Oxysterols
Friends, Foes, or Just Fellow Passengers?
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Abstract—Oxysterols are oxygenated derivatives of cholesterol that are intermediates or even end products in cholesterol excretion pathways. Because of their ability to pass cell membranes and the blood-brain barrier at a faster rate than cholesterol itself, they are also important as transport forms of cholesterol. In addition, oxysterols have been ascribed a number of important roles in connection with cholesterol turnover, atherosclerosis, apoptosis, necrosis, inflammation, immunosuppression, and the development of gallstones. According to current concepts, oxysterols are physiological mediators in connection with a number of cholesterol-induced metabolic effects. However, most of the evidence for this is still indirect, and there is a discrepancy between the documented potent effects of oxysterols under in vitro conditions and the studies demonstrating that they are of physiological importance in vivo. Oxysterol-binding proteins, such as liver X receptor-α (a nuclear receptor), do have a regulatory role in cholesterol turnover, but the physiological ligand of the protein has not yet been defined with certainty. Recently developed genetically engineered mouse models with markedly reduced or increased concentration of some of the oxysterols have exhibited surprisingly small changes in cholesterol turnover and homeostasis. The present review is a critical evaluation of the literature on oxysterols, in particular, the in vivo evidence for a role of oxysterols as physiological regulators of cholesterol homeostasis and as atherogenic factors.

Key Words: atherosclerosis ■ cholesterol homeostasis ■ CYP7A1 ■ CYP27A1 ■ CYP46

Oxysterols are oxygenated derivatives of cholesterol that are important as intermediates or end products in cholesterol excretion pathways. With few exceptions, introduction of an oxygen function in the cholesterol molecule drastically reduces the half-life of the molecule and directs it to excretion or to further oxidation to water-soluble bile acids. The rapid degradation and excretion of oxysterols are facilitated by their physical properties, allowing them to pass lipophilic membranes and to be redistributed in the cell at a much faster rate than cholesterol itself. In view of this, it is understandable that the metabolic control of cholesterol catabolic pathways is assigned to specific monoxygenases.

It should be emphasized that the oxysterols occurring in biological membranes and lipoproteins are normally present in trace amounts and are always together with a great excess (10³- to 10⁶-fold) of cholesterol. The addition of pure oxysterols to different in vitro systems is clearly a highly unphysiological situation, and all results of such studies must be evaluated with caution.

On the basis of their potent biological effects mainly studied in vitro, oxysterols have been ascribed a number of important roles in connection with cholesterol turnover, atherosclerosis, apoptosis, necrosis, inflammation, immunosuppression, and the development of gallstones (see reviews²—⁷).

More than 20 years ago, Kandutsch et al⁶ formulated the so-called oxysterol hypothesis, suggesting that most or all of the suppressive effect of cholesterol on its own synthesis may be mediated by oxysterols. According to current concepts, the effect of cholesterol on its own catabolism to bile acids may also be mediated by oxysterols. The discovery of nuclear receptors (the liver X receptors [LXRs]) with the ability to bind oxysterols with high affinity has given some recent support to the oxysterol hypothesis.²⁸ On the other hand, the evidence for important regulatory roles of oxysterols in vivo is largely indirect.

The present review is a general overview of the quantitatively most important oxysterols present in tissues and the circulation. In addition, we evaluate the oxysterol hypothesis in relation to recent work on nuclear receptors, transgenic animal models, and inherited metabolic disorders. We also discuss the possible role of oxysterols in connection with the development of atherosclerosis.

Definition and Structures of Oxysterols
In the present review, oxysterols are defined as oxygenated derivatives of cholesterol (or precursors to cholesterol) that may be formed directly by autoxidation or by the action of a specific monoxygenase or that may be secondary to enzymatic or nonenzymatic lipid peroxidation. The various structures that will be referred to in the text are depicted in Figure 1 and are referred to by trivial names.
Analysis of Oxysterols

With few exceptions (eg, atheromas), oxysterols are present in trace amounts; excess of cholesterol is seldom <1000-fold. Because of the great excess of cholesterol and the possibility of artifactual formation of several oxysterols during isolation and workup procedures, accurate analyses are difficult. In the past, different groups have reported levels of some oxysterols that differ by >2 orders of magnitude. Even when all possible precautions are taken to avoid autoxidation during the isolation, there is always some uncertainty with respect to the origin of, for example, 7β-hydroxycholesterol, 7-oxocholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol, and cholestane-3β,5α,6β-triol. That at least the first 3 of the above oxysterols may be formed in vivo has been convincingly shown by incorporation of $^{18}$O$_2$ in oxysterol after the exposure of rats to $^{18}$O$_2$.

The most accurate technique available for assay of the oxysterols involves isotope dilution–mass spectrometry, with the use of individual deuterium-labeled analytes as internal standards.

Oxysterols as Intermediates in Cholesterol Excretion Pathways: Origin of the Quantitatively Dominating Oxysterols in the Circulation

From a quantitative point of view, the most important oxygenation reactions are those involved in the early steps in the conversion of cholesterol into bile acids. An overview of these reactions is given in Figure 2. The products of these cytochrome P-450–mediated oxygenations are found in the circulation as dominating oxysterols. In addition to the critical hydroxylations of cholesterol leading to the normal bile acids, there is a cytochrome P-450–mediated oxygenation of cholesterol leading to a quantitatively important oxysterol in the circulation, 4β-hydroxycholesterol. The metabolic end product of this steroid has not yet been defined.
The major oxysterols in the circulation are transported by lipoproteins, and their distribution between different lipoprotein fractions is similar to that of cholesterol. An exception is cholestenoic acid, which is transported entirely in the lipoprotein-free fraction.

7α-Hydroxycholesterol

The classical and quantitatively most important pathway for bile acid synthesis starts with a 7α-hydroxylation of cholesterol. This pathway is under strict metabolic control, and there are multiple mechanisms involved in the regulation of the rate-limiting hepatic cytochrome P-450 enzyme, cholesterol 7α-hydroxylase (CYP7A1), by hormonal and dietary factors. Under normal conditions, the most important regulation of this enzyme is a negative-feedback suppression caused by bile acids reabsorbed from the intestine and reaching the liver via the portal vein. Details are now known about the transcription factors involved in this regulation (see Figure 2), although important, not-yet-defined, species-related differences seem to exist. One of the regulatory mechanisms possibly involving oxysterols (see Figure 3) will be discussed more in detail below.

The 7α-hydroxycholesterol present in the circulation corresponds to a “leakage” from the liver, and as judged by measurements in the hepatic artery and hepatic vein, the secretion of this oxysterol from the human liver seems to be about the same as the uptake. The concentration of 7α-hydroxycholesterol in the circulation reflects the activity of CYP7A in the liver and can, in fact, be used as an in vivo marker for this activity.

27-Hydroxycholesterol and Cholestenoic Acid

In addition to the 7α-hydroxylase pathway, there is an alternative “acidic” pathway, starting with the introduction of a hydroxyl group at the terminal methyl group (C27 position). In contrast to CYP7A1, the

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**Figure 2.** Major oxysterols in the circulation: formation and elimination.

**Figure 3.** Suggested mechanism for regulation of cholesterol 7α-hydroxylase and its relation to cholesterol synthesis and bile acid turnover (see Russell and Repa and Mangelsdorf). According to this suggested mechanism, the LXR is involved in an oxysterol-dependent feedforward regulation of CYP7A1 in rodents. Expansion of the bile acid pool activates the receptor FXR, which leads to a suppressed transcription of the CYP7A1 gene. This effect is not a direct interaction of FXR with a bile acid responsive element in the promoter of the CYP7A1 gene but is mediated by at least 2 other factors. Expansion of the cholesterol pool is assumed to lead to increased levels of oxysterols with subsequent reduced synthesis of cholesterol and increased degradation of cholesterol into bile acids. It should be emphasized that there is no direct experimental evidence for the role of oxysterols in this model.
cytochrome P-450 that is involved in this conversion, CYP27A1, has a broad substrate specificity and is present in most tissues and not only in the liver. The enzyme is capable of oxidizing the C27-methyl group not only into a CH3OH group but also into a carboxylic acid (cholestenoic acid). Although the alternative acidic pathway may be predominantly localized in the liver, there is a continuous flux of 27-oxygenated cholesterol metabolites from extrahepatic sources to the liver.21 It has been calculated that 5% to 10% of the total conversion of cholesterol into bile acids starts with an extrahepatic 27-hydroxylation.21,22 In addition to initiating the pathway starting with 27-hydroxylation, CYP27A1 is an important enzyme in connection with the conversion of the C-27 steroid side chain of cholesterol into the C-24 steroid side chain of bile acids. However, there are alternative mechanisms for degradation of the steroid side chain that are independent on the sterol 27-hydroxylase.23

Numerous regulatory mechanisms involving negative feedback by bile acids have also been described for CYP27A1.24–26 In a preliminary study from our laboratory, no clear effect on hepatic messenger RNA levels was seen in human subjects treated with chenodeoxycholic acid or chenodeoxycholic acid.27 That CYP27A1 is less sensitive to regulatory factors is also evident from the fact that the plasma levels of the product, 27-hydroxycholesterol, are relatively stable under different conditions (I. Björkhem, unpublished data, 2001).

### 24S-Hydroxycholesterol

The cytochrome P-450 responsible for formation of 24S-hydroxycholesterol, cholesterol 24S-hydroxylase (CYP46), is almost exclusively located in the brain in humans,28,29 and most or all of the 24S-hydroxycholesterol present in human circulation is thus derived from the brain. However, the situation is different in the mouse.30 There is a continuous flux of 24S-hydroxycholesterol across the blood-brain barrier into the circulation, and evidence has been provided that this flux is of importance for the homeostasis of brain cholesterol.31,32 Thus, the levels of 24S-hydroxycholesterol in the circulation can be used as a marker for the turnover of cholesterol in the brain.33 Although the mechanisms regulating the expression and activity of CYP46 have not been extensively studied, in general, the levels of 24S-hydroxycholesterol in the circulation appear to be remarkably stable.

It has recently been shown that only about half of the 24S-hydroxycholesterol formed in humans is converted into bile acids. The other half of it is excreted from the liver in conjugation with sulfuric acid and glucuronic acid. Part of it is ω-hydroxylated before conjugation and excretion.33

### 4β-Hydroxycholesterol

Very recently, it was shown that this oxysterol is formed by the action of cytochrome P-450 3A4 (CYP3A4) on cholesterol.15 At the present state of knowledge, the possibility that also other cytochrome P-450 enzymes are involved cannot be excluded. Preliminary experiments in our laboratory have shown that the rate of elimination of 4β-hydroxycholesterol is very low, much lower than that for the other circulating oxysterols. This may be the explanation for the relatively high concentration of this oxysterol in the circulation, in spite of a low rate of formation. The metabolic end products of cholesterol 4β-hydroxycholesterol have not yet been defined.

### Oxysterols Present in Minor Quantities in Circulation and/or Specific Tissues

25-Hydroxycholesterol is present in very low concentrations in the circulation and in tissues. It has been clearly shown that it is enzymatically formed in vivo,12,24 and the enzyme responsible for its formation has recently been characterized.33 Surprisingly, the cholesterol 25-hydroxylase is not a cytochrome P-450 but belongs to a smaller family of non-heme iron–containing proteins. The enzyme is expressed in most tissues, as judged by mRNA blot,35 24,25-Epoxysterols and 24,25-epoxylanosterol have been suggested to be potential regulatory oxysterols.36–38 According to 1 report, 24,25-epoxycholesterol is present in surprisingly high levels in the human liver (>2 μg/g liver).58 However, this has not been confirmed by other groups, and in an investigation from our group, the level of 24,25-epoxysterol in the mouse liver was found to be <25 ng/g.39

### Oxysterols as Regulators of Cholesterol Homeostasis

That cholesterol synthesis in isolated cultured cells is suppressed by cholesterol added to the medium is very well documented. Kandutsch et alβ found that purified cholesterol was only weakly inhibitory, and they suggested that most of the inhibitory actions on the cells are due to oxysterols rather than to cholesterol itself. The inhibitory potency of some oxysterols was found to be orders of magnitude higher than that of cholesterol itself. 25-Hydroxycholesterol was found to be the most efficient inhibitor, and since then, 25-hydroxycholesterol, alone or in combination with cholesterol, has been the most important tool to demonstrate steroid-sensitive biochemical effects in cultured cells. In most of these experiments, highly unphysiological concentrations of the free oxysterol have been used.

Under in vitro conditions, oxysterols are able to regulate key enzymes in cholesterol turnover at transcriptional and posttranscriptional levels (see review40). The genes affected at the transcriptional level by oxysterols all have a sterol-responsive element (SRE) within the 5′-flanking region. The SRE-containing genes are controlled by a sterol-regulated cleavage of the SRE-binding proteins (SREBP1a, SREBP1c, and SREBP2). Sterol-sensitive genes are responsible for the uptake (LDL receptor) and synthesis (3-hydroxy-3-methylglutaryl [HMG]-coenzyme A [CoA] synthase, HMG-CoA reductase, farnesyl diphosphate synthase, and squalene synthase) of cholesterol. Sterol-sensitive genes are also of importance for fatty acid synthesis and desaturation (fatty acid synthase, acetyl CoA carboxylase, and stearoyl CoA desaturase) as well as triglyceride synthesis (glycerol-3-phosphate acyltransferase). At the posttranscriptional level, oxysterols accelerate degradation of HMG-CoA reductase and activate acyl CoA:cholesterol acyltransferase by a mechanism that is independent of gene transcription and protein synthesis. The gene coding for the rate-limiting enzyme in degradation of cholesterol into bile acids, CYP7A1, is transcriptionally regulated by a mechanism that has been suggested to involve oxysterols, at least in the rat (see Figure 3).
Mutant cells of different classes have been shown to resist the regulation of HMG-CoA reductase by LDL and 25-hydroxycholesterol, consistent with a common mechanism for downregulation of cholesterol synthesis by cholesterol itself and oxysterols. This has been suggested to be integrated at the level of the SREBP sterol-sensing domain. In view of this, use of 25-hydroxycholesterol may provide useful information about steroid sensitivity in spite of the fact that the conditions are unphysiological.

Although the results of the above type of experiments are consistent with a role of oxysterols in the normal regulation of cholesterol homeostasis, they do not prove the hypothesis. According to some experiments with cultured cells, a cytochrome P-450 system seems to be involved in the LDL-induced suppression of cholesterol synthesis and uptake. However, the specificity of the cytochrome P-450 inhibitors used in these in vitro experiments may be questioned, and it is difficult to draw firm conclusions. In 1 of these experiments, support was obtained for the contention that 24,25-epoxylanosterol is metabolized into an important regulator of cholesterol synthesis by a ketocamazole-sensitive cytochrome P-450. In another study, support was obtained for the contention that LDL-induced downregulation of HMG-CoA reductase in cultured fibroblasts is dependent on the formation of 27-hydroxycholesterol by CYP27A1,43 and genetically CYP27A1-deficient fibroblasts had a markedly reduced response to LDL cholesterol. In another study, however, CYP27A1-deficient and control fibroblasts responded similarly to suppression by LDL cholesterol.44

Saucier et al45 have shown that several oxysterols accumulate in the mouse liver after cholesterol feeding, and they suggested that these oxysterols, in particular 24S-hydroxycholesterol, are responsible for the downregulation of HMG-CoA reductase. With use of data on the potential of the accumulated oxysterols to suppress HMG-CoA reductase in an in vitro system, it was concluded that the amount of oxysterol that had accumulated in the liver after cholesterol feeding was sufficient to explain the suppression of cholesterol synthesis. However, in view of the unphysiological conditions used for testing the suppressive potential, the conclusion may be questioned. It may be noted that the most important suppressive oxysterol accumulated was 24S-hydroxycholesterol. There is little or no formation of this oxysterol in human liver.

On the basis of recent experiments involving feeding rats an atherogenic diet or the administration of mevalonate, Zhang et al57 concluded that 25-hydroxycholesterol and 24(S),25-epoxycholesterol, but not 27-hydroxycholesterol, may be of regulatory importance.

Attempts in our laboratory to demonstrate a critical role of the sterol 27-hydroxylase and the cholesterol 24S-hydroxylase in the downregulation of HMG-CoA reductase activity in the mouse liver in vivo have failed.56 According to another in vivo study using different analogues and deuterium-labeled derivatives of cholesterol, the presence of the 3β-hydroxyl group and of the 5,6-double bond in cholesterol is essential for the downregulation.57 The results also suggest that hydroxylations at positions 7, 24, and 27 are not critical for the downregulation. According to similar older experiments by Erickson and Nes, even complete elimination of the steroid side chain of cholesterol does not prevent its inhibitory effect on the downregulation of hepatic cholesterol synthesis in mice.

**Oxysterol-Binding Proteins**

The early observation that several oxygenated cholesterol derivatives are able to regulate HMG-CoA reductase led to a search for proteins that could mediate these effects. Kandutsch and colleagues characterized a protein that had affinities for different oxysterols that were correlated with the potency of the oxysterol to suppress HMG-CoA reductase in fibroblasts. The protein seems to be present in most or all animal cells, suggesting that some vital function may be associated with it. Under normal conditions, the oxysterol-binding protein is mainly present in the cytosol. Addition of 25-hydroxycholesterol leads to a marked change in the distribution of the protein, with the majority of it bound to the Golgi apparatus.51 The latter finding is consistent with a function of the protein for intracellular transport of the oxysterol or even excretion of it. However, whether or not oxysterols are the natural and most important ligands for the protein is not known with certainty.

The above-mentioned, first-described, oxysterol-binding protein was later shown to belong to a family with several members occurring in mammals and also in yeast.52–54 Some of these proteins seem to be involved in Golgi-derived vesicle transport. Interestingly, at least 1 member of this family does not bind oxysterols but is able to bind phospholipids (in particular, phosphatidic acid) with high affinity.53

Recently, orphan nuclear receptors have been shown to be of importance for cholesterol homeostasis. The LXRα (LXRα and LXRβ) and the farnesoid X receptor (FXR) bind and are also activated by oxysterols and bile acids, respectively. These receptors are able to regulate the expression of a number of genes involved in cholesterol metabolism (see reviews17,18,55). A prerequisite for the transcriptional activation by LXR and FXR is that the receptor forms a heterodimer with the retinoid X receptor.56

LXRα has been shown to mediate the transcriptional induction of cholesterol 7α-hydroxylase, which is the rate-limiting enzyme in the major pathway from cholesterol to bile acids. Mice with a disruption of the gene coding for LXRα fail to upregulate the cholesterol 7α-hydroxylase when they are fed a high cholesterol diet, causing accumulation of cholesterol in the liver.57,58 This does not occur in LXRβ-deficient mice. Because the human gene coding for cholesterol 7α-hydroxylase lacks identified LXR-responsive elements in the promoter, this regulatory mechanism probably does not exist in humans. LXRα is also involved in the regulation of the cholesterol transporters ABCA1 and ABCG1, which are involved in the flux of cholesterol from enterocytes and macrophages, respectively.55,59–62 LXR also seems to have a role in the regulation of human cholesterol ester transfer protein, which translocates cholesterol ester between lipoproteins.62

According to a recent study, 27-hydroxylation of cholesterol in cholesterol-loaded skin fibroblasts and monocyte-derived macrophages may activate LXR, resulting in increased activity of the cholesterol transporters ABCA1 and ABCG1.63 According to another study, however, 27-hydroxycholesterol is not an efficient activator of human LXR.7
The human LXRα gene is itself a target of the LXR-signaling pathway, and autoregulation of LXRα has been suggested to be an important way to amplify the cholesterol catabolic cascade.55

Although the importance of the LXRα receptor in the regulation of cholesterol homeostasis has been well established (at least in experimental animals), the physiological ligand of the receptor has not been defined with certainty. Among the oxysterols tested, the receptor was found to have its highest affinity for 24S-hydroxycholesterol and 24,25-epoxycholesterol.64 The former steroid is present in relatively high concentrations in the brain, whereas the latter has been reported to occur in relatively high levels in the liver, in some66,68 but not all9 studies. At the present state of knowledge, there is no direct experimental evidence that the above 24- and 24,25-oxygenated steroids are the physiological ligands. Interestingly, it has been shown that activation of LXR can be antagonized by some unsaturated fatty acids65 and geranylgeranyl pyrophosphate.66 It should be emphasized that the different steroids that are potential ligands to the receptor are present in compartments containing a great excess of cholesterol (104- to 105-fold). It remains to be established that the receptors are able to sense and selectively bind the oxysterol when they are exposed to such mixtures.

The LXRβ receptor has a broader distribution than the LXRα receptor, with a particularly high concentration in the brain. In view of the efficient binding of 24S-hydroxycholesterol to this receptor and the high concentration of 24S-hydroxycholesterol in the brain, it is tempting to suggest that there may be an important oxysterol-signaling pathway in the brain involving LXRβ. However, direct evidence for this is still lacking.

Genetically Engineered Animal Models and Metabolic Diseases With Altered Levels of Oxysterols

A number of genetically engineered animal models have provided interesting information concerning the role of some of the oxysterols suggested to be of regulatory importance (for an excellent review, see Russell67).

Mice deficient in sterol 27-hydroxylase have a complete lack of 27-hydroxycholesterol and cholestenoic acid.68,69 These mice have normal levels of cholesterol in the circulation, suggesting that 27-hydroxycholesterol is not an obligatory factor for the regulation of cholesterol homeostasis. Because of the fact that the sterol 27-hydroxylase is needed for the normal synthesis of bile acids, there are a number of changes that are related to the reduced formation of bile acids, such as reduced cholesterol absorption and compensatory increase in cholesterol synthesis. Thus, the mRNA for the cholesterogenic transcription factor SREBP-2 and mRNAs for SREBP-2-regulated cholesterol biosynthetic genes are elevated in the livers of the transgenic animals. All these changes are reversed by feeding the animals cholic acid. Patients with the human disease cerebrotendinous xanthomatosis (CTX), in which sterol 27-hydroxylase is deficient, are also normolipidemic.70 In contrast to the situation in mice, there is an accumulation of cholesterol and cholesterol in the human disease, which is probably partially due to the lack of the transport function of the sterol 27-hydroxylase. Also, in humans, most or all of the biochemical changes due to the lack of CYP27A1 can be reversed by feeding with bile acids.

Mice deficient in the oxysterol-catabolizing enzyme oxysterol 7α-hydroxylase (Cyp7b) have markedly elevated levels of 27-hydroxycholesterol and 25-hydroxycholesterol.71 With the exception of a slight downregulation of cholesterol synthesis in the kidneys, the in vivo sterol biosynthetic rates were unaltered in several tissues in spite of the high levels of the oxysterols.71

The accumulated oxysterols were mainly in esterified form, and the possibility must be considered that an esterified oxysterol may be a less potent regulator than an oxysterol in the free form.71 The other possibility is that the well-documented ability of the side-chain oxidized oxysterols to downregulate genes in vitro is of little or no importance under in vivo conditions.

It may be mentioned that 1 fatal human case with a lack of the oxysterol 7α-hydroxylase has been reported.72 Also, in this case, there were high levels of 27-hydroxycholesterol and 25-hydroxycholesterol in the circulation. In addition, there were high levels of the bile acid 3β-hydroxy-5-cholenic acid. Whether the fatal outcome of the metabolic defect was due to the accumulation of this hepatotoxic bile acid or the hydroxysteroids is not possible to evaluate.

Although the above studies seem to exclude 27-hydroxycholesterol as an obligatory factor in cholesterol homeostasis, the possibility that 25-hydroxycholesterol is obligatory cannot be completely excluded at the present state of knowledge.

Oxysterols as Markers for Oxidative Stress

Oxidative modification of LDL is associated with an oxidation of cholesterol to yield 7- and 5,6-oxo-oxysterols. It has been suggested that the above oxysterols may be used as markers for in vivo lipoprotein oxidation. The great problems in connection with the analyses and the risk for artifactual formation of oxysterols from the parent cholesterol during the workup procedures make it difficult to draw conclusions from the results of several old studies with less accurate techniques. One recent study has demonstrated an association between plasma levels of 7β-hydroxycholesterol and the progression of carotid atherosclerosis.73 Long-term vitamin E supplementation reduced plasma levels of 7β-hydroxycholesterol.74 Plasma oxysterols have been reported to be higher in smokers than in nonsmokers.75 In view of the methodological problems, oxysterols do not appear to have clear merits in relation to other markers for oxidative modification of LDL.

Oxysterols and Atherosclerosis

On the basis of a great number of in vitro studies demonstrating cytotoxic effects on cultured endothelial cells and arterial smooth muscle cells, oxysterols have been suggested to be atherogenic. In most of the above-mentioned studies, highly unphysiological conditions have been used, and according to our opinion, it is not possible to draw valid conclusions from them about the role of the oxysterols under in vivo conditions.

In view of the fact that oxysterols mimic increasing concentrations of cholesterol, one would expect them to be atherogenic when they are fed to experimental animals. A great number of such studies have been carried out; most of these studies used highly unphysiological doses of the oxysterol (for an excellent review, see Brown and Jessup). Of 13 studies on the effect of dietary oxysterols, 6 indicated a proatherogenic effect, and 4
indicated an antiatherogenic effect, whereas 3 showed no clear-cut activity.\textsuperscript{2} It has been suggested that the markedly higher incidence of heart disease in Indians living in London compared with non-Indians in the same city may be due to their high consumption of ghee, which is known to contain very high concentrations of oxysterols.\textsuperscript{76} However, other explanations are possible, and there is a very low incidence of heart disease in India itself, where ghee is also an important part of the diet in many regions.\textsuperscript{2} At the present stage of knowledge, there is no direct evidence that dietary oxysterols contribute to atherogenesis in humans.

As pointed out above, oxidatively modified LDL contains elevated levels of 7- and 5,6-oxygenated oxysterols. The possibility cannot be excluded that some of these species may be of importance in atherogenesis. The primary 7-oxygenated product of cholesterol, 7-hydroperoxycholesterol, seems to be the most cytotoxic oxygenated lipid present in oxidized LDL.\textsuperscript{77} This oxysterol is rapidly decomposed into 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-oxocholesterol, which may be found in relatively high concentrations in foam cells and fatty streaks.\textsuperscript{78} Whether the 7-oxygenated steroids present in foam cells and atheromas are of pathogenetic importance or if they just reflect the uptake of oxidized LDL particles is not known. Interestingly, the accumulation of 7-oxocholesterol in macrophages seems to be prevented by the action of the sterol 27-hydroxylase.\textsuperscript{79}

7α-Hydroperoxycholesterol has been detected in low concentrations in atheromas. In view of the short half-life of this compound, the measurements are likely to underestimate the true levels. In view of its cytotoxicity, this oxysterol may be of pathogenetic importance.

The most dominant oxysterol in human atheromas is 27-hydroxycholesterol.\textsuperscript{80} As pointed out above, there is a continuous flux of 27-hydroxycholesterol and cholestenonic acid from peripheral cells to the liver; thus, the presence of 27-hydroxycholesterol in the atheromas may reflect a defense mechanism to prevent the accumulation of cholesterol.\textsuperscript{81,82} The fact that patients with CTX, lacking the sterol 27-hydroxylase, often develop premature atherosclerosis is in accordance with the hypothesis. The importance of the mechanism is underlined by the fact that CTX patients most often have normal levels of plasma cholesterol. The concentration of 27-hydroxycholesterol seems to be increased with the severity of the lesion, and the ratio between 27-hydroxycholesterol and cholesterol may be 100-fold higher in an atheroma than in the circulation. However, the “trapping” of 27-hydroxycholesterol and other oxysterols in the atheromas may be secondary to a high rate of esterification by the acyl CoA:cholesterol acyltransferase enzyme, and oxysterols are known to be a better substrate for this enzyme than cholesterol. Almost all of the oxysterols in the atheromas are thus esterified.

**General Conclusions**

Oxysterols are important intermediates in the elimination of cholesterol in the liver and in extrahepatic tissues. They are also important as transport forms of cholesterol over cell membranes and the blood-brain barrier.

At present, there is still only indirect evidence of the important role of oxysterols in the normal regulation of cholesterol homeostasis. Soluble and nuclear oxysterol-binding proteins exist with a very high affinity for oxysterols, but the physiological ligands for these proteins have not yet been defined with certainty. At the present state of knowledge, the possibility cannot be excluded that cholesterol itself binds or interferes with the binding of another compound to the receptor. Even if the affinity of the receptors to cholesterol is very low, this may be compensated for by the fact that cholesterol is present in concentrations 10\textsuperscript{-1} to 10\textsuperscript{-5} fold higher than that of the specific oxysterol. At least under some experimental conditions, the effect of a specific oxysterol has been shown to be reduced by moderate dilution with cholesterol.\textsuperscript{83} Recent experiments with transgenic animals seem to exclude some specific hydroxylations in the steroid side chain as being critical for cholesterol homeostasis.

With respect to the development of atherosclerosis, the CYP27A1-mediated flux of 27-hydroxycholesterol to the liver may be regarded as an antiatherogenic mechanism that is able to reduce the accumulation of cholesterol. However, the relative importance of this mechanism in relation to HDL-mediated reversed cholesterol transport is not known. There is a possibility that the cytotoxic and unstable oxysterol 7-hydroperoxycholesterol, formed in connection with oxidative modification of LDL, is of some importance as a pathogenetic factor in atherogenesis.

As judged from the results of animal experiments, the normal dietary intake of oxysterols is probably of little or no importance in the development of atherosclerosis.

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