Endogenous sterol synthesis is not required for regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by low density lipoprotein

Ernst Burki, Judy Logel, and Michael Sinensky

Eleanor Roosevelt Health Technologies, Inc., 1899 Gaylord Street, Denver, CO 80206

Abstract It has been proposed that an endogenously synthesized oxysterol mediates the regulation of cholesterol biosynthesis by low density lipoprotein in cultured mammalian cells. Studies in this report demonstrate that under conditions in which squalene conversion to sterols is blocked either by inhibition of squalene cyclization or lanosterol demethylation, or both, low density lipoprotein regulates 3-hydroxy-3-methylglutaryl coenzyme A reductase normally. These observations rule out the hypotheses that either an endogenously synthesized oxygenated cholesterol biosynthetic intermediate or epoxysterol is required to mediate the inhibition of this enzyme by low density lipoprotein.—**Burki, E., J. Logel, and M. Sinensky.** Endogenous sterol synthesis is not required for regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by low density lipoprotein. *J. Lipid Res.* 1987. **28:** 1199–1205.

Supplementary key words sterol synthesis • low density lipoprotein

Certain oxygenated derivatives of cholesterol are potent inhibitors of cholesterol biosynthesis and are regulators in cultured cells of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which catalyzes the synthesis of the cholesterol precursor mevalonic acid (1). Demonstration of the ubiquitous existence of a cytosolic binding protein for oxysterols but not for cholesterol (2) and the relatively greater potency of oxygenated sterols (1) as compared to cholesterol in regulating enzymes of cholesterol biosynthesis support the hypothesis that an oxygenated sterol rather than cholesterol itself is the immediate regulator of cellular cholesterol biosynthesis. Oxygenated sterols do occur naturally in mammalian tissue as intermediates in lanosterol demethylation (3, 4) and particularly as 24 (*S*),25-epoxycholesterol (5), a compound derived from the cyclization of squalene dioxide (6, 7). 25-Hydroxycholesterol has also been observed to be naturally occurring in biological tissue (8). It has been proposed that one or more of these oxygenated sterols, and specifically 24(*S*),25-epoxycholesterol, may also act as second messengers (9) mediating the inhibition of cholesterol biosynthesis produced by the uptake of low density lipoprotein from plasma. This hypothesis is supported by reports that compounds which inhibit endogenous cholesterol synthesis through the inhibition of squalene epoxide cyclase (10) or through the demethylation of lanosterol (11) also block the capacity of low density lipoprotein to regulate HMG-CoA reductase.

The specificity of these compounds as inhibitors of these reactions has not been completely assessed. However, the compound that has been utilized to block demethylation of lanosterol, ketoconazole (12, 13), also has other known effects on P450-mediated oxidation reactions. Therefore, an alternative hypothesis for the effect of this compound on the inhibition of low density lipoprotein-mediated regulation of HMG-CoA reductase is a possible block to some unknown reaction in which LDL cholesterol stimulates formation of a regulator through a P450-mediated process. In order to examine these possibilities further, we have utilized two alternative systems for studying the effects of inhibition of endogenous cholesterol synthesis on the capacity of low density lipoproteins to regulate HMG-CoA reductase activity in cultured mammalian cells. These two systems are the specific squalene oxide cyclase inhibitor 4,4,10-β-trimethyl-*trans*-decal-3β-ol (TMD) (14) and somatic cell mutants of the CHO-K1 (15, 16) and LM (17) cell lines which have been demonstrated to be blocked in lanosterol demethylation. Since an assessment of the formation of epoxysterols in these systems has not previously been reported, we describe studies that demonstrate the quantitative inhibition of all sterol synthesis after treatment of the 215 mutant with TMD. We utilized this system to test the hypothesis that an endogenously synthesized sterol is required for the activity of LDL in regulating HMG-CoA reductase.

Abbreviations: LDL, low density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; TMD, 4,4,10-β-trimethyl-*trans*-decal-3β-ol.

EXPERIMENTAL PROCEDURES

Medium and cells

Cells were routinely grown in Ham's F12 (18) supplemented with 1% fetal calf serum (F12FC1). The cell lines utilized were CHO-K1 (19), 215 (15), S2 (16), and LM (American Type Culture Collection #CCL1.2). The S2 and LM cells were gifts from Dr. David Silbert, Washington University in St. Louis, MO. The 215 mutant was a gift from Dr. T. Y. Chang, Dartmouth School of Medicine, Hanover, NH. TMD was a gift from Dr. Thomas Spencer, Dept. of Chemistry, Dartmouth College, Hanover, NH. In some experiments cells were derepressed for the cholesterol biosynthetic pathway by incubation in F12 supplemented with 5% organic solvent delipidized serum (F12DIPE5) prepared by the method of Cham and Knowles (20).

HMG-CoA reductase assays

Cells (5×10^6/150 mm petri dish) were incubated for 24 hr in F12FC1 and the medium was then switched to F12DIPE5 for 16 hr in the presence or absence of low density lipoprotein supplements. Cells were harvested and assayed for HMG-CoA reductase activity as previously described (21).

[^{3}H]Squalene labeling

Cells were seeded at 5×10^5 cells/60 mm dish in 3 ml of F12FC5 and incubated for 24 hr. The medium was then changed to 3 ml of F12DIPE5. After 16 hr the medium was discarded and replaced by 1.5 ml of labeling medium. Labeling medium (22) contained in phosphate-buffered saline (PBS ref. 21): 15 mM glucose, 1% BSA, 10% DMSO, and 133 nM of [^{3}H]squalene in ethanol (2.5 μCi/nmol). When utilized, TMD (20 μg/ml) was added for 5 hr prior to and throughout the pulse. The pulse was for 1 hr. Tritiated squalene (19.4 Ci/mmol, 4,8,12, 13,17,21-^{3}H), prepared biosynthetically, was obtained from New England Nuclear, Boston, MA.

The labeling medium was discarded and dishes were washed with 2 ml of cold PBS. Cells were harvested with a rubber policeman in 7 ml of cold PBS, pelleted at 1000 *g*, and resuspended in 1.1 ml of 0.05 M Tris-HCl, pH 7.4. Cells were disrupted by sonication and 0.1 ml of the homogenate was saved for determination of the protein content. Proteins were measured with a Bradford microassay (Bio-Rad, Richmond, CA) using beef γ-globulin as a standard.

Lipid extraction and saponification

Lipids were extracted from 1 ml of cell homogenate by the method of Bligh and Dyer (23), saponified with 2 N KOH in 50% ethanol for 2 hr at 70°C, and extracted twice with 3 ml of petroleum ether. Nonsaponifiable lipids were dried under nitrogen and stored under argon at −20°C.

HPLC analysis of nonsaponifiable lipids

Nonsaponifiable lipids were dissolved in 400 μl of methanol and passed through a 0.45-μm nylon filter (Cameo, Micron Separations, Inc., Honeoye Falls, NY). The solvent was evaporated under nitrogen and the lipids were redissolved in 30 μl of methanol containing, as mass standards: 0.5 μg of squalene, 0.5 μg of squalene-2,3-epoxide, 0.5 μg of squalene-2,3;22,23-dioxide, 1 μg of 24(S),25-oxidolanosterol, 2.5 μg of desmosterol, and 5 μg of lanosterol and cholesterol. Chemically synthesized (racemic) squalene 2,3 epoxide, squalene 2,3;22,23-dioxide, and 24(S)-oxidolanosterol mass standards were provided to us as part of collaborative studies with Dr. T. A. Spencer, Dept. of Chemistry, Dartmouth College, Hanover, NH. Cholesterol, lanosterol, and desmosterol mass standards were obtained commercially (Sigma, St. Louis, MO) and recrystallized three times from hot ethanol prior to use. Samples were loaded onto a reverse phase column (μBondapak C_{18}, 3.9 mm × 30 cm, Waters Chromatography Division, Millipore Corp., Milford, MA) and separations were achieved with a mobile phase of acetonitrile and water (93:7 for 10 min, 88:12 for 15 min, and 93:7 for 15 min) at a flow rate of 1.5 ml/min using a Beckman 110B solvent delivery system. Absorption of mass standards was measured at 210 nm using a Beckman 165 variable wavelength detector, and recorded with a Beckman 427 integrator. One ml/min of the column effluent was diverted into a Flo-one/Beta Radioactive Flow Detector, model CR (Radiomatic Instruments, Tampa, FL), mixed with 4 ml/min Scinti Verse LC (Fisher, Fair Lawn, NJ), and net cpm were counted in a 2.5-ml liquid cell with ^{3}H energy window settings of 1–35 and a background subtraction of 180 cpm. Counting efficiency was approximately 20%. Radioactive peaks were identified by reference to the simultaneously run UV chromatograms since the radioactive data acquisition system gives only approximate retention times. Data analysis was obtained by interfacing the radiodetector with a dedicated computer (Aspen ACS 2,000, Genesis Corp., Denver, CO).

LDL preparation

Human plasma LDL was prepared from the venous blood of a single donor who had fasted overnight. The plasma fraction was obtained by centrifugation at 600 *g* for 15 min. Lipoproteins were isolated according to the method of Havel, Eder, and Bragdon (24) as described by Goldstein, Basu, and Brown (25). Plasma was centrifuged in Quick-Seal tubes in a Ti 60 Beckman rotor at 45,000 rpm for 24 hr and the subnatant was removed through a side puncture. The subnatant density was adjusted to

1.063 g/ml with KBr and the sample was centrifuged in a SW41 Beckman rotor at 37,000 rpm for another 24 hr. The top layer was dialyzed against a total of 10 liters of buffer A (25) for 36–48 hr at 4°C.

The protein content was measured by the Markwell-Lowry method (26) and cholesterol content was determined by a cholesterol diagnostic kit (Sigma Diagnostics, St. Louis, MO).

RESULTS

Endogenously synthesized sterol regulators

If cholesterol or any other sterol regulator can be derived from endogenous synthesis rather than by uptake mediated by serum lipoproteins, it would be predicted that the activity of HMG-CoA reductase would be higher in cells in which synthesis of sterols is specifically inhibited. We compared wild type CHO-K1 cells with the 215 CHO-K1 mutant defective in lanosterol demethylation and with CHO-K1 cells that had been treated with TMD. We also compared HMG-CoA reductase activities of the LM cell line and another previously described demethylation mutant (S2) of this cell line. In all three instances we found that inhibition of endogenous cholesterol synthesis resulted in an elevation of cellular HMG-CoA reductase activity of the order of two- to threefold **(Fig. 1)**. We also found that treatment with TMD failed to stimulate HMG-CoA reductase activity in the 215 mutant (Fig. 1) which would be expected if the stimulatory effects of TMD on cellular HMG-CoA reductase activity are mediated through inhibition of endogenous sterol synthesis.

Regulation of HMG-CoA reductase activity by low density lipoprotein in the absence of endogenous sterol synthesis

The possibility that endogenously synthesized sterol regulators are required for the inhibitory activity of low density lipoprotein was then examined. Since we wished to minimize a priori assumptions about either the nature or the amount of endogenously synthesized regulator that might be required to mediate the effects of low density lipoprotein, we re-examined the extent of inhibition of sterol biosynthesis in CHO-K1 cells treated with TMD and in the 215 mutant. These studies were necessary in that prior reports did not carefully examine the extent of inhibition of epoxidation and cyclization in these systems particularly with regard to the formation of epoxysterols. We utilized a more vigorous set of techniques for these studies than were used in the original characterization of these cell lines. The conversion of radioactive squalene to sterols was determined rather than the conversion of labeled acetate previously employed. The conversion of

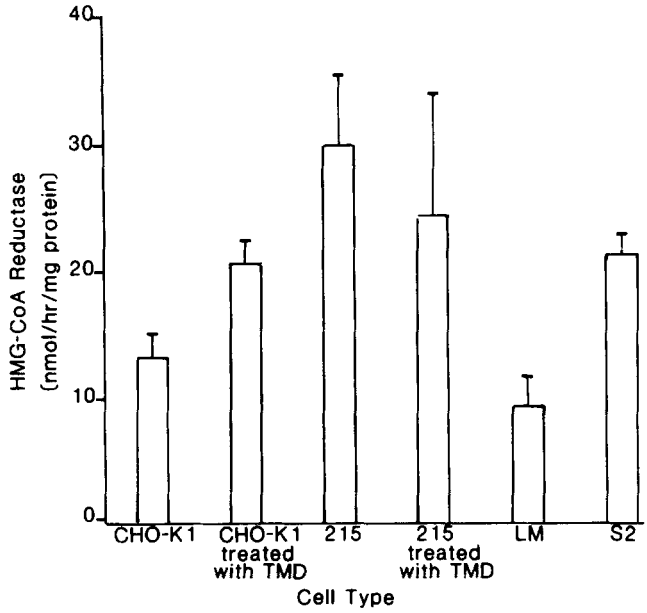

Fig. 1. Effect of inhibition of endogenous cholesterol synthesis on cellular HMG-CoA reductase activity. Cells (5×10^6/150 mm plate) were derepressed for HMG-CoA reductase activity by incubation for 16 hr in F12DIPE5, as described in Methods. TMD (20 μg/ml) is an inhibitor of squalene epoxide and squalene dioxide cyclization and the two mutant lines used (S2 and 215) are blocked in lanosterol demethylation. Results are expressed as the mean ± SEM for six determinations. HMG-CoA reductase activities for the cell lines used under repressed conditions (F12FC1) were (in nmol/hr per mg protein): CHO-K1, 1.09 ± 0.14; 215, 1.05 ± 0.24; LM, 0.74 ± 0.15; S2, 1.14 ± 0.16.

squalene to products was monitored by means of a high pressure liquid chromatograph equipped with an on-line liquid scintillation radioactivity detector. Squalene labeling was chosen for initial studies because, aside from the squalene oxides, all products derived from squalene could be assumed to be sterols, whereas this is not the case when labeling with either acetate or mevalonate. We utilized high pressure liquid chromatography as the isolation technique for products rather than thin-layer chromatography since identification of products on thin-layer chromatography gives misleading results. Incorporation of squalene during a 2-hr pulse in CHO-K1 cells resulted in a diverse group of products which are most likely sterol intermediates of cholesterol biosynthesis **(Fig. 2A)**. It is of interest to note that very little cholesterol (retention time, 19 min) is actually formed under these conditions. Similar results were observed with tracer labeling with mevalonate (data not shown), a method that is in general use. The major triplet of peaks (retention time, 10–15 min) was found to chromatograph closely with cholesterol on several thin-layer chromatography systems, suggesting that cholesterol is frequently misidentified in short-term labeling studies that employ thin-layer chromatography. A similar observation has previously been reported (27). Treatment with TMD for 5 hr was found to effectively block sterol synthesis in CHO-K1 cells (Fig. 2B), leading

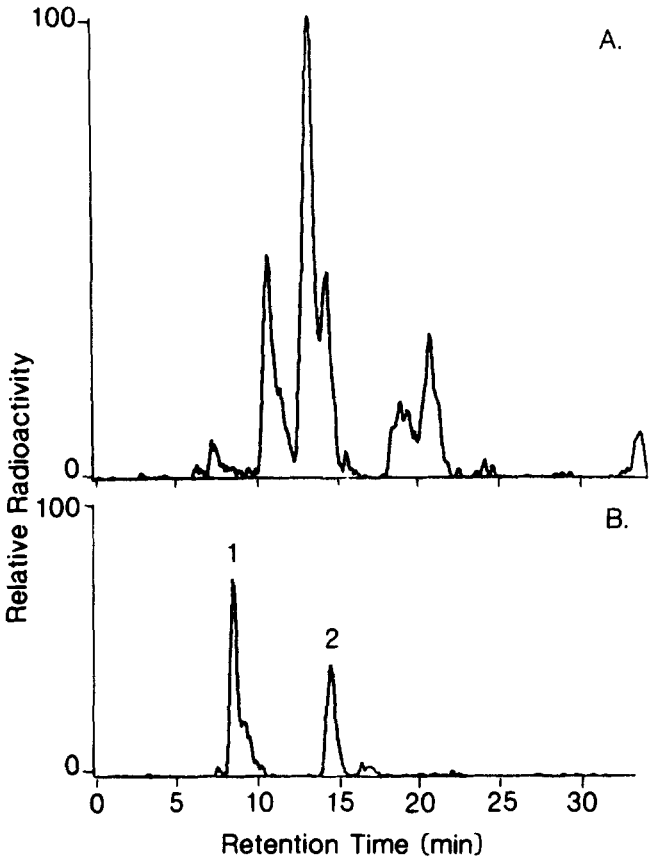

Fig. 2. Formation of products from tritiated squalene in the absence (panel A) or presence (panel B) of TMD (20 μg/ml). Squalene labeling and analysis of radioactive products by high pressure liquid chromatography was performed as described in Experimental Procedures. Dimethyl sulfoxide permeabilized cells (5×10^5/60 mm dish) were incubated for 1 hr with tritiated squalene (0.5 μCi/dish) at a specific activity of 2.5 μCi/nmol. Peak 1 cochromatographs with racemic squalene-2,3;22,23 dioxide and peak 2 cochromatographs with racemic squalene-2,3 epoxide. The total counts per minute analyzed in the samples were 13,816 for panel A and 7,055 for panel B.

primarily to the production of squalene-2,3(S);22,23(S) dioxide (peak 1) and squalene-2,3(S) epoxide (peak 2), as has previously been reported (14). There is, however significant (15% of total incorporation) formation of another product that runs with slightly longer retention time as a shoulder on peak 1. This material is not observed in CHO-K1 cells not treated with TMD. The 215 mutant was observed to accumulate only lanosterol from squalene **(Fig. 3A)**, consistent with the previously described characterization of this mutant cell line. When this cell line was treated with TMD (Fig. 3B), no other significant products (greater than 5% of total incorporation) were formed besides squalene-2,3(S);22,23(S) dioxide and squalene-2,3(S) epoxide. It should be noted that the incorporation studies shown in Figs. 2 and 3 validate the assumption that, in CHO-K1 cells, all major products derived from squalene are, in fact, sterols. The distribution of products observed was highly reproducible in triplicate repeats in all these studies.

To test the effect of inhibition of sterol synthesis on regulation of HMG-CoA reductase by low density lipoprotein, it was desirable to measure activity after a 16-hr exposure to LDL in order to assure maximal suppression of enzyme activity. It was, therefore, also necessary to assess the effect of TMD on formation of products under these conditions. We elected to employ tracer labeling with high specific activity tritiated mevalonate to determine the intracellular products formed over the 16-hr period. Squalene labeling is not suitable for this purpose because of the requirement for treatment with demethyl sulfoxide to effect efficient uptake of squalene by the cells. The main intracellular product formed under these conditions was squalene dioxide **(Fig. 4)**. No sterol products were observed. The effect of LDL on regulation of HMG-CoA reductase was then examined in CHO-K1 cells and CHO-K1 cells treated with TMD **(Fig. 5A)**. An identical experiment was performed on 215 cells (Fig. 5B) since product identification from squalene was clearer in this cell line (Fig. 3B). LDL was observed to down-regulate HMG-CoA reductase to the same extent under all conditions.

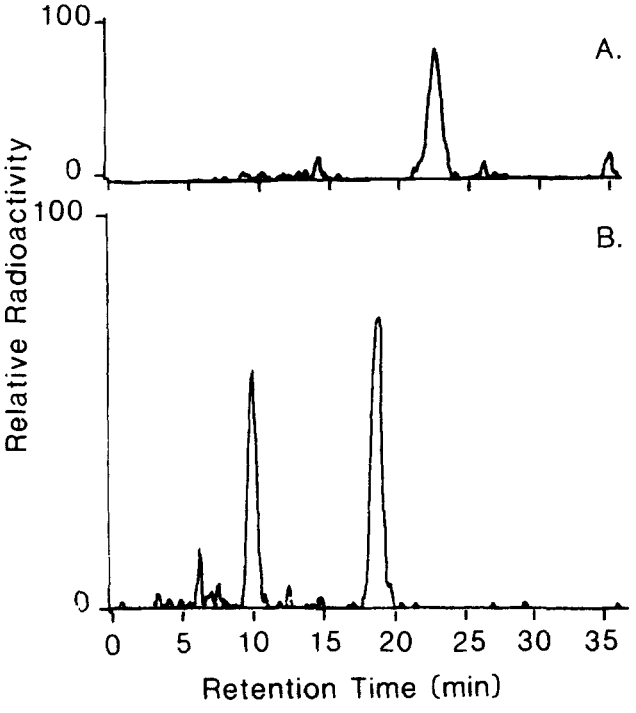

Fig. 3. Formation of products from tritiated squalene in the 215 mutant in the absence (panel A) or presence (panel B) of TMD (20 μg/ml). Labeling conditions were identical to those described in the legend to Fig. 2. Analysis of products was the same as that described in Methods except that the products were separated isocratically using 92% acetonitrile–8% water as the mobile phase. The sole product formed from squalene in the absence of TMD (panel A) cochromatographs with lanosterol. In the presence of TMD the products formed are squalene-2,3(S);22,23(S) dioxide and squalene-2,3(S) oxide which are the major products formed in the wild-type CHO K1 cell, as well (Fig. 2B). The total counts per minute analyzed in the samples were 4,059 for panel A and 2,286 for panel B.

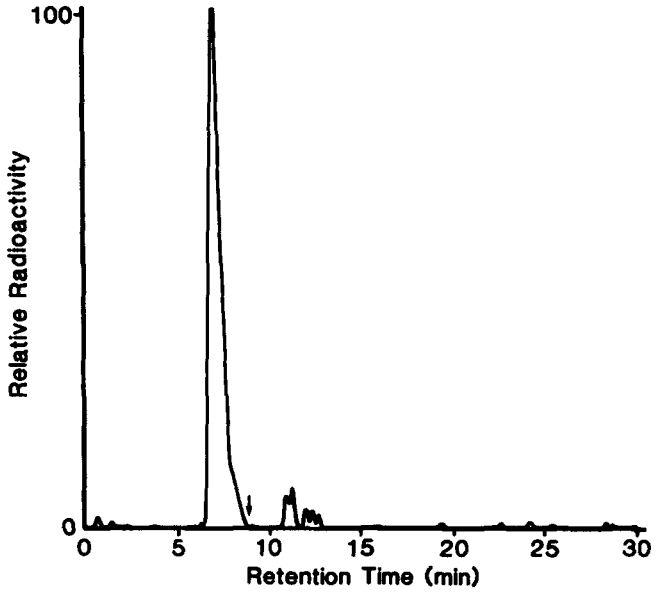

Fig. 4. Effect of TMD on the conversion of mevalonate to sterols in 215 cells. Cells (1×10^6/150 mm dish) were incubated for 2 hr in F12FC1 in the presence of 20 μg/ml TMD. The medium was then changed to F12DIPE5 containing the same concentration of TMD and an LDL supplement that was sufficient to inhibit HMG-CoA reductase by over 95% (20 μg LDL protein/ml medium). Cells were labeled for 16 hr in this medium with trace amounts of R,S-5-tritiated mevalonate (New England Nuclear, Boston, MA. 0.16 μM, sp act 30 Ci/mmol). Extraction and analysis of nonsaponifiable lipids were as described in Experimental Procedures except that the products were separated isocratically on HPLC using 94% acetonitrile–6% water as the mobile phase. The two major compounds coelute with authentic mass standards for squalene-2,3;22,23 dioxide (retention time: 6.9 min) and 2,3-squalene expoxide (11.1 min). Retention times for authentic mass standards for 24(S)25 oxidolanosterol (↓) and lanosterol were 8.8 and 16.4 min, respectively. Total radioactivity recovered was 4000 cpm for SDO and 180 cpm for SO.

DISCUSSION

A number of reports have appeared describing endogenously biosynthesized oxysterols of mammalian cells and postulating a significant role for these compounds in the regulation of cellular cholesterol biosynthesis by cholesterol 3–5,8,9,11,28,29), particularly cholesterol supplied to the cell by low density lipoprotein (9–11,28). Data presented in this report show that endogenous sterol synthesis is not required for regulation of HMG-CoA reductase activity by LDL. Our studies on the effect of inhibition of endogenous synthesis by the squalene oxide cyclase inhibitor TMD and on two lanosterol demethylation mutants show that endogenously synthesized sterol products can regulate HMG-CoA reductase activity as does the well-characterized supplementation with exogenous sterols (30). However, a complete inhibition of endogenous sterol synthesis by TMD in the lanosterol demethylation mutant 215 did not lead to any perturbation of the ability of LDL supplementation to inhibit HMG-CoA reductase activity. This model is particularly easy to interpret since TMD produces no stimulation of HMG-CoA reductase activity in this line, giving rise to the virtually identical dose-response curves of enzyme activity to LDL supplementation in the presence or absence of TMD seen in Fig. 5B. The interpretation of our results in Fig. 5 hinge on the effectiveness of our methods for inhibition of cholesterol synthesis and so we have examined these methods very carefully. Utilization of on-line radioactivity detection of products after HPLC provides a rigorous assessment of the inhibition of sterol biosynthesis in our studies. The number of sterol intermediates that appear with precursor labeling that may not be properly identified by thin-layer chromatography suggests that TLC analysis of such products may be problematic. The utilization of TMD as an inhibitor of sterol synthesis in CHO-K1 cells has previously been reported (14). In our experiments, the accumulation of squalene epoxide and squalene dioxide from squalene, supplemented at levels which produce maximal sterol synthesis from squalene in CHO-K1 cells (22), indicates the effectiveness of this compound in inhibiting the conversion of these products to lanosterol and 24(S), 25-oxidolanosterol (6, 7). The effective cyclization of squalene dioxide to 24(S), 25-oxidolanosterol has been reported in both cell-free liver homogenates (6, 7) and upon incubation of squalene dioxide with cultured rat intestinal epithelial cells (10). In unpublished experiments we have made similar observations in CHO cells (Burki, E., J. Logel, and M. Sinensky). In

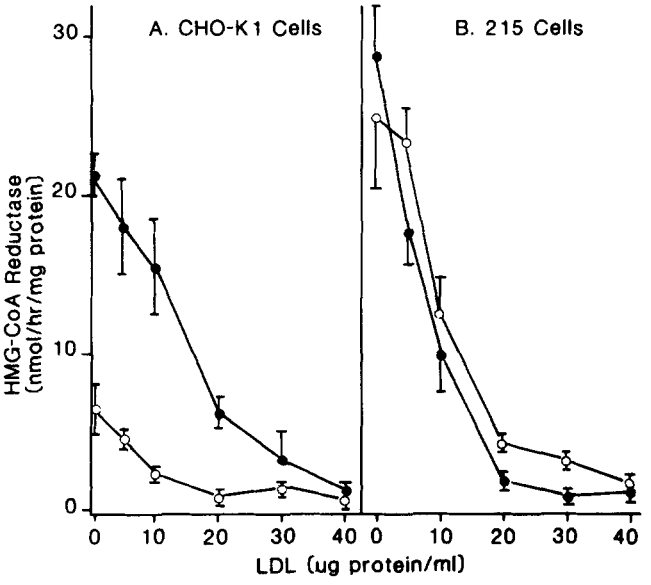

Fig. 5. The regulation of HMG-CoA reductase by human LDL in CHO K1 cells (panel A) and the 215 mutant (panel B) in the presence (●) or absence (○) of TMD. Cells (5×10^6/150 mm plate) were incubated for 16 hr in the presence of various human LDL supplements as shown. The cells were then harvested and assayed for HMG-CoA reductase activity as described in Methods. Results are shown as the mean ± SEM for three determinations. TMD (20 μg/ml) was added 2 hr prior and throughout the entire incubation period of 16 hr.

Fig. 4 we showed that neither lanosterol nor 24(S), 25-oxidolanosterol was detected after overnight incubation of cells labeled with mevalonate in the presence of LDL and TMD. The lack of observed formation of the oxidolanosterol is especially noteworthy in view of the large amount of squalene dioxide that accumulates under these conditions, and again is consistent with activity of TMD in inhibiting this alternative cyclization reaction. Taken together, these data conclusively demonstrate that endogenous sterol synthesis is not required for LDL to act as a regulator of HMG-CoA reductase activity in cultured cells. Such a requirement could be considered a modification of the oxysterol hypothesis that an endogenously synthesized oxygenated sterol rather than cholesterol itself is the primary feedback regulator of cholesterol synthesis. Our data indicate that if the oxysterol hypothesis is valid, the oxysterol regulator must be derived from the cholesterol supplied to the cell by LDL or must be contained within the LDL particle itself.

A critical review of the nature and abundance of biologically active oxygenated sterol has been reported (31). Cogent arguments were presented in this review against the notion that LDL harbors significant amounts of regulatory oxysterol. If these arguments are correct, then the only remaining mechanism by which an oxysterol may mediate the effect of LDL on cholesterol synthesis is through the formation of an oxysterol through catabolism of cholesterol delivered to the cell by LDL. Such considerations point to the desirability of further studies to elucidate the metabolism of exogenously supplied cholesterol by mammalian cells in culture.

Funding for these studies was provided by Somatogenetics International, Inc. This paper is contribution no. 1 from Eleanor Roosevelt Health Technologies, Inc.

Manuscript received 19 February 1987 and in revised form 18 March 1987.

REFERENCES

1. Kandutsch, A. A., H. W. Chen, and H. J. Heiniger. 1978. Biological activity of some oxygenated sterols. *Science.* **201:** 498–501.
2. Taylor, F. R., S. E. Saucier, E. P. Shown, E. J. Parish, and A. A. Kandutsch. 1984. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Biol. Chem.* **259:** 12382–12387
3. Gibbons, G. F., C. R. Pullinger, H. W. Chen, W. K., Cavenee, and A. A. Kandutsch. 1980. Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols. *J. Biol. Chem.* **255:** 395–400.
4. Tabaesk, C., S. Aliau, B. Serror, and A. Crastes de Paulet. 1981. Post-HMG-CoA reductase regulation of cholesterol biosynthesis in normal human lymphocytes lanosten-3β-ol-32-al, a natural inhibitor. *Biochem. Biophys. Res. Commun.* **101:** 1087–1095.
5. Spencer, T. A., A. K. Gayen, S. Phirwa, J. A. Nelson, F. R. Taylor, A. A. Kandutsch, and S. K. Erickson. 1985. 24(*S*), 25-Epoxycholesterol. Evidence consistent with a role in the regulation of hepatic cholesterogenesis. *J. Biol. Chem.* **260:** 13391–13394.
6. Nelson, J. A., S. R. Steckbeck, and T. A. Spencer. 1981. Biosynthesis of 24,25-epoxycholesterol from squalene 2,3: 22,23-dioxide. *J. Biol. Chem.* **256:** 1067–1068.
7. Nelson, J. A., S. R. Steckbeck, and T. A. Spencer. 1981. 24(*S*),25-epoxycholesterol is a natural product of mammalian steroid biosynthesis. *J. Am. Chem. Soc.* **103:** 6974–6975.
8. Saucier, S. E., A. A. Kandutsch, F. R. Taylor, T. A. Spencer, S. Phirwa, and A. K. Gayen. 1985. Identification of regulatory oxysterols, 24(*S*), 25-epoxycholesterol and 25-hydroxycholesterol in cultured fibroblasts. *J. Biol. Chem.* **260:** 14571–14579.
9. Fielding, C. J., and P. E. Fielding. 1986. Metabolism of cholesterol and lipoproteins. *In* Biochemistry of Lipids and Membranes. D. E. Vance and J. E. Vance, editors. Benjamin/Cummings, Menlo Park, CA. 421–422.
10. Panini, S., R. C. Sexton, and H. Rudney. 1984. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by oxysterol by-products of cholesterol biosynthesis. Possible mediators of low density lipoprotein action. *J. Biol. Chem.* **259:** 7767–7771.
11. Gupta, A., R. C. Sexton, and H. Rudney. 1986. Modulation of regulatory oxysterol formation and low density lipoprotein suppression of HMG-CoA reductase activity by ketoconazole. *J. Biol. Chem.* **261:** 8348–8356.
12. Murray, M., A. J. Ryan, and P. J. Little. 1982. Inhibition of rat hepatic microsomal aminopyrine N-demethylase activity by benzimidazole derivatives. Quantitative structure-activity relationships. *J. Med. Chem.* **25:** 887–892.
13. Santen, R. J., H. Van den Bossche, J. Symoens, J. Brugmans, and R. De Coster. 1983. Site of action of low dose ketoconazole on androgen biosynthesis in men. *J. Clin. Endocrinol. Metab.* **57:** 732–736.
14. Chang, T-Y., E. S. Schiwoni, Jr., K. R. McCrae, J. A. Nelson, and T. A. Spencer. 1979. Inhibition of cholesterol biosynthesis in Chinese hamster ovary cells by 4, 4, 10 β-trimethyl-*trans*-decal-3β-ol. A specific 2,3-oxidosqualene cyclase inhibitor. *J. Biol. Chem.* **254:** 11258–11263.
15. Chang, T-Y., C. Telakowski, W. Vandenheuvel, A. W. Alberts, and P. R. Vagelos. 1977. Isolation and partial characterization of a cholesterol requiring mutant of Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. USA.* **74:** 832–836.
16. Berry, D. J., and T-Y. Chang. 1982. Further characterization of a Chinese hamster ovary cell mutant defective in lanosterol demethylation. *Biochemistry.* **21:** 573–580.
17. Saito, Y., S. M. Chou, and D. F. Silbert. 1977. Animal cell mutants defective in sterol metabolism. Specific selection procedure and partial characterization of the defects. *Proc. Natl. Acad. Sci. USA.* **74:** 3730–3734.
18. Ham, R. G. 1965. Clonal growth of mammalian cells in a chemically defined synthetic medium. *Proc. Natl. Acad. Sci. USA.* **53:** 288–293.
19. Kao, F. T., and T. T. Puck. 1975. Mutagenesis and genetic analysis with Chinese hamster auxotrophic cell markers. *Genetics.* **79:** 343–352.
20. Cham, B. E., and B. R. Knowles. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. *J. Lipid Res.* **17:** 176–181.
21. Sinensky, M., G. Duwe, and F. Pinkerton. 1979. Defective regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in a somatic cell mutant. *J. Biol. Chem.* **254:** 4482–4486.

22. Eilenberg, H., and I. Schechter. 1984. A possible regulatory role of squalene epoxidase in Chinese hamster ovary cells. *Lipids.* **19:** 539–543.
23. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
24. Havel, R., H. Eder, and J. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
25. Goldstein, J., S. Basu, and M. Brown. 1983. Receptor-mediated endocytosis of low density lipoprotein in cultured cells. *Methods Enzymol.* **98:** 241–260.
26. Markwell, M., S. Hars, L. Bieber, and N. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
27. Pinkerton, F. D., A. Izumi, C. M. Anderson, L. R. Miller, A. Kisic, and G. J. Schroepfer, Jr. 1982. 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, a potent inhibitor of sterol biosynthesis, has two sites of action in cultured mammalian cells. *J. Biol. Chem.* **257:** 1929–1936.
28. Rachal, E., C. M. Haval, T. J. Scallen, and J. A. Watson. 1985. Lipoprotein-mediated synthesis of lanosterol 24,25 epoxide. *Federation Proc.* **44:** 656 (abstract).
29. Trzaskos, J. M., R. T. Fischer, and M. F. Favata. 1986. Mechanistic studies of lanosterol C-32 demethylation. Conditions which promote oxysterol intermediate accumulation during the demethylation process. *J. Biol. Chem.* **261:** 16937–16942.
30. Goldstein, J. L., and M. S. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46:** 897–930.
31. Chang, T-Y. 1983. Mammalian HMG-CoA reductase and its regulation. *In* The Enzymes. P. D. Boyer, editor. Academic Press, New York. 491–521.