best explanation for this paradoxical increase in 25r,26-hydroxycholesterol is that it is derived from the 7- and 8-dehydro intermediates rather than from cholesterol. Thus, 25r,26-hydroxycholesterol might be of dual origin, derived from both cholesterol and intermediates in cholesterol synthesis.

The need for this type of information becomes apparent when one considers the varied phenotypic expressions in individuals with mutations in CYP27A1. An extensive analysis of the clinical and molecular genetic characteristics of patients with mutations in the gene revealed 29 different mutations in 79 homozygous patients comprising 45 families [22]. The mutations ranged from deletions or insertions in several different exons to nonsense, splice site and missense mutations. Although accelerated atherosclerosis, particularly affecting the coronary arteries, is a well-known consequence in some families [23], the variety of neurologic manifestations ranging from peripheral neuropathies to epilepsy and dementia was the focus of this study [22]. The authors conclude that no genotype–phenotype correlation can currently be made, which is consonant with previous findings that indicated striking intrafamilial phenotypic variability [24].

From studies in mice, we are aware that induced mutations in the expression of both nuclear receptors [25] and oxysteroids [26] by gene disruption (knockout) mimic in part the phenotypic expression found in humans. As the same mutation inserted into animals with different genetic backgrounds greatly modifies the phenotypic expression, the variability found in humans with mutations in CYP27A1 might represent a similar phenomenon. However, it is also reasonable to suspect that the broad substrate specificity [5] of the enzyme generates many different ligands that locally modulate the transcription of other genes.

The activation of the LXR family of nuclear receptors by 25r,26-hydroxycholesterol and 25r,26-hydroxy-7-dehydrocholesterol provides a cellular basis for understanding the phenotypic expression of three genetically determined diseases.

Cerebrosideinious xanthomatosis (CTX)
The accelerated atherosclerosis in some patients with mutations in CYP27A1 (Table 1) can be attributed to a
decrease in reverse cholesterol transport [27]. Macrophages in the arterial wall that accumulate cholesterol return it to the circulation through the ATP-binding cassette transporter, ABCA1, which participates in a complex efflux pattern with phospholipid and Apo AI to generate high-density lipoproteins (HDL). Uproadation of ABCA1 is dependent on the binding of these oxysteroids to LXR, which forms a heterodimer with RXR (9-cis retinoic acid receptor) that binds to response elements on the target gene [28]. Low to absent levels of 25r,26-hydroxycholesterol in CTX reduce transporter activity and favor macrophage foam cell formation and atherosclerosis. Because accumulation of cholesterol in macrophages is dependent on many variables including diet, variations in phenotypic expression can be expected.

**Tangier Disease (TD)**
The non-functional ATP-binding cassette transporter ABCA1 that occurs as a consequence of the mutation in the ABCA1 gene (Table 1) that underlies TD, further illustrates the importance of this transporter in cholesterol homeostasis [29]. Reduced efflux from intestinal cells enhances cholesterol absorption with accumulation of cholesterol in many tissues, expressed phenotypically as peripheral neuropathies and accelerated atherosclerosis in some but not all patients.

**SLOS**
The converse occurs in SLOS, a genetically determined deficiency in 7-dehydrocholesterol-7-reductase [18] that leads to increased levels of 25r,26-hydroxycholesterol [21] and 25r,26–7 and 8-dehydrocholesterols in plasma and presumably also in tissues [1]. The high levels of these three oxysteroids indicate maximal expression of LXR-mediated ABCA1 transporter activity consonant with the low rates of intestinal cholesterol absorption that have been found in balance studies [30]. Thus, although the phenotypic expressions of the syndrome (Table 1) relate to altered morphogen formation during fetal development, consideration also needs to be given to the biologic effects of the oxysteroids that are generated.

A corollary of the findings in SLOS is the possibility that relative deficiencies in the many enzymes that carry forward the transformation of lanosterol to cholesterol will generate increased levels of some intermediates that are substrates for CYP27A1. These transient elevations might be part of the modulation of gene expression that occurs during normal development. Drugs that affect specific enzymes in the cholesterol biosynthetic pathway might also generate relatively large amounts of oxysteroids.

**Conclusion**
The oxysteroids detected in SLOS together with the knowledge that other intermediates in cholesterol synthesis are substrates for the multifunctional CYP27A1 enzyme provide the rationale for believing that a large family of ligands exist for ‘orphan’ nuclear receptors. Adding to the potential of their biologic roles is the knowledge that the CYP27A1 gene is expressed in many tissues, beginning in fetal life, and that many enzyme-regulated steps are needed for the transformation of lanosterol to cholesterol. Thus the framework exists for the transient generation of potent oxysteroids during development that modulate gene expression locally. Of the known metabolic errors in cholesterol synthesis, only the oxysteroids generated in SLOS have been studied. Detecting other oxysteroids that might occur under physiologic or pathophysiologic conditions will be aided greatly by the chemical synthesis of authentic compounds that will provide known parameters for their analysis and that can also be used to determine their biologic effects in cell culture and in animals, a traditional approach for evaluating steroid hormones.

**Acknowledgements**
The author’s research cited in this report was funded in part from grants from the National Institutes of Health and from the Jerome and Joan Jakubovitz Foundation. I wish to thank Suzanne Javitt for her editorial help in preparing the manuscript.

**References**
27 Fu, X. et al. (2001) 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. J. Biol. Chem. 276, 38378–38387

Important information for personal subscribers
Do you hold a personal subscription to a Trends journal? As you know, your personal print subscription includes free online access, previously accessed via BioMedNet. From now on, access to the full-text of your journal will be powered by ScienceDirect and will provide you with unparalleled reliability and functionality. Access will continue to be free; the change will not in any way affect the overall cost of your subscription or your entitlements.

The new online access site offers the convenience and flexibility of managing your journal subscription directly from one place. You will be able to access full-text articles, search, browse, set up an alert or renew your subscription all from one page.

In order to protect your privacy, we will not be automating the transfer of your personal data to the new site. Instead, we will be asking you to visit the site and register directly to claim your online access. This is one-time only and will only take you a few minutes.

Your new free online access offers you:
• Quick search • Basic and advanced search form • Search within search results • Save search • Articles in press • Export citations • E-mail article to a friend • Flexible citation display • Multimedia components • Help files • Issue alerts & search alerts for your journal

http://www.trends.com/claim_online_access.htm