Regulation of 3-hydroxy-3-methylglutaryl-CoA reductase mRNA contents in human hepatoma cell line Hep G2 by distinct classes of mevalonate-derived metabolites

Louis H. COHEN and Marieke GRIFFIOEN

TNO Gaubius Institute for Cardiovascular Research, Herenstraat 5d, 2313 AD Leiden, The Netherlands

Hep G2 cells were incubated under conditions known to influence the HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase activity, e.g. in the presence of compactin (a competitive inhibitor of HMG-CoA reductase itself) and U18666A (a squalene-2,3-epoxide cyclase inhibitor). We studied the effects of these conditions both on the HMG-CoA reductase activity and on the reductase mRNA content. In the presence of compactin the mRNA content increased, but less than the enzyme activity, as determined after removal of the inhibitor. The increase in mRNA could be prevented by addition of mevalonate or by a combination of low-density lipoprotein (LDL) plus a low concentration of mevalonate. LDL alone prevented the compactin-induced increases in mRNA and activity only partially. The effect of U18666A on reductase mRNA content and activity was biphasic, i.e. a slight decrease at low (0.3–0.5 μM) concentrations, with a concomitant formation of polar sterols [Boogaard, Griffioen & Cohen (1987) *Biochem. J.* **241**, 345–351], and an increase at high (20–30 μM) concentrations, with complete blockage of sterol formation. At these high concentrations of U18666A, additional compactin (2 μM) increased the reductase activity, but not the mRNA content. We conclude that non-sterol metabolites of mevalonate regulate exclusively at the enzyme level, whereas sterol metabolites regulate at the reductase mRNA level. In the latter group of regulators we distinguish mevalonate metabolites which can, and metabolites which cannot, be replaced by exogenous LDL.

INTRODUCTION

It has been shown that the human hepatoma cell line Hep G2 is a suitable model for the study of regulation of cholesterol synthesis in the human hepatocyte (Cohen *et al.*, 1984, 1985; Boogaard *et al.*, 1987; Erickson & Fielding, 1987). Also in Hep G2 cells HMG-CoA reductase (EC 1.1.1.34), the rate-limiting enzyme in cholesterol biosynthesis, is regulated by endogenous formed metabolites of mevalonate (Cohen *et al.*, 1984). By blocking the sterol synthesis with the squalene-2,3-epoxide cyclase (EC 5.4.99.7) inhibitor U18666A {3 β -[2-(diethylamino)ethoxy]androst-5-en-17-one} (Sexton *et al.*, 1983), we were able to show that the sterol components of these metabolites, which seem to be polar sterols, influenced the reductase activity and that additionally non-sterol mevalonate-derived suppressors play a role in the regulation of the enzyme (Boogaard *et al.*, 1987). The existence of at least two different classes of mevalonate-derived effectors followed also from experiments in which low-density lipoprotein (LDL) could only partially prevent the rise in reductase activity induced by incubation with compactin, a competitive inhibitor of the reductase itself (Endo *et al.*, 1976). Additionally, mevalonate had to be added to the cells for complete abolition of the compactin effect (Cohen *et al.*, 1985). However, from these experiments it was not clear whether the additional mevalonate-derived suppressors, which could not be replaced by LDL, belong to the non-sterol class, or whether a sterol component is also involved.

To discriminate better between the apparent distinct regulators of the reductase activity derived from mevalonate, and to gain more insight into the levels on which these regulators act, we compared the changes in enzyme activity with the effects on HMG-CoA reductase mRNA content in Hep G2 cells, after incubations under different conditions. Evidence is provided that non-sterol mevalonate-derived suppressors act at the enzyme level, whereas the endogenous reductase mRNA concentration is regulated by sterol(s), which can be replaced by exogenous LDL and by another sterol class, for which LDL cannot substitute.

MATERIALS AND METHODS

Materials

U18666A was obtained from Dr. P. W. O'Connell, Upjohn Co., Kalamazoo, MI, U.S.A. Compactin was given by Dr. A. Endo, Tokyo Nōkō University, Tokyo, Japan. [3-¹⁴C]HMG-CoA (sp. radioactivity 47–57 Ci/mol) and [α -³²P]dCTP (sp. radioactivity 3×10^6 Ci/mol) were purchased from New England Nuclear. Human serum albumin (fraction V) (subsequently termed 'albumin') was obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. It was freed of fatty acids (Chen, 1967) and loaded with palmitate (Fluka A.G.; 10 μmol of palmitate/g of albumin; Spector & Hoak, 1969). Picofluor and dioxan scintillator were from United Packard Industries. Human LDL (density 1.03–

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low-density lipoprotein; DMEM, Dulbecco's modified Eagle's medium; SSC, standard saline citrate (0.15 M-NaCl/0.015 M-sodium citrate, pH 7.0).

1.05 g/ml) was isolated from freshly collected pooled blood by density-gradient ultracentrifugation (Redgrave *et al.*, 1975). Restriction enzymes *Hind*III and *Pst*I were purchased from Promega and Boehringer Mannheim respectively. *Escherichia coli* polymerase I was from Pharmacia. All other chemicals were of analytical grade.

Human hepatoma cell line Hep G2

The cells were cultured in 10 cm² multiwell dishes in DMEM, supplemented with 10% (w/v) foetal-calf serum (Havekes *et al.*, 1983). At 18 h before harvest, the medium was replaced with DMEM, supplemented with 1% (w/v) palmitate-loaded albumin and the different constituents as indicated in the Results section. In incubations with U18666A the concentration of the solvent (ethanol) in the medium was 0.15%, which has no significant effect on the reductase activity.

HMG-CoA reductase assay

HMG-CoA reductase activity was determined in the Hep G2 cell homogenate as described previously (Cohen *et al.*, 1984; the [¹⁴C]HMG-CoA concentration was 0.5 mM). Values were obtained from duplicate determinations in two homogenates of identically treated cells. The average value for the individual homogenates agreed within 10%. Protein concentrations were determined in the cell homogenate by the method of Lowry *et al.* (1951).

cDNA probes

The plasmid pHRed-102 containing human HMG-CoA reductase cDNA (Luskey & Stevens, 1985) was kindly given by Dr. K. L. Luskey, Department of Molecular Genetics and Internal Medicine, University of Texas, Dallas, TX, U.S.A. The 1136-base-pair *Hind*III fragment, isolated by electro-elution from a 1%-agarose gel, was used as a hybridization probe. The human serum albumin cDNA clone (referred to as pHSA) was obtained from Dr. H. Pannekoek, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. We used the 740-base-pair *Hind*III/*Pst*I fragment, located at the 3' end of the cDNA as described by Dugaiczky *et al.* (1982). Both fragments were labelled by nick-translation (Maniatis *et al.*, 1982) and were shown to hybridize specifically with Hep G2 HMG-CoA reductase and albumin mRNA, respectively, in a Northern-blot analysis (cf. Cohen *et al.*, 1987).

Isolation of RNA from Hep G2 cells

After being washed at 37 °C three times with phosphate-buffered saline (0.15 M-NaCl/10 mM-Na₂HPO₄/1.5 mM-KH₂PO₄, pH 7.4), the cells were lysed by gently shaking at 37 °C for 10 min in 0.3 ml (per well) of a buffer containing 50 mM-Tris/HCl, pH 7.5, 0.1 M-NaCl, 7.5 mM-EDTA, 1% (w/v) SDS and 150 µg of proteinase K/ml (a minor modification of the procedure of Lizardi & Engelberg, 1979). The lysates of two 10 cm² wells were combined, frozen in liquid N₂ and stored at -80 °C. RNA was extracted by a hot-phenol method as described by Warner *et al.* (1966), with minor modifications. The lysates were extracted twice with phenol at 58 °C and subsequently with phenol/chloroform (2:1, v/v) and diethyl-ether at 20 °C. The RNA pellet, obtained by precipitation with ethanol in the presence of 0.15 M-

sodium acetate and successive centrifugation at 7000 g for 90 min at -20 °C, was dissolved in 60 µl of 10 mM-Tris/HCl, pH 7.5. The concentration was determined by measuring the A₂₈₀ and A₂₆₀, and subsequently the RNA solution was stored at -20 °C.

Determination of mRNA by dot-blot hybridization

RNA was immobilized on nitrocellulose filters (BA 85; Schleicher & Schüll) by the procedure described by Van Zonneveld *et al.* (1986). For hybridization with the reductase probe, a series of six dots per RNA sample containing between 1.8 and 14 µg of RNA was loaded. For hybridization with the albumin probe, these series contained between 0.55 and 4.15 µg of RNA/dot. Prehybridization was performed for at least 6 h at 42 °C in 4 × SSC/1% SDS/5 × Denhardt's solution/50% (v/v) formamide containing denatured salmon sperm DNA (0.1 mg/ml) (Maniatis *et al.*, 1982). Hybridization took place for 16–20 h at 42 °C in the same solution (10 ml) after addition of 0.1 µg of the nick-translated heat-denatured probe. The average specific radioactivity of the HMG-CoA reductase cDNA fragment was 2 × 10⁸ c.p.m./µg of DNA, and that of the albumin cDNA fragment was 10⁸ c.p.m./µg of DNA. After hybridization, the blots were washed twice with 2 × SSC (5 min at 20 °C), 2 × SSC/0.1% SDS (30 min at 65 °C), 0.1 × SSC/0.1% SDS (30 min at 65 °C) and 0.1 × SSC (at 20 °C) successively. The RNA dots hybridized with the albumin cDNA probe were cut out and counted for radioactivity in 4 ml of dioxan scintillator (200–3000 c.p.m./dot). Because the radioactivity bound to the dot-blot hybridized with the reductase cDNA probe was too low for direct counting, these blots were exposed to Hyperfilm MP (Amersham) for 8 h–1 week together with an intensifying screen (Kodak) at -80 °C, and the autoradiograms were scanned with a Shimadzu CS-910 chromatogram scanner. For each sample the amount of probe hybridized per µg of RNA was calculated as the regression coefficient of the linear part of the hybridization curve. In general, all six dots per sample were within this linear part. The albumin-mRNA values were used as an internal mRNA recovery standard; the values for the HMG-CoA reductase mRNA contents are expressed as HMG-CoA-reductase-probe-derived blackening (arbitrary) units per µg of RNA divided by c.p.m. of hybridized albumin probe per µg of RNA. S.E.M. values were calculated for the mRNA values from separate identical incubations, performed in two to three incubation series. The number of identical incubations (*n*) is mentioned in the legends of the Figures.

RESULTS

Effect of compactin and mevalonate on HMG-CoA reductase mRNA contents in Hep G2 cells

Total cellular RNA was isolated from Hep G2 cells, which had been incubated for 18 h with different concentrations of compactin, the inhibitor of the reductase itself. As an example, the results of a dot-blot hybridization of a concentration series of this RNA with a fragment of the human HMG-CoA reductase plasmid pHRed-102 and with a cDNA probe for human serum albumin (pHSA) are depicted in Fig. 1. In none of the experiments described in this paper did the quantity of hybridizable albumin mRNA change significantly, so we considered the quantity of this mRNA to be constant

Fig. 1. Dot-blot hybridization of Hep G2 RNA with human HMG-CoA reductase cDNA (a) and with human serum albumin cDNA (b)

Total cellular RNA was isolated from Hep G2 cells, which had been incubated for 18 h at 37 °C with DMEM, supplemented with 1 % albumin and the indicated concentrations of compactin in duplicate incubations, as described in the Materials and methods section. The indicated quantities of RNA were loaded on a nitrocellulose filter and hybridized for 20 h with a ³²P-labelled cDNA fragment of either (a) pHRed-102 (4×10^8 c.p.m./μg of DNA), coding for human HMG-CoA reductase, or (b) pHSA (10^8 c.p.m./μg of DNA), coding for human serum albumin. The radioactivity hybridized was detected by autoradiography for 16 h.

Fig. 2. Effect of compactin (a) and mevalonate (b) on HMG-CoA reductase mRNA contents in Hep G2 cells

Hep G2 cells were incubated for 18 h at 37 °C with DMEM, supplemented with 1 % albumin and either the indicated compactin concentrations (a; ●) or the indicated concentrations of mevalonate with (b; ▲) or without (b, ■) 2 μM-compactin. RNA was isolated and hybridized by the dot-blot procedure as described in the Materials and methods section. Hybridization with the reductase probe was quantified by scanning the autoradiograph, whereas the dots hybridized with the albumin probe were counted for radioactivity. The values, reductase-probe-derived blackening (arbitrary units) per μg of RNA/c.p.m. of hybridized albumin probe per μg of RNA, are expressed as mean percentages of control ($\pm$ S.E.M.; $n = 4$).

under the different incubation conditions and felt free to use the values obtained as an internal mRNA-recovery standard. The data obtained for the HMG-CoA reductase mRNA are expressed relative to the values for the albumin mRNA measured in the same RNA preparations. Quantification of results such as those depicted in Fig. 1 yielded the values as shown in Fig. 2(a). Reductase mRNA contents increased at increasing compactin concentrations, indicating that mevalonate or mevalonate-derived metabolites suppress the mRNA contents.

This is supported by the observation that simultaneously added mevalonate was able to abolish the compactin-induced increase (Fig. 2b).

Effect of U18666A on HMG-CoA reductase mRNA contents

We showed previously that, by dividing the cholesterol-biosynthesis pathway into a sterol and a non-sterol part with U18666A (an inhibitor of squalene-2,3-epoxide cyclase), both non-sterol and (polar) sterol effectors

Fig. 3. Effect of U18666A on HMG-CoA reductase mRNA contents in Hep G2 cells

Cells were incubated for 18 h at 37°C in DMEM, supplemented with 1% albumin and the indicated U18666A concentrations. The mRNA contents were determined as described in the Materials and methods section. The values are expressed as percentage of controls as means $\pm$ S.E.M. ($n = 6$).

Fig. 4. Effect of U18666A combined with compactin and mevalonate on the HMG-CoA reductase mRNA content in Hep G2 cells

Cells were incubated for 18 h at 37°C with DMEM, supplemented with 1% albumin and the indicated (+) combination of 30 μ M-U18666A, 2 μ M-compactin and 3 mM-mevalonate. HMG-CoA reductase mRNA contents were determined as described in the Materials and methods section. The values are expressed as percentages of control values, as means $\pm$ S.E.M. ($n = 4-6$).

derived from mevalonate were involved in the feedback regulation of the reductase (Boogaard *et al.*, 1987). The effect of U18666A on HMG-CoA reductase mRNA contents is depicted in Fig. 3. A slight decrease occurred

Fig. 5. Effect of LDL combined with compactin and mevalonate on HMG-CoA reductase activity in Hep G2 cells

Cells were incubated for 18 h at 37°C with DMEM, supplemented with 1% albumin, the indicated concentrations of LDL and the following additions: $\square$, none; $\circ$, 3 mM-mevalonate; $\blacksquare$, 2 μ M-compactin; $\bullet$, 2 μ M-compactin + 3 mM-mevalonate. HMG-CoA reductase activity was determined in the cell homogenate as described in the Methods section. Values are expressed as percentages of control (122 ± 21.9 pmol/min per mg of cell protein), as means $\pm$ S.E.M. ($n = 4$).

at 0.3–0.5 μ M U18666A, whereas an increase was detected at 20–30 μ M. Comparable effects on the enzyme activity were observed (Boogaard *et al.*, 1987). The simultaneous appearance of polar sterols at the low U18666A concentrations (Boogaard *et al.*, 1987) seems to suggest that these compounds are involved in the decrease in reductase mRNA. The elevation of the mRNA content at high U18666A concentration is thought to be the result of total blockage of sterol formation, indicating that sterols are effective as suppressors at the mRNA level.

The existence of a non-sterol suppressor was shown by determining the reductase activity in cells which had been totally blocked in sterogenesis by U18666A and incubated additionally with compactin (2 μ M) and/or mevalonate (3 mM). Under such conditions compactin was still able to induce the reductase activity (2.5 times the activity obtained after incubation with U18666A alone), which could be prevented by mevalonate (Boogaard *et al.*, 1987). In identically performed experiments the effect on the mRNA content was studied; the results are shown in Fig. 4. As discussed above, an U18666A-induced increase in mRNA was observed (cf. bars a and c); however, additional compactin in the incubation medium did not lead to a further increase in the quantity

of reductase mRNA (cf. bars *c* and *d*). The combined effects of U18666A/compactin and mevalonate resulted in the same values (bar *e*). The control values obtained from incubations with 3 mM-mevalonate (bar *f*) and with compactin plus mevalonate (bar *g*) agreed with the results discussed above (cf. Fig. 2*b*). These results support the existence of one or more mevalonate-derived non-sterol suppressors, which are effective at the enzyme level, but not at the mRNA level.

Effects of LDL and mevalonate on the compactin-induced increase in the HMG-CoA reductase activity and mRNA contents

In order to investigate whether exogenous cholesterol can substitute for the mevalonate-derived sterol suppressors, not formed in the presence of compactin, cells were incubated with medium containing 2 μ M-compactin and different quantities of LDL. Subsequent determinations of the HMG-CoA reductase activity resulted in the data presented in Fig. 5 (a preliminary result was reported previously; Cohen *et al.*, 1985), and the values obtained with the HMG-CoA reductase mRNA determinations are depicted in Fig. 6. LDL, even at high concentrations, can only partially prevent the compactin-induced increase at the level of enzyme activity (Fig. 5), whereas exogenous mevalonate has to be present additionally for complete abolition of the compactin effect. From this experiment one cannot discriminate whether the mevalonate-derived suppressor, which could not be replaced by LDL, has a non-sterol or a sterol character. However, that is possible from the mRNA measurements (Fig. 6), because, as shown by the data in Fig. 4, non-sterol effectors do not play a role at the mRNA level. Fig. 6 shows that also at the mRNA level LDL, at high concentrations, could only partially prevent the increase induced by compactin and that additional mevalonate is needed to return to the same basal mRNA amounts as those obtained with LDL alone.

Fig. 6. Effect of LDL combined with compactin and mevalonate on the HMG-CoA reductase mRNA contents in Hep G2 cells

Cells were treated as described in the legend of Fig. 5. The HMG-CoA reductase mRNA contents were determined as described in the Materials and methods section. Values are expressed as percentages of control, as means $\pm$ S.E.M. ($n = 4$).

DISCUSSION

The present study has yielded more insight into the different classes of mevalonate-derived metabolites which play a role in the feedback regulation of HMG-CoA reductase in Hep G2 cells and their level of action. The results arise from comparing the amounts of reductase mRNA with the enzyme activities, measured after incubation of the cells under different conditions. To our knowledge, this is the first description of such experiments in cells of human origin.

Upon incubation of Hep G2 cells with 2 μ M-compactin the increase in mRNA compared with control values (about 2–3-fold; Figs. 1, 4 and 6) is smaller than the increase in reductase activity (5–9 times the control value; Cohen *et al.*, 1984; Boogaard *et al.*, 1987; cf. Fig. 5). This observation seems to indicate that, in Hep G2 cells, regulation by mevalonate-derived effectors takes place at the mRNA as well as at the enzyme level. This is further extended by the results discussed below, but is also in agreement with effects observed in rat liver, where mevinolin (an analogue of compactin; Alberts *et al.*, 1980) increased (Clarke *et al.*, 1983) and mevalonate decreased (Clarke *et al.*, 1984) the reductase mRNA content, whereas mevalonate enhanced the enzyme degradation (Edwards *et al.*, 1983). Further, it was noted that mevinolin prevented HMG-CoA reductase enzyme degradation in Chinese-hamster ovary cells (Sinensky & Logel, 1983). Comparable effects of compactin and mevalonate on reductase mRNA contents were measured in UT-1 cells (Luskey *et al.*, 1983), a Chinese-hamster ovary mutant cell line, resistant to compactin (Chin *et al.*, 1982), by a stable amplification of the reductase gene (Luskey *et al.*, 1983).

With the aid of the squalene-2,3-epoxide cyclase inhibitor, U18666A (Sexton *et al.*, 1983; Boogaard *et al.*, 1987), we were able to show that the mevalonate metabolites, responsible for the feedback regulation, can be divided into at least two classes with a distinguishable way of action: one class, the non-sterol suppressors, which, at total blockade of sterologenesis by 30 μ M-U18666A, are still influencing the enzyme activity (Boogaard *et al.*, 1987) but not the mRNA contents (Fig. 4); the other class is the sterol repressors, which are not synthesized during the cell incubation with compactin and/or high concentrations of U18666A and are effective at the mRNA level (cf. Figs. 1–4).

From experiments performed in a different way with Mev-1 cells, a Chinese-hamster ovary mutant lacking HMG-CoA synthase, it was also concluded that a non-sterol effector does not regulate at the mRNA level (Peffley & Sinenski, 1985). These authors reported regulation by a translational control mechanism. From our experiments we are unable to discriminate between regulation of either the synthesis or the degradation of the enzyme.

The experiments described here supply only limited evidence to conclude that the endogenously formed sterol suppressors are additionally effective at the enzyme level in Hep G2 cells. The larger decreasing effect of LDL on the compactin-induced activity (Fig. 5) compared with that effect on the mRNA content (Fig. 6) seems to indicate that exogenous LDL is also active at the enzyme level. It was suggested previously (Boogaard *et al.*, 1987) that the decrease in enzyme activity at low concentrations of U18666A was due to the formation of

polar sterols. Also in this case the decrease in activity (50% of control values at 0.5 μ M-U18666A; Fig. 3a in Boogaard *et al.*, 1987) was larger than the decrease in mRNA (Fig. 3). For comparison, it was reported that in UT-1 cells exogenous LDL or 25-hydroxycholesterol (Faust *et al.*, 1982) and in chicken myeloblasts exogenous 25-hydroxycholesterol (Tanaka *et al.*, 1983) increased the rate of enzyme degradation. Furthermore, Chin *et al.* (1985) showed an increase in enzyme degradation by added sterols in a Chinese-hamster mutant cell line, transfected with a HMG-CoA reductase cDNA construct, transcription of which was insensitive to exogenous sterols.

Further distinction in the sterol suppressor class could be made: as shown in Fig. 5, LDL could only partly compensate for absent mevalonate-derived suppressors when their formation was blocked by compactin. A small quantity of exogenous mevalonate, which by itself could also only in part suppress the effect of compactin, totally abolished, together with LDL, the compactin-induced increase in HMG-CoA reductase activity. From similar experiments performed with human fibroblasts (Brown & Goldstein, 1980) and with bovine endothelial cells (Cohen *et al.*, 1982) it was concluded that exogenous LDL was compensating for all mevalonate-derived sterol effectors, whereas the exogenous mevalonate should provide the non-sterol effectors. If that would be the correct interpretation, LDL should compensate totally for the compactin-induced increase in the mRNA contents, and additional mevalonate should not have any effect on the amount of mRNA at high LDL concentrations in the medium. As shown in Fig. 6, that is not the case. At the mRNA level too, LDL could only partly compensate for the missing suppressors, and only together with exogenous mevalonate did it totally prevent the compactin-induced increase in the reductase mRNA.

We concluded that in Hep G2 cells non-sterol metabolites of mevalonate regulate HMG-CoA reductase at the enzyme level, whereas mevalonate-derived sterol suppressors are additionally active at the reductase mRNA level. In the second-mentioned group we distinguish sterols which act in the same way as exogenous LDL and compounds which do not. The latter compounds may be polar sterol intermediates or side products of the cholesterol biosynthetic pathway, which cannot be formed from LDL cholesterol. For instance, the formation of a polar sterol fraction in the presence of low concentrations of U18666A (Boogaard *et al.*, 1987) coincided with a decrease in reductase mRNA (Fig. 3) and activity (Boogaard *et al.*, 1987). H.p.l.c. analysis performed in the laboratory of Dr. T. A. Spencer showed that this fraction is most probably 24(S),25-epoxycholesterol (results not shown), which can be formed as a side product via squalene 2,3:22,23-diepoxy (Nelson *et al.*, 1981). Such 24(S),25-epoxy sterols are thought to play a role in the regulation of HMG-CoA reductase (Spencer *et al.*, 1985; Taylor *et al.*, 1986; Panini *et al.*, 1986). On the other hand, oxygenated lanosterol 14 α -demethylation intermediates are also mentioned as putative repressors of the HMG-CoA reductase activity (Trzaskos *et al.*, 1987). The study of the effect of these oxysterols combined with LDL on HMG-CoA reductase activity and mRNA contents in Hep G2 cells may give more insight in the distinct mevalonate-derived feedback regulators.

Dr. K. L. Luskey and Dr. H. Pannekoek are gratefully acknowledged for their gifts of cDNA probes for human HMG-CoA reductase and human serum albumin respectively. We thank Dr. A. Endo for providing us with compactin and Dr. P. W. O'Connell for the gift of U18666A. Dr. A. K. Gayen and Dr. T. A. Spencer are thanked for performing the h.p.l.c. analysis of Hep G2 sterols. We are grateful to Dr. B. B. Knowles for providing us with the Hep G2 cell line, and Mrs. L. de Pagter and Dr. H. M. G. Princen for excellent handling of the cell culture. We thank Mrs. M. Horsting for preparing the manuscript.

REFERENCES

- Alberts, A. W., *et al.* (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3957-3961
- Boogaard, A., Griffioen, M. & Cohen, L. H. (1987) *Biochem. J.* **241**, 345-351
- Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 505-517
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173-181
- Chin, D. J., Luskey, K. L., Anderson, R. G. W., Faust, J. R., Goldstein, J. L. & Brown, M. S. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1185-1189
- Chin, D. J., Gil, G., Faust, J. R., Goldstein, J. L., Brown, M. S. & Luskey, K. L. (1985) *Mol. Cell. Biol.* **5**, 634-641
- Clarke, C. F., Edwards, P. A., Lan, S.-F., Tanaka, R. D. & Fogelman, A. M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3305-3308
- Clarke, C. F., Fogelman, A. M. & Edwards, P. A. (1984) *J. Biol. Chem.* **259**, 10439-10477
- Cohen, D. C., Massoglia, S. L. & Gospodarowicz, D. (1982) *J. Biol. Chem.* **257**, 11106-11112
- Cohen, L. H., Griffioen, M., Havekes, L., Schouten, D., Van Hinsbergh, V. W. M. & Kempen, H. J. (1984) *Biochem. J.* **222**, 35-39
- Cohen, L. H., Griffioen, M. & Boogaard, A. (1985) in *Cholesterol Metabolism in Health and Disease: Studies in the Netherlands* (Beynen, A. C., Geelen, M. J. H., Katan, M. B. & Schouten, J. A., eds.), pp. 53-66, Ponsen and Looijen, Wageningen
- Cohen, L. H., Princen, H. M. G., Kwekkeboom, J., Havekes, L. M. & Kempen, H. J. (1987) *Biochem. Soc. Trans.* **15**, 339-340
- Dugaiczky, A., Law, S. W. & Dennison, O. E. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 71-75
- Edwards, P. E., Lan, S.-F., Tanaka, R. D. & Fogelman, A. M. (1983) *J. Biol. Chem.* **258**, 7272-7275
- Endo, A., Kuroda, M. & Tanzawa, K. (1976) *FEBS Lett.* **72**, 323-326
- Erickson, S. K. & Fielding, Ph. E. (1986) *J. Lipid Res.* **27**, 875-883
- Faust, J. R., Luskey, K. L., Chin, D. J., Goldstein, J. L. & Brown, M. S. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5205-5209
- Havekes, L., Van Hinsbergh, V. W. M., Kempen, H. J. & Emeis, J. (1983) *Biochem. J.* **214**, 951-958
- Lizardi, P. M. & Engelberg, A. (1979) *Anal. Biochem.* **98**, 116-122
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Luskey, K. L. & Stevens, B. (1985) *J. Biol. Chem.* **260**, 10271-10277
- Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S. & Goldstein, J. L. (1983) *J. Biol. Chem.* **258**, 8462-8469
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, New York

- Nelson, J. A., Steckbeck, S. R. & Spencer, T. A. (1981) *J. Biol. Chem.* **256**, 1067–1068
- Panini, S. R., Sexton, R. C., Gupta, A. K., Parish, E. J., Chitrakorn, S. & Rudney, H. (1986) *J. Lipid Res.* **27**, 1190–1204
- Peffley, D. & Sinenski, M. (1985) *J. Biol. Chem.* **260**, 9949–9952
- Redgrave, T. G., Roberts, D. C. K. & West, C. E. (1975) *Anal. Biochem.* **65**, 42–49
- Sexton, R. C., Panini, S. R., Azran, F. & Rudney, H. (1983) *Biochemistry* **22**, 5687–5692
- Sinenski, M. & Logel, J. (1983) *J. Biol. Chem.* **258**, 8547–8549
- Spector, A. A. & Hoak, J. C. (1969) *Anal. Biochem.* **32**, 297–302
- Spencer, T. A., Gayen, A. K., Phirwa, S., Nelson, J. A., Taylor, F. R., Kandutsch, A. A. & Erickson, S. K. (1985) *J. Biol. Chem.* **260**, 13391–13394
- Tanaka, R. D., Edwards, P. A., Lan, S.-F. & Fogelman, A. M. (1983) *J. Biol. Chem.* **258**, 13331–13339
- Taylor, F. R., Kandutsch, A. A., Gayen, A. K., Nelson, J. A., Steckbeck, S., Phirwa, S. & Spencer, T. A. (1986) *J. Biol. Chem.* **261**, 15039–15044
- Trzaskos, J. M., Favata, M. F., Fischer, R. T. & Stam, S. H. (1987) *J. Biol. Chem.* **262**, 12261–12268
- Van Zonneveld, A. J., Chang, G. T. G., Van den Berg, J., Kooistra, T., Verheijen, J. H., Pannekoek, H. & Kluft, C. (1986) *Biochem. J.* **235**, 385–390
- Warner, J. R., Soeiro, R. & Birnboim, H. C. (1966) *J. Mol. Biol.* **19**, 349–361

Received 6 January 1988/25 April 1988; accepted 12 May 1988