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# Regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in human hepatoma cell line Hep G2

## Effects of inhibitors of cholesterol synthesis on enzyme activity

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Incubating Hep G2 cells for 18 h with triparanol, buthiobate and low concentrations ($< 0.5\ \mu$M) of U18666A, inhibitors of desmosterol $\Delta^{24}$-reductase, of lanosterol 14$\alpha$-demethylase and of squalene-2,3-epoxide cyclase (EC 5.4.99.7) respectively, resulted in a decrease of the HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase activity. However, U18666A at concentrations higher than 3 $\mu$M increased the HMG-CoA reductase activity in a concentration-dependent manner. None of these inhibitors influenced directly the reductase activity in Hep G2 cell homogenates. Analysis by t.l.c. of $^{14}$C-labelled non-saponifiable lipids formed from either [$^{14}$C]acetate or [$^{14}$C]mevalonate during the cell incubations confirmed the sites of action of the drugs used. Beside the $^{14}$C-labelled substrates of the blocked enzymes and $^{14}$C-labelled cholesterol, another non-saponifiable lipid fraction was observed, which behaves as polar sterols on t.l.c. This was the case with triparanol and at those concentrations of U18666A that decreased the reductase activity, suggesting that polar sterols may play a role in suppressing the reductase activity. In the presence of 30 $\mu$M-U18666A (sterol formation blocked) the increase produced by simultaneously added compactin could be prevented by addition of mevalonate. This indicates the existence of a non-sterol mevalonate-derived effector in addition to a sterol-dependent regulation. LDL (low-density lipoprotein), which was shown to be able to decrease the compactin-induced increase in reductase activity, could not prevent the U18666A-induced increase. On the contrary, LDL enhanced the U18666A effect, showing that the LDL regulation is not merely the result of introducing cholesterol to the cells.

## INTRODUCTION

The human hepatoma cell line Hep G2 seems to be a good model for the study of the regulation of HMG-CoA reductase (EC 1.1.1.34), the rate-limiting enzyme in cholesterol biosynthesis (Rodwell *et al.*, 1976), in human hepatocytes (Cohen *et al.*, 1984, 1985). It was shown that in Hep G2 cells compactin, an inhibitor of the reductase itself (Endo *et al.*, 1976), gave an induction of the reductase activity (measured after removal of the drug; Cohen *et al.*, 1984). This induction could be partially prevented by LDL, and the remaining activity was abolished by simultaneous addition of mevalonate (Cohen *et al.*, 1985). The same observation was made in other cell types (Brown & Goldstein, 1980; Cohen *et al.*, 1982). These findings suggest the existence of at least two classes of mevalonate-derived effectors involved in the regulation of HMG-CoA reductase activity, i.e. effectors compensated by LDL uptake (possibly sterol effectors) and effectors not introduced into the cell by LDL (possibly non-sterol effectors) (Brown & Goldstein, 1980; Cohen *et al.*, 1982, 1985). In order to gain more insight into the nature of these effectors, we investigated the effect of several inhibitors of the cholesterol-biosynthetic pathway on the reductase activity. The accumulation of intermediates before the site of inhibition, and/or the depletion of metabolites behind the blockade, may influence the HMG-CoA reductase activity. In the experiments described here we used U18666A {3$\beta$-[2-(diethylamino)ethoxy]androst-5-en-17-one} as an inhibitor of squalene-2,3-epoxide cyclase (EC 5.4.99.7) (Sexton *et al.*, 1983). The use of buthiobate (*S*-n-butyl *S'*-*p*-t-butylbenzyl *N*-3-pyridyldithiocarbonimidate) in mammalian cell-culture studies was not previously reported. It was found to block lanosterol 14$\alpha$-demethylase in yeast cells (Kato & Kawase, 1976; Aoyama *et al.*, 1983) and in rat liver microsomal preparations (Yoshida & Aoyama, 1985). Triparanol {1-[*p*-($\beta$-diethylaminoethoxy)phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)ethanol} was used as an inhibitor of desmosterol $\Delta^{24}$-reductase (Avigan *et al.*, 1960; Gibbons & Pullinger, 1977).

## MATERIALS AND METHODS

### Materials

U18666A was obtained from Dr. P. W. O'Connell, Upjohn Co., Kalamazoo, MI, U.S.A. Buthiobate was a gift from Dr. T. Kato, Sumitomo Chemical Co., Takatsukasa, Japan. Dr. W. J. Hudak, Merrell Dow Pharmaceuticals, Cincinnati, OH, U.S.A., provided us with triparanol. Compactin was given by Dr. A. Endo, Tokyo Nōkō University, Tokyo, Japan. Squalene 2,3-epoxide and 2,3:22,23-diepoxide were given by Dr. S. Panini, University of Cincinnati, OH, U.S.A. Sigma

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; DMEM, Dulbecco's modified Eagle's medium.

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Chemical Co. supplied DL-mevalonic acid, DL-HMG-CoA, glucose-6-phosphate dehydrogenase and desmosterol. Glucose 6-phosphate and $NADP^+$ were purchased from Boehringer, Mannheim, West Germany. [2-$^{14}$C]Acetate (sp. radioactivity 54 Ci/mol), [3-$^{14}$C]HMG-CoA (sp. radioactivity 47–57 Ci/mol), *RS*-[2-$^{14}$C]mevalonate (sp. radioactivity 47 Ci/mol), [7(n)-$^3$H]cholesterol (sp. radioactivity 5000 Ci/mol) and En$^3$hance were supplied by New England Nuclear. Cholesterol and lanosterol were obtained from Merck. Human serum albumin (fraction V) (further referred to as albumin) was obtained from the Centraal Laboratorium voor de Bloedtransfusiedienst, Amsterdam, the Netherlands. It was freed of fatty acids (Chen, 1967) and loaded with palmitate (Fluka A. G.; 10 $\mu$mol of palmitate/g of albumin; Spector & Hoak, 1969). Instafluor and Picofluor were from United Packard Industries. LDL (density 1.03–1.05 g/ml) was isolated from freshly collected pooled blood by density-gradient ultracentrifugation (Redgrave *et al.*, 1975). All other chemicals were of analytical grade.

### Human hepatoma cell line Hep G2

The cells were cultured in 10 cm$^2$ multiwell dishes in DMEM, supplemented with 10% (w/v) foetal bovine serum (Havekes *et al.*, 1983). At 18 h before harvesting, the medium was replaced with DMEM, supplemented with 1% (w/v) palmitate-loaded albumin and the different inhibitors at concentrations as indicated in the Results section. The final concentrations of the solvents of the different inhibitors were: 0.015–0.1% ethanol for U18666A, 0.2% dimethyl sulphoxide for buthiobate, 2% propane-1,2-diol for triparanol, and 0.12% ethanol in experiments with compactin. At these concentrations the solvents did not show a significant effect on the HMG-CoA reductase activity.

### HMG-CoA reductase assay

HMG-CoA reductase activity was determined in the cell homogenate as described previously (Cohen *et al.*, 1984). Values were obtained from duplicate determinations in two homogenates of identically treated cells. The average values for the individual homogenates agreed within 10%. Protein concentrations were determined in the cell homogenate as described by Lowry *et al.* (1951). The data presented in the Figures are expressed as percentages of the control values, as means ± S.E.M., obtained from at least three separately performed experiments.

### Determination of the incorporation of [2-$^{14}$C]acetate or [2-$^{14}$C]mevalonate into non-saponifiable lipids

After the cells were incubated for 3 h with the medium containing the agents under investigation, either [$^{14}$C]acetate or [$^{14}$C]mevalonate was added (0.3 $\mu$Ci/well containing 1 ml of medium). After 15 h the medium was removed and the cells were scraped in 400 $\mu$l of cold buffer (0.1 M-potassium phosphate/0.1 M-NaCl/10 mM-EDTA, pH 7.4). Medium and cells were stored at −20 °C. After thawing and rupture of the cells by sonication (Branson sonifier B12, 70 W output, for 5 s at 0 °C), samples were taken for protein determination (Lowry *et al.*, 1951). Thereafter, total lipids were extracted from cell homogenate and medium together, as described by Bligh & Dyer (1959), in the presence of 0.008% (w/v) butylated hydroxytoluene (Sigma) as an antioxidant and 0.01 $\mu$Ci of [$^3$H]cholesterol as a recovery standard (in the experiments with triparanol, 250 $\mu$g of desmosterol was added as carrier). After evaporating the chloroform from the lipid extract under $N_2$, saponification was conducted in 0.2 ml of ethanolic (96%) 0.5 M-NaOH for 1½ h at 60 °C. After cooling, 0.2 ml of water was added and the non-saponifiable lipids were extracted with 2 × 0.5 ml of hexane. The combined hexane extracts were washed with 0.8 ml of ethanolic (48%) 0.25 M-NaOH, followed by evaporation of the hexane under nitrogen. The non-saponifiable lipids were analysed by t.l.c. In t.l.c. system I, separation was on silica-coated plates (Merck DC 60), as described by Sexton *et al.* (1983), with minor modifications, i.e. development in light petroleum (b.p. 40–60 °C)/acetone (8:2, v/v). $R_F$ values of lipid standards in this system were: cholesterol 0.42; lanosterol 0.53; desmosterol 0.42; squalene diepoxide 0.67; squalene epoxide 0.87; squalene 0.91.

In t.l.c. system II, separation was on reversed-phase plates (Whatman KC18) as described by Chang *et al.* (1979). The plates were developed twice in acetonitrile/chloroform (2:1, v/v). Cholesterol ($R_F$ 0.44), desmosterol ($R_F$ 0.51) and lanosterol ($R_F$ 0.61) were used as standards.

Standards run in separate lanes were detected by heating after spraying with $H_2SO_4$. After spraying with En$^3$hance, the $^{14}$C-labelled non-saponifiable lipids were detected by fluorography.

The $^{14}$C-labelled non-saponifiable lipids from control cells (incubated without inhibitor) migrated in only one spot, corresponding to cholesterol in both t.l.c. systems. Radioactively labelled spots were scraped into scintillation vials containing 0.5 ml of methanol and counted for radioactivity after addition of 10 ml of Instafluor. Values in the Figures are expressed as $^{14}$C c.p.m./mg of cellular protein, corrected for loss of [$^3$H]cholesterol during the extraction procedure, which was 10–30%. The data presented are obtained from duplicate incubations and are representative of at least two separately performed experiments.

## RESULTS

### Effect of triparanol on HMG-CoA reductase activity and on [$^{14}$C]mevalonate incorporation into non-saponifiable lipids

Blocking cholesterol biosynthesis at the desmosterol $\Delta^{24}$-reductase site by triparanol decreases the reductase activity in Hep G2 cells. Fig. 1(*a*) shows the HMG-CoA reductase activity as determined in the cell homogenate after incubation of the cells for 18 h at 37 °C with medium supplemented with the indicated triparanol concentration. The reductase activity was suppressed in a concentration-dependent manner finally to 20% of the control value at 20 $\mu$M drug. Triparanol had no effect on the reductase activity in the cell homogenate (result not shown). In order to confirm the site of action of triparanol, the accumulation of lipid intermediates of the pathway to cholesterol was determined. This was performed by determination of [$^{14}$C]mevalonate incorporation into non-saponifiable lipids, extracted from cells incubated for 18 h with the different triparanol concentrations indicated in Fig. 1. In t.l.c. system I the labelled lipids migrated as two spots, with $R_F$ values of 0.35 and 0.42. The second spot contained desmosterol

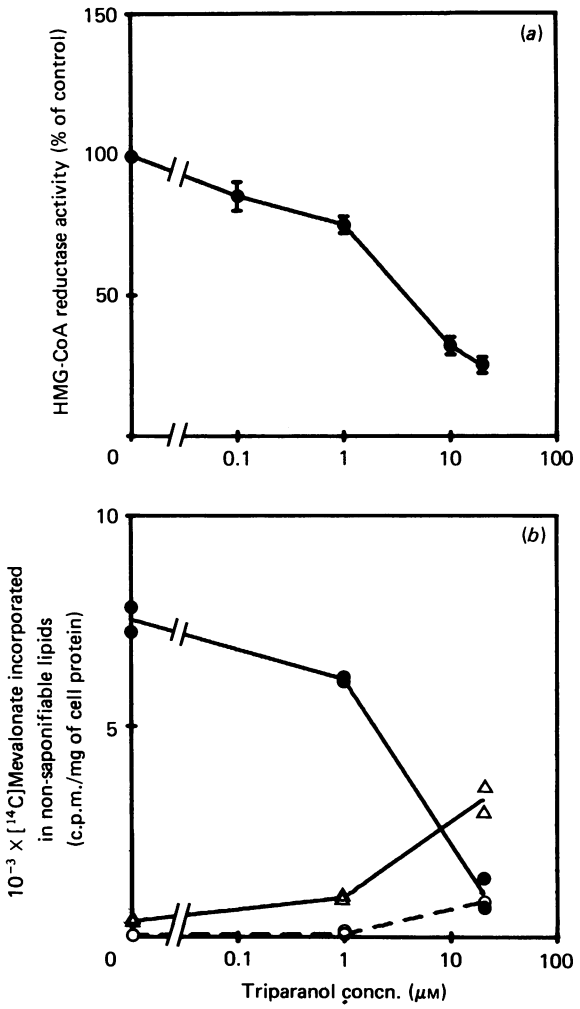


**Fig. 1. Effect of triparanol on HMG-CoA reductase activity (*a*) and on incorporation of [$^{14}$C]mevalonate into non-saponifiable lipids (*b*)**

Hep G2 cells were incubated for 18 h at 37 °C with DMEM, supplemented with 1% albumin and the indicated triparanol concentrations. (*a*) HMG-CoA reductase activity was determined in the cell homogenates as described in the Materials and methods section. Values are expressed as percentages of control (58.7 ± 10.6 pmol/min per mg of cell protein); means ± S.E.M. ($n = 3$). (*b*) For determination of [$^{14}$C]mevalonate incorporation, 0.3 μCi of [$^{14}$C]mevalonate was added to the medium at 15 h before harvesting the cells. Non-saponifiable lipids were extracted from the cells and medium as described in the Materials and methods section. Half of the lipid preparation was analysed in t.l.c. system I, and the other half in system II. The $^{14}$C-labelled sterols detected were cholesterol (●), desmosterol (△) and polar sterols (○).

and cholesterol, which could be separated by chromatography in system II. The other spot ($R_F$ 0.35) was considered to contain polar sterols, according to its behaviour in system I (Sexton *et al.*, 1983). As depicted in Fig. 1(*b*), at increasing triparanol concentrations a considerable decrease in the [$^{14}$C]mevalonate incorporation into cholesterol was observed, accompanied by an increase in $^{14}$C-labelled desmosterol, confirming the site of inhibition. Incorporation into polar sterols was substantial at 20 μM-triparanol in the incubation medium.

### Effect of buthiobate on HMG-CoA reductase activity and on [$^{14}$C]mevalonate incorporation into non-saponifiable lipids

To investigate whether inhibition of the metabolic pathway of cholesterol at the lanosterol 14α-demethylase site has any effect on the reductase activity, the reductase activity and [$^{14}$C]mevalonate incorporation into non-saponifiable lipids of Hep G2 cells treated with buthiobate were determined. The reductase activity of cells incubated for 18 h with the buthiobate concentrations indicated in Fig. 2 decreased at higher drug

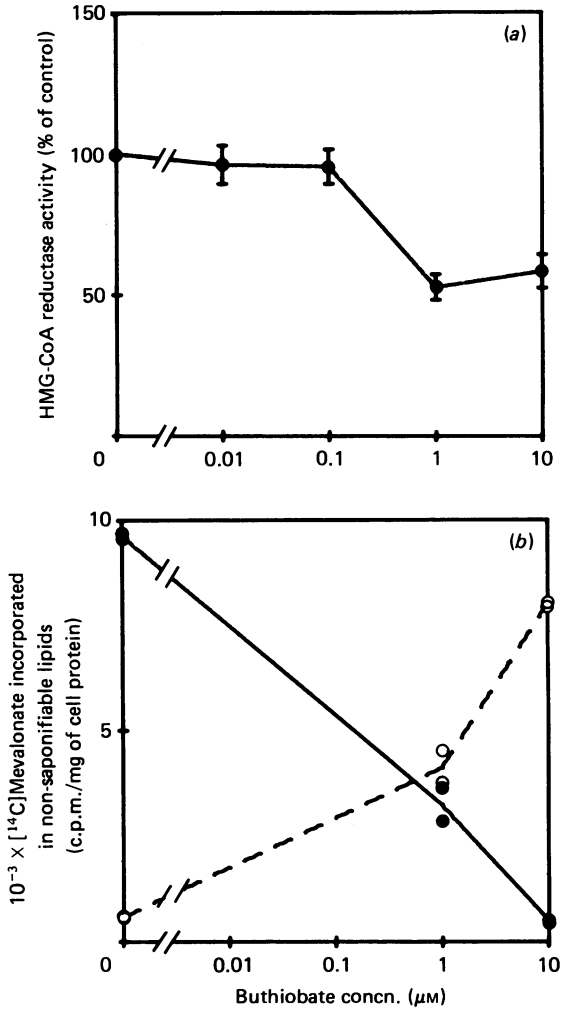


**Fig. 2. Effect of buthiobate on HMG-CoA reductase activity (*a*) and on incorporation of [$^{14}$C]mevalonate into non-saponifiable lipids (*b*)**

Cells were incubated for 18 h at 37 °C with DMEM, supplemented with 1% albumin and the indicated buthiobate concentrations. (*a*) HMG-CoA reductase activity was determined as described in the Materials and methods section. Values are expressed as percentages of control (55.8 ± 4.1 pmol/min per mg of cell protein); means ± S.E.M. ($n = 3$). (*b*) For determination of the [$^{14}$C]mevalonate incorporation in the lipid fraction, 0.3 μCi of [$^{14}$C]mevalonate was added to the medium 15 h before harvesting the cells. Non-saponifiable lipids were isolated from the cells and medium as decribed in the Materials and methods section and further analysed in t.l.c. system I. The only labelled lipids detected were cholesterol (●) and lanosterol (○).

concentrations (Fig. 2*a*). At 10 $\mu$M-buthiobate the enzyme activity was about half the control value. Buthiobate had no effect on the reductase activity in the cell homogenate (results not shown).

Fig. 2(*b*) shows the results of the incorporation of [$^{14}$C]mevalonate into non-saponifiable lipids in cells incubated with the indicated buthiobate concentrations. Separation by means of t.l.c. system I indicated that only $^{14}$C-labelled cholesterol and lanosterol were detectable in the lipid extracts. As shown in Fig. 2(*b*), buthiobate caused a concentration-dependent decrease in the labelling of cholesterol, which led to an almost complete abolition of this incorporation at 10 $\mu$M drug. On the other hand, the incorporation of label into lanosterol strongly increased at the concentrations tested. This observation confirms the inhibitory effect of buthiobate on lanosterol 14$\alpha$-demethylase in Hep G2 cells.

### Effect of U18666A on HMG-CoA reductase activity and on [$^{14}$C]acetate incorporation into non-saponifiable lipids

The squalene-2,3-epoxide cyclase inhibitor U18666A was used to investigate whether a decrease in sterologenesis and the subsequent accumulation of non-sterols has an effect on the reductase activity in Hep G2 cells. HMG-CoA reductase activity was determined after incubation of the cells for 18 h with medium containing different concentrations of U18666A. The results are depicted in Fig. 3(*a*). At low U18666A concentrations the reductase activity was concentration-dependently sup-

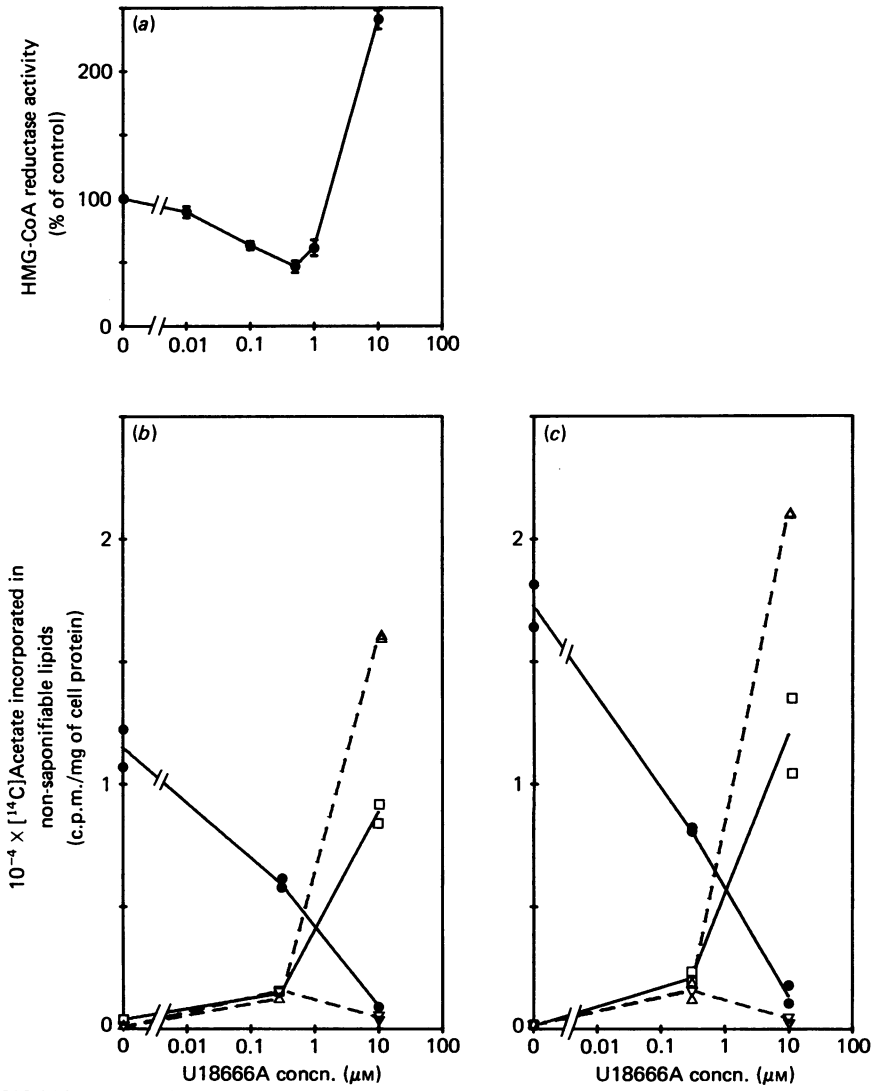


**Fig. 3. Effect of U18666A on the HMG-CoA reductase activity (*a*) and on incorporation of [$^{14}$C]acetate into non-saponifiable lipids either in the absence (*b*) or in the presence (*c*) of LDL**

Hep G2 cells were incubated for 18 h at 37 °C with DMEM, supplemented with 1% albumin and the indicated U18666A concentrations. (*a*) HMG-CoA reductase activity was determined in the cell homogenates as described in the Materials and methods section. Values are expressed as percentages of control (83.4 ± 13.9 pmol/min per mg of cell protein); means ± S.E.M. ($n$ = 3). (*b*) At 15 h before harvesting the cells, 0.3 $\mu$Ci of [$^{14}$C]acetate was added to the medium. See the Materials and methods section for the subsequent extraction of the $^{14}$C-labelled non-saponifiable lipids from cells and medium. Chromatography in t.l.c. system I showed the presence of cholesterol (●), squalene 2,3-epoxide (△), squalene 2,3:22,23-diepoxide (□) and polar sterols (▽). (*c*) As described at (*b*), except that additionally 200 $\mu$g of LDL/ml was present during the cell incubation.

pressed, with a minimum (40% of control activity) around 0.5 μM. However, at higher concentrations (> 3 μM) the enzyme activity was stimulated (Fig. 3*a*; cf. also Table 1 and Fig. 4). U18666A had no effect on the reductase activity in the cell homogenate (result not shown).

Four lipid fractions labelled from [$^{14}$C]acetate in Hep G2 cells in the presence of different concentrations of U18666A were detected by chromatography in t.l.c. system I. Besides a polar sterol fraction and labelled cholesterol, two radioactive spots were detected, which in this system behaved as squalene 2,3-epoxide and squalene 2,3:22,23-diepoxide. As shown in Fig. 3(*b*), U18666A blocked the $^{14}$C-labelling of cholesterol and induced concomitantly the $^{14}$C incorporation into the squalene epoxides. The polar sterol fraction was only detected at a U18666A concentration (0.3 μM) at which the HMG-CoA reductase activity was strongly inhibited (cf. Figs. 3*a* and 3*b*).

### Effect of U18666A in the presence of LDL on HMG-CoA reductase activity and on [$^{14}$C]acetate incorporation into non-saponifiable lipids in Hep G2 cells

Low-density lipoprotein (LDL) was able to decrease the compactin-induced rise in HMG-CoA reductase activity in Hep G2 cells, probably by compensating for missing mevalonate-derived metabolites (Cohen *et al.*, 1984, 1985). Analogous to this observation, it was investigated whether LDL had the same effect on the U18666A-induced increase in the reductase activity, supposing that LDL influx could compensate for a missing sterol regulator.

In Table 1 the relative effect of LDL (200 μg/ml) on the reductase activity in either the absence or the presence of 20 μM-U18666A is depicted. The HMG-CoA reductase activity was suppressed by LDL alone; however, unexpectedly, at 20 μM-U18666A, the reductase activity was additionally stimulated by LDL. The presence of LDL in the medium did not result in a significant change in the incorporation pattern of [$^{14}$C]acetate into non-saponifiable lipids in cells treated with U18666A (compare Fig. 3*c*, LDL present, with Fig. 3*b*, without LDL). Here also polar sterols were only detectable in the lipid extracts from cells incubated with 0.3 μM-U18666A, at which concentration the reductase activity was maximally depressed also in the presence of LDL (results not shown).

Cholesterol influx from LDL was not influenced by the presence of U18666A, as shown by g.l.c. analysis of the lipid content of the cells after the incubation (increase in cholesterol content of 10 μg/mg of cellular protein by incubation with 200 μg of LDL/ml, independent of different U18666A concentrations in the incubation medium; cholesterol content was 15 μg/mg of cellular protein in untreated cells).

### Effect of U18666A combined with compactin and mevalonate on HMG-CoA reductase activity in Hep G2 cells

It was previously shown (Cohen *et al.*, 1985) that in Hep G2 cells mevalonate-derived effectors in the regulation of HMG-CoA reductase can be divided

**Table 1. Effect of LDL on basal and U18666A-induced HMG-CoA reductase activity**

Hep G2 cells were incubated for 18 h without or with 200 μg of LDL/ml in either the absence or the presence of 20 μM-U18666A. Reductase activity was determined as described in the Materials and methods section. Values are given as means ± S.E.M. ($n = 5$) of the percentage of those obtained from the incubations without LDL, which were at 0 μM-U18666A 110.8 ± 10.7 and at 20 μM-U18666A 255.7 ± 52.9 (S.E.M., $n = 5$) pmol/min per mg of cell protein.

| U18666A present | HMG-CoA reductase (%) | |
|---|---|---|
| | No LDL | +LDL |
| − | 100 | 50.2 ± 3.9* |
| + | 100 | 144.8 ± 3.3* |

* $P < 0.001$ in Student's $t$ test for paired observations.

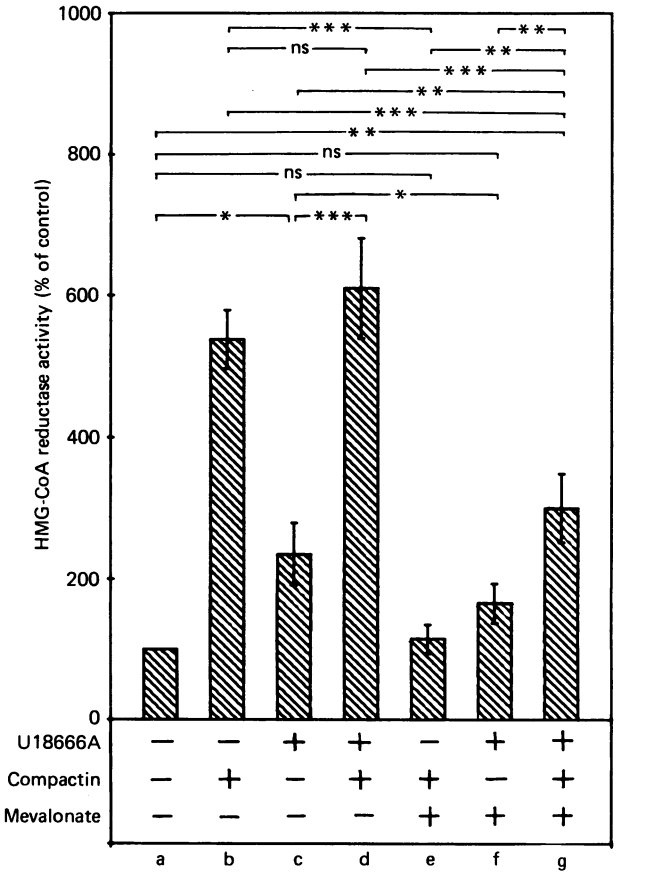


**Fig. 4. Effect of U18666A combined with compactin and mevalonate on the HMG-CoA reductase activity**

Hep G2 cells were incubated for 18 h at 37 °C with DMEM, supplemented with 1% albumin and the indicated (+) combinations of 30 μM-U18666A, 2 μM-compactin and 3 mM-mevalonate. The HMG-CoA reductase assay was performed as described in the Materials and methods section. The values are expressed as percentages of control values (116.9 ± 9.9 pmol/min per mg of cell protein); means ± S.E.M. ($n = 7$). Statistical analysis was performed with a paried Student's $t$ test: ***$P < 0.001$; **$P < 0.005$; *$P < 0.025$; n.s., not significant ($P \geq 0.05$).

into effectors compensatable by LDL (possibly sterol effectors) and non-LDL-related (possibly non-sterol) effectors. With the aid of U18666A, which blocks sterologenesis (see, e.g., Fig. 3*b*; 92% inhibition at 10 $\mu$M-U186666A), we are able to discriminate more clearly between sterol and non-sterol effectors. Therefore we investigated the effect of blocking sterologenesis with 30 $\mu$M-U18666A, combined with the additional effects of compactin and mevalonate. The results are shown in Fig. 4. Mevalonate decreased the reductase activity relatively much more in compactin-treated cells (Fig. 4; cf. bars b and e) than in U18666A-treated cells (Fig. 4; cf. bars c and f). The additional increase in activity in U18666A-treated cells caused by compactin (Fig. 4; cf. bars c and d) could almost totally be prevented by addition of mevalonate (Fig. 4, bar g). From these experiments it was concluded that, in total blockade of sterologenesis by U18666A, mevalonate or mevalonate-derived products still influenced the reductase activity, indicating the existence of a non-sterol effector.

## DISCUSSION

It was shown that, in the human hepatoma cell line Hep G2, the drugs triparanol, buthiobate and U18666A were strong inhibitors of cholesterol biosynthesis (Figs. 1*b*, 2*b* and 3*b* respectively). The blockade at the desmosterol reductase site by triparanol resulted in a decrease in HMG-CoA reductase activity (Fig. 1). The opposite effect, i.e. reductase stimulation by triparanol, was observed by Havel *et al.* (1979). However, in that case, with a primary rat hepatocyte culture and a high concentration of triparanol (30 $\mu$M), the drug treatment resulted in a strong inhibition of squalene-2,3-epoxide cyclase. Their findings are in agreement with our observations with high concentrations of U18666A (Fig. 3), indicating that blockage of sterol formation caused an increase in HMG-CoA reductase activity, confirming the existence of a sterol effector.

In treating Hep G2 cells with triparanol, we observed the formation of polar sterols. It is not likely that these sterols are formed from the accumulated [$^{14}$C]desmosterol during the extraction procedure, because of the large quantity of carrier desmosterol added. Although the nature of these polar sterols is not yet known, the possibility exists that they are involved in decreasing the reductase activity. It was shown that certain polar sterols (oxysterols) act as potent inhibitors of the reductase activity (Kandutsch *et al.*, 1978; Schroepfer, 1981). Although we cannot exclude that in this case the accumulation of desmosterol might have contributed to the decrease in reductase activity, limited desmosterol accumulation in rat hepatocytes (induced by a short incubation with triparanol) had no effect on the rate of $C_{27}$ sterol synthesis (Gibbons & Pullinger, 1977; Pullinger & Gibbons, 1983). Our observations seem to be in conflict with the results published by the last-mentioned authors, who did not observe an effect of triparanol on the incorporation of [$^{14}$C]acetate or $^3H_2O$ into $C_{27}$ sterols in rat liver cell suspensions. However, their experiments were performed at a low concentration of the drug (2 $\mu$M) and for much shorter incubation times (maximally 4 h).

Because of the decrease in synthesis of endogenous mevalonate at 20 $\mu$M-triparanol, an increase in incorporation of exogenous [$^{14}$C]mevalonate into total non-saponifiable lipids was expected. However, a slight decrease in the incorporation was observed (Fig. 1*b*). At present we cannot exclude that this concentration of triparanol influences in another way the specific radioactivity of the endogenous mevalonate pool, for instance by decreasing the uptake of [$^{14}$C]mevalonate into the cells, or that the drug has an effect on (an)other enzymes(s) of mevalonate metabolism in Hep G2 cells.

HMG-CoA reductase activity was also decreased when cholesterol synthesis was inhibited at the lanosterol 14$\alpha$-demethylase site by buthiobate (Fig. 2). Besides cholesterol, the only accumulating sterol detected was lanosterol, suggesting that this sterol may be involved in the decrease in reductase activity. On the one hand, the observed relationship between the accumulation of methyl sterols and the decrease in HMG-CoA reductase activity in primary rat hepatocyte culture cells (Havel *et al.*, 1979) and the inhibition of the reductase by lanosterol in human fibroblasts (Brown & Goldstein, 1974) seem to support this suggestion. However, on the other hand it was reported more recently (Gupta *et al.*, 1986) that lanosterol accumulation in rat intestinal epithelial cells (caused by inhibition of lanosterol 14$\alpha$-demethylase with the drug ketoconazole) was not accompanied by a decrease in reductase activity at drug concentrations higher than 15 $\mu$M. On the contrary, a stimulation of enzyme activity was observed, which was found to correlate with a decrease in polar sterol formation. Although not detected in the t.l.c. system used, we cannot exclude the formation of very minor amounts of other, possibly inhibitory, sterols in the cells treated with buthiobate.

The effect of incubating Hep G2 cells with the lanosterol 14$\alpha$-demethylase inhibitor ketoconazole (Kempen & Cohen, 1985) was similar to that described here for buthiobate. Even after incubation with ketoconazole in concentrations up to 100 $\mu$M the reductase activity in Hep G2 cells remained suppressed (H. J. Kempen, K. van Son, L. H. Cohen, M. Griffioen, H. Verboom & L. Havekes, unpublished work). It is possible that the hepatic origin of the human hepatoma cell line plays a role in the difference in the ketoconazole effect in Hep G2 cells compared with that in the rat intestinal cells, as observed by Gupta *et al.* (1986).

Polar sterols were also detected in the non-saponifiable lipid extracts from the Hep G2 cells treated with low concentrations of U18666A, whereas squalene-2,3-epoxide cyclase was partially blocked (accumulation of the squalene epoxides and cholesterol still formed) and reductase activity was decreased (Fig. 3). At high concentrations of U18666A, when the squalene 2,3-epoxide cyclase was blocked almost completely, the formation of polar sterols was much decreased, accompanied by a strong stimulation of the reductase activity. Again, these results suggest that polar sterols are involved in the suppression of the reductase activity. Similar observations were made by Panini *et al.* (1984), by incubating rat intestinal epithelial cells with U18666A. They concluded that when squalene epoxide cyclase is partially blocked, the diepoxide, formed from squalene epoxide, but not an intermediate of cholesterol biosynthesis, is converted into polar 24,25-epoxide sterols, which inhibit the reductase activity. When the cyclase is blocked completely, this conversion does not take place and the inhibition of the reductase activity is subsequently abolished. They did not report a strong stimu-

lation of the reductase activity at high U18666A concentrations, as was observed in the experiments described here.

LDL enhanced the stimulating effect of U18666A on HMG-CoA reductase activity at high drug concentrations. Thus LDL could not replace the deficit of sterols caused by the blockade of sterologenesis, as effector in the inhibition of the reductase activity. The decrease in reductase activity caused by LDL alone was not accompanied by a detectable accumulation of $^{14}C$-labelled polar sterols (Fig. 3*c*). This contradicts Panini *et al.* (1984), who observed an abolition of the LDL-induced inhibition of the reductase activity by U18666A and suggested that LDL should influence the squalene 2,3-epoxide cyclase in the same way as low concentrations of U18666A. No preferential binding of U18666A to LDL was detected, making an enhanced influx of U18666A by LDL unlikely (results not shown). The cholesterol content of cells incubated with LDL increased markedly, but this was independent of the U18666A concentration, indicating that the LDL influx was not impaired by the drug. The possibility remains that the LDL effector is derived from the cholesterol introduced, but that its synthesis is blocked by U18666A. The exact mechanism is as yet unclear.

When mevalonate was added to Hep G2 cells together with U18666A in a sterologenesis preventing concentration, the U18666A-induced elevation of the reductase activity could not be abolished. However, when mevalonate was added to Hep G2 cells in the presence of the same concentration of U18666A and 2 $\mu$M-compactin, the additional increase in reductase activity caused by compactin could be prevented by mevalonate (Fig. 4). This observation supports the existence of non-sterol effectors derived from mevalonate, in addition to a sterol-dependent regulation which was blocked by U18666A.

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