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Regulation of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity by Side-Chain Oxysterols and Their Derivatives

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I. INTRODUCTION

The biosynthesis of oxysterols in mammalian systems has been intensely studied for decades.[1–3] The continuing interest in the cholesterol biosynthetic pathway has intensified following clinical observations that the incidence of cardiovascular disease is greater in individuals with higher than normal levels of serum cholesterol.[4,5] More recently, the results of numerous clinical studies have indicated that the lowering of serum cholesterol levels may reduce the risk of coronary heart disease[6] and even promote the regression of atherosclerotic lesions.[6–8] Serum cholesterol levels can often be controlled by restricting the dietary intake of cholesterol. However, with a significant number of patients, this approach does not result in the reduction of serum cholesterol concentrations to lower levels. This makes the development of other approaches to control serum cholesterol levels an imperative goal of cardiovascular research.[4,9]

A more recent approach to this problem has been the development of inhibitors of cholesterol or sterol biosynthesis. A key regulatory enzyme of cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.134) which catalyzes the reductive deacylation of HMG-CoA to yield mevalonate (Figure 1).[9,10] The addition of highly purified cholesterol to cultured cells does not affect the activity of HMG-CoA reductase or the rate of sterol biosynthesis.[11,12] However, various oxysterols have been shown to be potent suppressors of HMG-CoA reductase activity resulting in marked reductions of sterol biosynthesis.[13] These observations led Kandutsch and coworkers[13,14] to hypothesize that oxysterols, rather than cholesterol, may function as the natural regulators of HMG-CoA reductase activity and sterol biosynthesis.

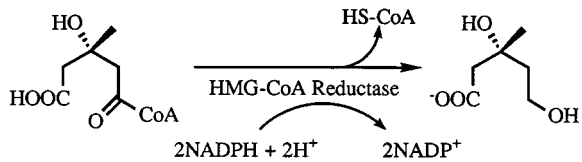

Figure 1 The enzymatic reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate by HMG-CoA reductase.

Sterols bearing a second oxygen function in addition to that at carbon-3 are termed oxysterols and have been demonstrated to possess diverse biological activities.[9,15–17] Some of these include cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia, and various effects on specific enzymes. In addition, several oxysterols have been isolated from drugs used in folk medicine for the treatment of cancer.[18–20] Other studies have shown that certain oxysterols have significant activity in the inhibition of DNA synthesis in cultured cells.[21,22]

A significant property of many oxysterols is their ability to repress HMG-CoA reductase activity in cultured mammalian cells.[9,15,23] This suppression of activity has been found to vary over a wide range, depending on the structural features of the oxysterol. As a general trend, inhibitory activity increases as the distance between carbon-3 and the second oxygen group becomes greater. Sterols with an additional oxygen function in ring D and the side chain have been shown to have the highest activity. An intact side chain is a requirement for potent activity a decrease in the length of the (iso-octyl) side chain results in decreased activity.[24] Other noticeable trends indicate a relationship between inhibitory activity and the extent to which the second oxygen function is sterically hindered. In general, axial hydroxyl groups are more hindered and possess lower activities than the less hindered equatorial conformation.[9,24,25] Steric hindrance from other parts of the steroid molecule can result in diminished activity (i.e., effect of carbon-14 alkyl substituents on the carbon-15 hydroxyl group).[26] It has been suggested that oxygen functions in conformationally flexible positions, such as those in ring D and in the side chain, produce more inhibitory steroids due to increased effectiveness of hydrogen bonding or hydrophilic interactions with receptor molecules.[17]

Oxysterols having the second oxygen function in the side-chain region of the sterol structure are termed side-chain oxysterols. As a class of compounds, side-chain oxysterols are known to be potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key regulatory enzyme in the biosynthesis of sterols. Structural variations in the side-chain oxysterols influence enzyme inhibition. Under certain conditions biological systems have been induced to produce side-chain oxysterols, adding support to the hypothesis that oxysterols may be natural regulators of sterol biosynthesis in the intact cell. The specific inhibition of sterol biosynthesis is of special interest since it may prove useful in the prevention of reversal of certain cardiovascular diseases and may also prove to be useful in the control of normal and abnormal cell growth.

II. THE OXYSTEROL REGULATION HYPOTHESIS

In the preceding section we have described evidence which suggests a regulatory mechanism which, by analogy to steroid hormone receptors and bacterial induction-repression systems, requires a binding protein to recognize oxysterols and to mediate subsequent cellular events. There is evidence for the existence of a specific cytosolic receptor protein for oxysterols.[24,27] After the activities of a number of sterols were evaluated, a good correlation was found between the actions of certain oxysterols on HMG-CoA reductase in L cells and their affinity for an oxysterol binding protein.[24] Moreover, the actions of oxysterols which depress the rate of cholesterol biosynthesis from lanosterol and possibly inhibit the 14-demethylation of lanosterol are also postulated to exert their actions by an oxysterol binding protein.[23,28,29]

The most frequently utilized side-chain oxysterol, in the study of HMG-CoA reductase regulation, is cholest-5-ene-3β,25-diol (25-hydroxycholesterol). This oxysterol is a potent inhibitor of enzymatic activity (IC_{50} <1μ*M*).[11,13,14,25] Repression of the synthesis of immunoprecipitable HMG-CoA reductase by this oxysterol has been demonstrated by Faust et al.[30] in a Chinese hamster ovary (CHO)-derived compactin-resistant cell (UT-1) line that overproduces the enzyme and by Sinensky et al.[31] in CHO cells. Luskey et al.[32] have shown that the level of mRNA for the reductase in UT-1 cells is reduced in the presence of the oxysterol. Similar results were obtained by Kandutsch et al. in wild-type CHO cells.[11,13,24] These results were utilized by Kandutsch and Thompson,[33] who proposed the following model for the regulation of HMG-CoA reductase activity by oxysterols: The oxysterol binds to a cellular protein and the resultant oxysterol-protein complex then acts to repress transcription of the gene for reductase production. In support of this model, Kandutsch *et al*. have isolated a protein which exhibits high affinity and low capacity for 25-hydroxycholesterol and other oxysterols which are known to suppress reductase activity.[24,25,27,34] Although 25-hydroxycholesterol has been isolated from cells, there is no direct evidence that this oxysterol functions as the natural regulator of cholesterol biosynthesis.[35] This model (Figure 2) was in direct contrast to the prevailing view that cholesterol, accumulating in cells after endocytosis of low density lipoprotein (LDL) (containing cholesteryl esters), is the regulatory messenger molecule.[36]

III. THE DIOXIDOSQUALENE PATHWAY

Primary metabolism in mammalian systems is known to produce side-chain oxysterols. Derivatives of cholesterol hydroxylated in the 25- or 26-positions are produced in liver during bile acid biosynthesis and side-chain hydroxylation at the 20α– and 22 *R*-positions in the initial step of the conversion of

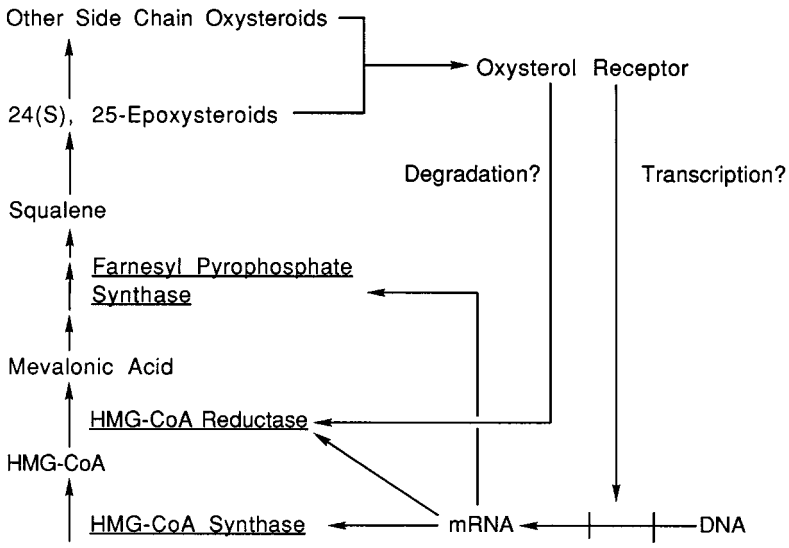

Figure 2 The Kandutsch model for the oxysterol regulation of sterol biosynthesis. (Adapted from References 45 and 46.)

cholesterol to steroid hormones in endocrine organs.[37–39] Another mode of oxysterol biosynthesis has been described which utilizes the isopentenoid pathway to produce side-chain derivatives of cholesterol and lanosterol (Figure 3).[40,41] Such compounds are derived from squalene 2,3-epoxide by the introduction of a second oxygen function to form 2,3,22,23-dioxidosqualene prior to cyclization. Thus, this intermediate has been shown to form 24(*S*),25-epoxylanosterol, 24(*S*),25-epoxycholesterol, and 25-hydroxycholesterol in mammalian systems.[42–45] 24(*S*),25-Epoxycholesterol has been isolated from cultured mouse L cells, Chinese hamster lung fibroblasts, and human liver.[35] These oxygenated side-chain derivatives have been shown to be potent inhibitors of HMG-CoA reductase and sterol biosynthesis, and possess a high affinity for the oxysterol binding protein.[35,41–44] These results add further support to the hypothesis that oxysterols may be natural regulators of cholesterol biosynthesis in mammalian cells.[10,13] This dioxidosqualene pathway has recently been reviewed.[45–47] Also, its occurrence in plants, animals, and microorganisms[48,49] and its evolution in a variety of organisms have been reviewed.[50]

IV. REGULATION OF HMG-CoA REDUCTASE

Sterol biosynthesis is controlled and catalyzed by the major and regulatory enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Therefore, much of the effort directed towards the development of inhibitors of sterol biosynthesis has focused on this enzyme.[4,51,52]

Collectively, oxysterols are not known to be significant inhibitors of the HMG-CoA reductase found in plant tissues.[53–55] However, in intact animal cells, oxysterols are potent inhibitors of reductase activity.[9,12,24] This response requires intact cells and active protein synthesis.[56] It has recently been attributed to a decrease in the rate of HMG-CoA reductase synthesis[30,31,57] and under some,[30,58] but not all, conditions to an increase in the rate of enzyme degradation. Side-chain oxysterols, which are products of primary metabolism, are among the most potent inhibitors of enzymatic activity. This high activity may be a result of their proposed role as natural regulators of cholesterol biosynthesis in mammalian systems.

It has been shown that the inhibitory activity of these compounds may vary in different cell lines. In this report we have attempted to combine the results from a number of different studies and organize these results according to the type of reported cell line.

The suppression or inhibition of HMG-CoA reductase actively L cells (a subline of NCTC clone 929 mouse fibroblasts) by a number of side-chain oxysterols is presented in Table 1, as collected from published reports.[23,35,60] In general, sterols with an intact iso-octal side chain and a 3β-hydroxyl group showed more potent inhibitory activity.[24,35] Also in general terms, sterols with a 3-ketone group showed somewhat less activity than those with a 3β-hydroxyl group.[24] It is known that 3-keto sterols with a

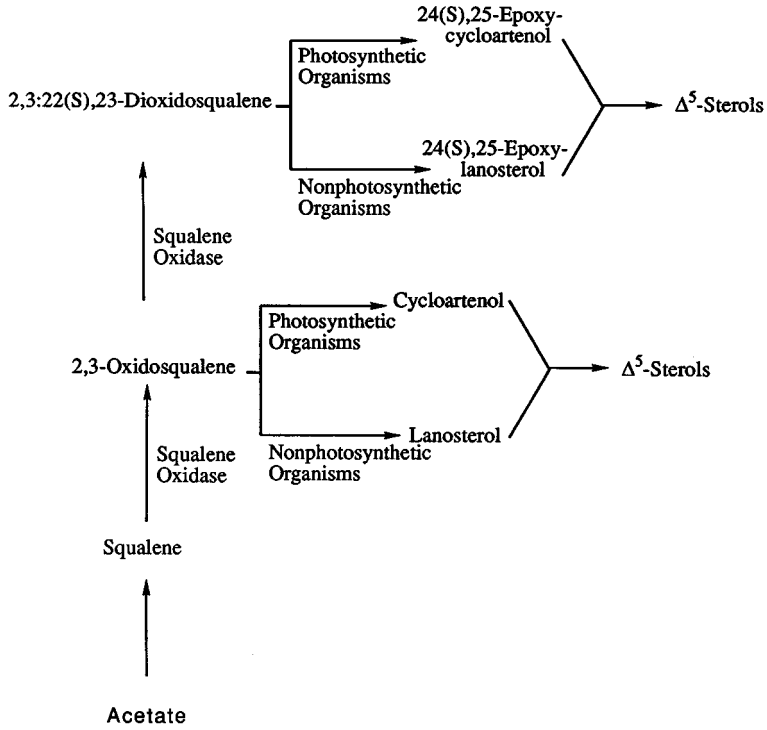

Figure 3 The biosynthesis of sterols and side-chain oxysterols in plants, animals, and microorganisms.

saturated A ring are efficiently reduced in L cell cultures to the alcohols while Δ^4-3-ketones are not reduced and accumulate as the conjugated ketones exhibiting less activity.[24]

These types of results have been reported in other cell lines. Table 2 presents the results of inhibition studies carried out with primary cultures of fetal mouse liver cells.[35] Although the overall values for reductase inhibition are somewhat lower than those reported for L cells (Table 1), the general trends in inhibition activity remain similar. Side-chain oxysterols with intact iso-octyl side chains exhibit the greatest inhibition. The most potent oxysterol in these series was cholest-5β-ene-3,25-diol (15-hydroxycholesterol).

In a like manner, the effect of side-chain oxysterols on the reductase found in rat intestinal epithelial cells (IEC-6, CRL-1592) are presented in Table 3.[43,61] In these studies, lanost-8-ene-3β,25-diol was the most potent inhibitor of enzymatic activity. Chemical oxidation to the 3-keto derivative resulted in a significant reduction in reductase activity.[43] The authors also reported that dioxidosqualene was cyclized to 24(*S*),25-epoxylanost-8-en-3β-ol and metabolized to 24(*S*),25-epoxylanost-8-en-3β-ol in this cell line.[43]

Recently, (25*R*)-5α-cholest-8(14)-ene-3β,26-diol-15-one was found to be a potent inhibitor of reductase CHO-K1 cells.[62] At a concentration of 0.1 μ*M*, this oxysterol caused a 50% reduction in enzymatic activity. Its potency was indistinguishable from that of its analog devoid of the carbon-26 hydroxyl group, 3β-hydroxy-5α-cholest-8(14)-en-15-one. Interestingly, 3β-hydroxy-5α-cholest-8(14)-en-15-one and (25*R*)-cholest-5α-ene-3β,26-diol (both potent inhibitors of reductase) have been found to shown synergism in the reduction of the levels of reductase activity in CHO-K1 cells.[63] When equimolar concentrations of these were added together, synergistic reduction of enzyme activity was observed at total oxysterol concentrations of 0.1 μ*M*, 0.2 μ*M*, and 0.5 μ*M*. Maximal synergistic effect in lowering of reductase activity (28% greater than that predicted) was observed at 0.1 μ*M* total oxysterol concentrations.

In other recent studies, 25-fluorocholesterol was prepared by treatment of 25-hydroxycholesterol with hydrogen fluoride-pyridine and its inhibition of HMG-CoA reductase in CHO-K1 cell studied.[64] It was thought that the 25-fluoresterol might behave like a hydroxyl group but unlike a proton. In this study 25-hydroxycholestrol caused a 66% lowering of reductase activity at 0.1 μ*M*, whereas the 25-fluorosterol had no effect. At a concentration 25 times higher (2.5 μ*M*), the 25-fluorosterol caused only a 43% suppression of enzymatic activity.

In a related report, 25,20,20,26,27,27,27-heptafluorocholesterol was prepared in eight steps from 3α,6α-diacetoxy-5β-cholanic acid.[65] It had little or no effect on the levels of HMG-CoA reductase

Table 1 The Side-Chain Oxysterol Inhibition of HMG-CoA Reductase Activity in L Cells

Oxysterols	Inhibition of Reductase Activity (μ*M*, IC_{50})
Cholest-5-ene-3β,25-diol	0.17
(25*S*)-Cholest-5-ene-3β,26-diol	0.16
(25*R*)-Cholest-5-ene-3β,26-diol	0.26
Cholest-5-ene-3β,20α-diol	0.30
3β,25-Dihydroxycholest-5-en-7-one	0.48
27-Norcholest-5-ene-3β,20α-diol	0.77
27-Norcholest-5-ene3β,25-diol	0.86
24(*S*),25-Epoxycholest-5-en-3β-ol	0.89
3β-Hydroxycholest-5-en-24-one	1.0
3β-Hydroxy-27-norcholest-5-en-25-one	1.2
26,27-Bisnorcholest-5-ene-3β,20α-diol	1.2
3β-Hydroxycholest-5-en-22-one	1.4
25-Hydroxycholesta-4,6-dien-3-one	1.8
3β-Hydroxylanost-8-en-24-one	1.8
Cholest-5-ene-3β,22(*S*)-diol	1.9
25-Hydroxycholest-4-en-3-one	3.5
20-Pentylprega-5-ene-3β,20α-diol	5.4
20-Butylprega-5-ene-3β,20α-diol	8.2
25,26,27-Trinorcholest-5-ene-3β,20α-diol	9.7
25-Hydroxycholesta-3,5-dien-7-one	10
20-Propylpregn-5-ene-3β,20α-diol	>30.0
20-Ethylpregn-5-ene-3β,20α-diol	>30.0
20-Methylpregn-5-ene-3β,20α-diol	—
Pregn-5-ene-3β,20α-diol	—

Data from References 24, 35, and 60.

Table 2 The Side-chain Oxysterol Inhibition of HMG-CoA Reductase Activity in Primary Cultures of Liver Cells

Oxysterol	Inhibition of Reductase Activity (μ*M*, IC_{50})
Cholest-5-ene-3β,25-diol	3.0
Cholest-5-ene-3β,20α-diol	3.2
Cholest-5-ene-3β,22(S)-diol	5.8
Cholest-5-ene-3β,22(R)-diol	7.5
20-Pentylprega-5-ene-3β,20α-diol	10.0
27-Norcholest-5-ene-3β,25-diol	26.0
3β-Hydroxycholest-5-en-24-one	62.0
20-Butylprega-5-ene-3β,20α-diol	75.0
3β-Hydroxy-27-norchcholest-5-en-25-one	>75.0

Data from Reference 35.

Table 3 The Side-Chain Oxysterol Inhibition of HMG-CoA Reductase Activity in Rat Intestinal Epithelial Cells

Oxysterol	Inhibition of Reductase Activity (μ*M*, IC_{50})
Lanost-8-ene-3β,25-diol	0.10
24(*S*),25-Epoxylanost-8-en-3β-ol	0.24
24(*S*),25-Epoxycholest-5-en-3β-ol	0.30
25-Hydroxylanost-8-en-3-one	0.30

Data from References 44 and 59.

activity in CHO-K1 cells over the range of concentrations studied (0.1 μ*M* to 2.5 μ*M*). The F_7-cholesterol only showed a marginal (12%) lowering of reductase activity at 2.5 μ*M*.

V. CONCLUSIONS

We have reviewed studies on the side-chain oxysterol repression of HMG-CoA reductase activity in various mammalian cell lines. We have also included examples of a number of derivatives. These results have provided much useful information concerning the possible role of side-chain oxysterols as natural regulators of reductase activity and thus sterol biosynthesis. These studies have contributed to our basic knowledge concerning the regression of cholesterol biosynthesis in general. Continued basic research in this area may provide further leads for the development of new technology useful in the control of cholesterol biosynthesis and the reduction of serum cholesterol levels.

REFERENCES

1. **Nes, W. R. and McKean, M. L.,** *Biochemistry of Steroids and Other Isopentenoids*, University Park Press, Baltimore, MD, 1981, 1.
2. **Gibbons, G. F., Mitropoulos, K. A., and Myant, N. B.,** *Biochemistry of Cholesterol*, Elsevier Biomedical, Amsterdam, 1982, 122.
3. **Goldstein, J. L. and Brown, M. S.,** Biological activities of oxygenated sterols, *Nature*, 243, 425, 1990.
4. **Brown, M.S., Faust, J. R., Goldstein, J. L., Kaneko, I., and Endo, A.,** Induction of 3-hydroxy--3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase, *J. Biol. Chem.*, 253, 1121, 1978.
5. Lipid Research Clinics Program, *JAMA*, 251, 351, 1984.
6. Lipid Research Clinics Program, *JAMA*, 251, 365, 1984.
7. **Blankenhorn, D. H., Nessim, S. A., Johnson, R. L., Sanmarco, M. E., Azen, S. P., and Cashin-Hemphill, L.,** Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous by-pass grafts, *JAMA*, 257, 3233, 1987.
8. **Parish, E. J., Nanduri, U. B. B., Seikel, J. M., Kohl, H. H., and Nusbaum, K. E.,** Synthesis and hypocholesterolemic activity of 3β-hydroxycholest-8-en-7-one and 3β-hydroxycholest-8-en-11-one, *Steroids*, 48, 407, 1986.
9. **Schroepfer, G. J., Jr.,** Sterol biosynthesis, *Annu. Rev. Biochem*., 50, 585, 1981.
10. **Schroepfer, G. J., Jr.,** Sterol biosynthesis, *Annu. Rev. Biochem*., 51, 555, 1981.
11. **Kandutsch, A. A. and Taylor, F. R.,** Control of de novo cholesterol biosynthesis, in *Lipoprotein and Cholesterol Metabolism in Steroidogenic Tissues*, Straus, J. F. and Menon, K. M. J., Eds, George F. Stickley Co., Philadelphia, 1985, 194.
12. **Gibbons, G. F.,** Molecular control of *3-Hydroxy-3-methylglutaryl Coenzyme A Reductase*: The role of oxygenated sterols, in *3-Hydroxy-3-methylglutaryl Coenzyme A Reductase*, Sabine, J. R., Ed, CRC Press, West Palm Beach, FL, 1983, 153.
13. **Kandutsch, A. A., Chin, H. W., and Heiniger, H.-J.,** Biological activity of some oxygenated sterols, *Science*, 201, 498, 1978.
14. **Kandutsch, A. A. and Chin, H. W.,** Inhibition of sterol synthesis in cultured mouse cells by 7α–hydroxycholesterol, 7β–hydroxycholesterol, and 7-ketocholesterol, *J. Biol. Chem.*, 248, 8408, 1973.
15. **Smith, L. L.,** *Cholesterol Autoxidation*, Plenum Press, New York, 1981, 231.
16. **Parish, E. J., Nanduri, U. B. B., Kohl, H. H., and Taylor, F. R.,** Oxysterols: Chemical synthesis, biosynthesis and biological activities, *Lipids*, 21, 27, 1980.
17. **Gibbons, G. F.,** The role of oxysterols in the regulation of cholesterol biosynthesis, *Biochem. Soc. Trans.* (*London*), 11, 649, 1983.
18. **Cheng, K. P., Nagano, N., Bang, L., and Ourisson, G.,** Chemistry and biochemistry of Chinese drugs. I. Sterol derivatives cytotoxic to hepatoma cells, *J. Chem. Res.*, (S) 217 M(M) 2521, 1977.
19. **Nagano, J., Poyser, J. P., Cheng, K-P., Luu, B., Ourisson, G., and Beck, J. P.,** Chemistry and biochemistry of Chinese drugs. II. Cytotoxicity of hydroxysterols on tumor cells. Synthesis and biological activity, *J. Chem. Res*., (S)218 (M) 2522, 1977.
20. **Zander, M., Patrick, K., Bang, L., and Ourisson, G.,** Chemistry and biochemistry of Chinese drugs. III. Mechanism of action of hydroxylated sterols in cultured hepatoma cells, *J. Chem. Res.*, (S) 219, (M) 2572, 1977.
21. **DeFay, R., Astruc, M. E., Roussillon, S., Decomps, B., and Crastes de Paulet, A.,** DNA synthesis and HMG-CoA reductase activity in PHA stimulated human lymphocytes: a comparative study of the inhibitor effects of some oxysterols with specific reference to side chain hydroxylated derivatives, *Biochem. Biophys. Res. Commun*., 106, 362, 1982.
22. **Astruc, M., Laporte, M., Tabacik, C., and Crastes de Paulet, A.,** Effect of oxysterols on reductase and DNA synthesis in phytohemagglutinin-stimulated human lymphocytes, *Biochem. Biophys. Res. Commun.*, 85, 691,1978.

23. **Moriasaki, M., Sonoda, Y., Makino, T., Ohihara, H., Ikekawa, H., and Sato, Y.,** Inhibitory effect of 15-oxygenated sterols on cholesterol synthesis from 24,25-dihydrolanosterol, *J. Biochem.*, 99, 597, 1986.
24. **Taylor, F. R., Saucier, S. E., Shown, E. P., Parish, E. J., and Kandutsch, A. A.,** Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase, *J. Biol. Chem.*, 259, 12382, 1984.
25. **Gibbons, G. F., Pullinger, C. R., Chen, H. W., Cavernee, W. K., and Kandutsch, A. A.,** Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols, *J. Biol. Chem.*, 255, 395, 1980.
26. **Schroepfer, G. J., Jr., Parish, E. J., Tsuda, M., Raulston, D. L., and Kandutsch, A. A.,** Inhibition of sterol biosynthesis in animal cells by 14α–alkyl-substituted 15-oxygenated sterols, *J. Lipid Res.*, 20, 994, 1979.
27. **Kandutsch, A. A., Taylor, F. R., and Shown, E. P.,** Different forms of the oxysterol-binding protein, *J. Biol. Chem.*, 259, 12388, 1984.
28. **Sonoda, Y. and Sato, Y.,** Effects of oxygenated lanosterol analogs on cholesterol biosynthesis from lanosterol, *Chem. Pharm. Bull. Jpn.*, 31, 1698, 1983.
29. **Sato, Y., Sonoda, Y., Morisaki, M., and Ikekawa, N.,** Oxygenated sterols as inhibitors of enzymatic conversion of dihydrolanosterol into cholesterol, *Chem. Pharm. Bull. Jpn.*, 32, 3305, 1984.
30. **Faust, J. R., Luskey, K. L., Chin, D. J., Goldstein, J. L., and Brown, M.S.,** Regulation of synthesis and degradation of HMG-CoA reductase by low density lipoprotein and 25-hydroxycholesterol in UT-1 cells, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 5205, 1982.
31. **Sinensky, M., Torget, R., and Edwards, P. A.,** Radioimmune precipitation of HMG-CoA reductase from Chinese hamster fibroblasts, *J. Biol. Chem.*, 256, 11744, 1981.
32. **Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S., and Goldstein, J. L.,** Amplification of the gene for HMG-CoA reductase, but not for the 53-KDa protein, in UT-1 cells, *J. Biol. Chem.*, 258, 8462, 1982.
33. **Kandutsch, A. A. and Thompson, E. B.,** Cytosolic proteins that bind oxygenated sterols, *J. Biol. Chem.*, 255, 10813, 1980.
34. **Kandutsch, A. A., Chen, H. W., and Shown, E. P.,** Binding of 25-hydroxycholesterol and cholesterol to different cytoplasmic proteins, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2500, 1977.
35. **Saucier, S. E., Kandutsch, A. A., Taylor, F. R., Spencer, T. A., Phirwa, S., and Gayen, A. K.,** Identification of regulatory oxysterols, 24(S),25-epoxycholesterol and 25-hydroxycholesterol in cultured fibroblasts, *J. Biol. Chem.*, 260, 14571, 1985.
36. **Gould, R. G.,** Lipid metabolism and atherosclerosis, *Am. J. Med.*, 11, 209, 1951.
37. **Vlahcevic, Z. R., Schwartz, C. C., Gustaffson, J., Halloran, L. G., Danielson, H., and Swell, L.,** Biosynthesis of bile acids in man, *J. Biol. Chem.*, 255, 2925, 1980.
38. **Pederson, J. L., Bjorkhem, I., and Gustaffson, J.,** 26-Hydroxylation of C_{27}-steroids by soluble liver mitochondrial cytochrome P-450, *J. Biol. Chem.*, 254, 6464, 1979.
39. **Smith, L. O., Teng, J. L., Lin, Y. Y., Seitz, P. K., and McGehee, M. F.,** Sterol metabolism. Oxidized cholesterol esters in human tissues, *J. Steroid Biochem.*, 14, 889, 1981.
40. **Schroepfer, G. J., Jr., Parish, E. J., Pascal, R. A., Jr., and Kandutsch, A. A.,** Inhibition of sterol biosynthesis by 14α-hydroxymethyl sterols, *J. Lipid Res.*, 21, 571, 1980.
41. **Nelson, J. A., Stackbeck, S. R., and Spencer, T. A.,** Biosynthesis of 24,25-epoxycholesterol from squalene 2,3:22,23-dioxide, *J. Biol. Chem.*, 256, 1067, 1981.
42. **Panini, S. R., Sexton, R. C., and Rudney, H.,** Regulation of HMG-CoA reductase by oxysterol by-products of cholesterol biosynthesis. Possible mediators of low density lipoprotein action, *J. Biol. Chem.*, 259, 7767, 1984.
43. **Paini, S. R., Sexton, R. C., Gupta, A. K., Parish, E. J., Chitrakorn, S., and Rudney, H.,** Regulation of HMG-CoA reductase activity and cholesterol biosynthesis by oxylanosterols, *J. Lipid Res.*, 27, 1290, 1986.
44. **Spencer, T. A. Gayen, A. K., Phirwa, S., Nelson, J. A., Taylor, F. R., Kandutsch, A. A., and Erickson, S.,** 24(S),25-Epoxycholesterol. Evidence consistent with a role in the regulation of hepatic cholesterogenesis, *J. Biol. Chem.*, 260, 13391, 1985.
45. **Spencer, T. A.,** The squalene dioxide pathway of steroid biosynthesis, *Acc. Chem. Res.*, 27, 83, 1994.
46. **Taylor, F. R.,** Oxysterol regulation of cholesterol biosynthesis, in *Regulation of Isopentenoid Metabolism*, Nes, W. D., Parish, E. J., and Trzaskos, M., Ed., ACS Symposium Series 497, American Chemical Society, Washington, D.C., 1992, 81.
47. **Parish, E. J., Parish, S. C., and Li, S.,** Side-chain oxysterol regulation of HMG-CoA reductase activity, *Lipids*, 30, 247, 1995.
48. **Parish, E. J.,** The biosynthesis of oxysterols in plants and microorganisms, in *Physiology and Biochemistry of Sterols*, Patterson, G. W. and Nes, W. D., Eds., American Oil Chemists' Society, Champaign, 1991, 324.
49. **Parish, E. J.,** Biosynthesis of oxysterols in plants, animals, and microorganisms, in *Regulation of Isopentenoid Metabolism*, Nes, W. D. and Parish, E. J., Eds., ACS Symposium Series 497, American Chemical Society, Washington, D.C., 1992, 146.
50. **Parish, E. J.,** Evolution of the oxysterol pathway, in *Evolution of Natural Products*, Nes, W. D., Ed., ACS Symposium Series 562, American Chemical Society, Washington, D.C., 1994, 109.
51. **Brown, M. S. and Goldstein, J. L.,** *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed., Pergamon Press, New York, 1990, 874.

52. **Nankanishi, M., Goldstein, J. L., and Brown, M. S.,** Multivalent control of HMG-CoA reductase. Mevalonate-derived product inhibits translation of mRNA and accelerates degradation of enzyme, *J. Biol. Chem*., 1263, 8929, 1988.
53. **Bach, T. J. and Lichtenthaler, H. K.,** Plant growth regulation by mevinolin and other sterol biosynthesis inhibitors, in *Ecology and Metabolism of Plant Lipids*, Fuller, G. and Nes, W. D., Eds., ACS Symposium Series 325, American Chemical Society, Washington, D.C., 1987, 109.
54. **Bach, T. J., Wettstein, A., Boronat, A., Ferrer, A., Enjuto, M., Gruissem, W., and Narita, J. O.,** Properties and molecular cloning of plant HMG-CoA reductase, in *Physiology and Biochemistry of Sterols*, Patterson, G. W. and Nes, W. D., Eds., *American Oil Chemists*' Society, Champaign, IL, 1991, 29.
55. **Stermer, B. A., Bianchini, G. M., and Korth, K. L.,** Regulation of HMG-CoA reductase activity in plants, *J. Lipid Res*., 35, 1133, 1994.
56. **Cavenee, W. K., Chen, H. W., and Kandutsch, A. A.,** Regulation of cholesterol biosynthesis in enucleated cells, *J. Biol. Chem*., 256, 2675, 1981.
57. **Chen, H. W., Richards, B. A., and Kandutsch, A. A.,** Inhibition of protein synthesis blocks the response to 25-hydroxycholesterol by degradation of HMG-CoA reductase, *Biochim. Biophys. Acta*, 712, 484, 1982.
58. **Tanaka, R. D., Edwards, P. A., Lan, S.-F., and Fogelman, A. M.,** Regulation of HMG-CoA reductase activity in avian myeloblasts, *J. Biol. Chem.*, 258, 13331, 1983.
59. **Sinesky, M., Torget, R., Schnitzer-Polokoff, R., and Edwards, P. A.,** Analysis of regulation of HMG-CoA reductase in a somatic cell mutant auxotropic for mevalonate, *J. Biol. Chem*., 257, 7284, 1982.
60. **Kandutsch, A. A. and Chen, H. W.,** Inhibition of sterol synthesis in cultured mouse cells by cholesterol derivatives in the side chain, *J. Biol. Chem.*, 249, 6057, 1974.
61. **Panini, S. R., Gupta, A., Sexton, R. C., Parish, E. J., and Rudney, H.,** Regulation of sterol biosynthesis and HMG-CoA reductase activity in cultured cells by progesterone, *J. Biol. Chem*., 262, 14435, 1987.
62. **Schroepfer, Jr., G. J., Kim, H.-S., Vermilion, J. L., Stephens, T. W., Pinkerton, F. D., Needleman, D. H., Wilson, W. K., and St. Pyrek, J.,** *Biochem. Biophys. Res. Commun*., 151, 130, 1988.
63. **Pinkerton, F. D., Pelley, R. P., and Schroepfer, G. J., Jr.,** Synergestic action of two oxysterols in the lowering of HMG-CoA reductase activity in CHO-K1 cells, *Biochem. Biophys. Res. Commun.*, 186, 569, 1992.
64. **Wilson, W. K., Swaminathan, S., Pinkerton, F. D., Gerst, N., and Schroepfer, G. J., Jr.,** Inhibitors of sterol synthesis. Effects of fluorine substitution at carbon 25 of cholesterol on its spectral and chromatographic properties and on HMG-CoA reductase activity in CHO-K1 cells, *Steroids*, 59, 310, 1994.
65. **Swaminathan, S., Wilson, W. K., Pinkerton, F. D., Gerst, N., Ramser, M., and Schroepfer, G. J., Jr.,** Inhibitors of sterol synthesis. Chemical synthesis and properties of 3β-hydroxy-25,26,26,27,27-heptafluoro-5α– cholest-8(14)-en-15-one and 25,26,26,27,27-heptaflurorcholesterol and their effects on HMG-CoA reductase activity in cultured mammalian cells, *J. Lipid Res*., 34, 1805, 1993.