Parameters of cholesterol metabolism in the human hepatoma cell line, Hep-G2

Sandra K. Erickson[1] **and Phoebe E. Fielding**

Cardiovascular Research Institute and Department of Medicine, University of California School of Medicine, San Francisco, CA 94143

Abstract The human hepatoma cell line Hep-G2 has been shown to express the major enzymes of intra- and extracellular cholesterol metabolism. These include lecithin:cholesterol acyltransferase, acyl coenzyme A:cholesterol acyltransferase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and cholesterol-7α-hydroxylase. Regulatory mechanisms that have been described in other hepatic systems also appear to be active in Hep-G2 cells: perturbations of cholesterol and triglyceride metabolism affected the enzyme activities and the accumulation of specific apolipoproteins in the culture media. The results indicate that studies of Hep-G2 cells may provide useful information for the elucidation of mechanisms of regulation of human hepatocyte cholesterol, lipoprotein, and biliary metabolism. — **Erickson, S. K., and P. E. Fielding.** Parameters of cholesterol metabolism in the human hepatoma cell line, Hep-G2. *J. Lipid Res.* 1986. **27:** 875–883.

Supplementary key words LCAT • ACAT • HMG-CoA reductase • cholesterol 7α-hydroxylase • apolipoproteins

The human hepatoblastoma-derived cell line Hep-G2 expresses many of the functions attributed to a normal human hepatocyte. It secretes a variety of plasma proteins (1) including apolipoproteins B, E, A-I, A-II, A-IV, C-II, and C-III (2–6). Only apolipoprotein B-100 is synthesized and secreted by this cell line (3) in accord with the report (7) that apoB-100 is the sole apoB species secreted by the human liver. Hep-G2 cells can synthesize and secrete lipoproteins in the very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) density ranges (2, 3, 6). They express receptors for insulin and transferrin (8), estrogen (6), and LDL (9–12), and bind HDL (13).

Of the hepato-specific functions, these cells retain the ability to express receptors for asialoglycoproteins (8, 14) and are reported to synthesize and secrete bile acids including chenodeoxycholate and cholate (15). Morphologically, they have an hepatocyte-like differentiated plasma membrane including a bile canalicular region (16). Thus, this is a highly differentiated cell line although some chromosomal abnormalities have been described (17).

For these reasons, Hep-G2 cells appear to be a reasonable model for the study of human hepatocyte functions. Therefore, this cell line was used to examine enzymes of intra- and extrahepatic cholesterol metabolism, which have not been previously described for Hep-G2 cells, and their regulatory responses, specifically lecithin:cholesterol acyltransferase (LCAT), the enzyme responsible for extracellular cholesterol esterification; acyl coenzyme A:cholesterol acyltransferase, responsible for intracellular cholesterol esterification; and cholesterol 7α-hydroxylase, a rate-limiting enzyme in bile acid synthesis. In addition, regulatory aspects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme for cholesterol synthesis [described previously in Hep-G2 cells by Cohen et al. (18)] were studied.

Enzyme activity and apoprotein measurements were made after culturing cells in minimal essential medium (MEM). The other culture conditions chosen for investigation included well-documented perturbations known to alter hepatic lipid metabolism in order to test whether these cells could express regulatory mechanisms similar to those described in other systems (19, 20). These included: *1*) change in endogenous cholesterol availability induced by exposure of the cells to mevalonate, a cholesterol precursor which enters the pathway distal to the rate-limiting step for sterol synthesis (19); *2*) change in exogenous fatty acid availability by exposure of the cells to physiological concentrations of fatty acid complexed to albumin (20); and *3*) culture in physiological concentrations of hormones

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl coenzyme A:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MEM, minimal essential meduim.

[1] Dr. Erickson was a Visiting Scientist in the Cardiovascular Research Institute, University of California, San Francisco, 1984–1985, and was on leave from the Division of Gastroenterology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305. Dr. Erickson's present address is: Metabolism Section, Department of Medicine, University of California School of Medicine, Veterans Administration Medical Center (111F), 4150 Clement Street, San Francisco, CA 94121.

designed to mimic the in vivo environment of the hepatocyte. This culture condition has been reported to maintain specific hepatocyte function in primary culture (21).

MATERIALS AND METHODS

[1-^{14}C]Oleoyl coenzyme A (40–60 mCi/mmol) was obtained from Amersham, Arlington Heights, IL; [1,2-^{3}H]cholesterol (40–60 Ci/mol), [1,2,6,7,-^{3}H]cholesteryl oleate (60–100 Ci/mol), D,L-3-[^{14}C-glutaryl-3]hydroxy-3-methylglutaryl coenzyme A (40–60 mCi/mmol) and *R,S*-[5-^{3}H]mevalonolactone (2–10 Ci/mol) were from New England Nuclear, Boston, MA. Oleoyl coenzyme A, NADP, glucose-6-phosphate, human serum albumin, β-mercaptoethanol, dithiothreitol, mevalonic acid, (Na salt), phosphatidylcholine, oleic acid, linoleic acid, D-α-tocopherol succinate, testosterone, 17β-estradiol, hydrocortisone, D-thyroxine, glucose-6-phosphate dehydrogenase, and cholesterol oxidase were from Sigma, St. Louis, MO. 3-Hydroxy-3-methylglutaryl coenzyme A was from PL Biochemicals, Milwaukee, WI, and 7α-hydroxycholesterol was from Steraloids, Wilton, NH. Insulin and glucagon were obtained from Eli Lilly, Indianapolis, IN. Cholesterol esterase was purchased from Boehringer-Mannheim, Indianapolis, IN. Disposable tissue culture supplies were from Corning, Corning, NY.

Cell culture

Experiments were carried out using a human hepatoma-derived cell line, Hep-G2. Cells were obtained from the Cell Culture facility at the University of California, San Francisco. Cell stocks were grown in T75 flasks containing 12 ml of MEM supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml), containing 10% fetal calf serum, and incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Fresh medium was added every third day. For experiments, cells were plated in 10-cm dishes or T175 flasks and grown to late log phase. Nearly confluent monolayers were washed three times with MEM and then incubated for 10 hr at 37°C in one of the four culture media described. Plates contained 6 ml of medium and T175 flasks contained 20 ml. Following incubation, media and cells were harvested separately as described in the following sections.

Culture media

Hep-G2 cells were incubated for 10 hr in one of the four following media: *1*) MEM; *2*) MEM supplemented with oleic acid (18 μM), linoleic acid (18 μM), D-α-tocopherol succinate (0.6 μM), testosterone (1 μM), 17β-estradiol (1 μM), hydrocortisone (10 μM), human serum albumin (30 μM), D-thyroxine (10 μM), insulin (0.02 units/ml), and glucagon (50 nM), designated as hormone media; *3*) MEM supplemented with linoleic acid (0.3 mM) and human serum albumin (0.11 mM); *4*) MEM supplemented with mevalonolactone (10 mM).

Immunoassay of plasma apoproteins

Levels of apoproteins A-I, E, B, D, and A-II were determined in Hep-G2 cell culture media and cell homogenates by specific radial immunodiffusion assays, as previously described (22). Hep-G2 cell culture media were harvested and centrifuged 30 min at 20,000 *g* at 4°C in a Sorvall RC-5B centrifuge to remove any floating cells and debris. The clear supernatant was then concentrated at 4°C in an Amicon pressure cell containing a YM-10 membrane. Concentration was routinely 20- to 30-fold as determined by protein analysis. Intracellular apoprotein levels were measured in portions of whole cell homogenates prepared as described below.

Pure apoprotein antigens for antibody production and the immunoassays were prepared as described previously (23) from human plasma lipoproteins isolated by ultracentrifugation (24). Briefly, the lipoproteins were delipidated with ethanol and diethyl ether and the protein was dissolved in 6 M urea and fractionated using molecular sieve and DEAE-cellulose chromatography. Purity was assessed by gel electrophoresis in sodium dodecyl sulfate (25) and at basic pH in urea (26). Polyclonal antibodies to each apoprotein were raised in New Zealand white rabbits. Immunoassays were carried out in 1% wt/vol agarose in 0.05 M barbital buffer, pH 8.2 (22).

Preparation of cell homogenates and a subcellular membrane or microsomal fraction

After removal of media, cells were washed twice with cold MEM and harvested by scraping into cold MEM. All manipulations were at 4°C. Cells were collected by centrifugation for 5 min at 2000 rpm and resuspended by vortexing in ice-cold homogenizing buffer containing 0.12 M sucrose, 0.15 M KCl, 0.03 M EDTA, and 0.05 M potassium phosphate, pH 7.4. Samples were removed for protein, cholesterol, and apoprotein determinations. The portions for apoprotein determinations were made 1% in Triton X-100, frozen and thawed once, vortexed vigorously, and stored on ice until use. At this point, no intact cells were visible by light microscopy.

After standing 15 min on ice, the remaining cells were homogenized with a Dounce homogenizer by 40 strokes of pestle B. Portions were removed for assay of HMG-CoA reductase and the remainder was used to prepare a crude microsomal fraction. The homogenate was centrifuged for 10 min at 2000 rpm to remove unbroken cells and nuclei. The supernatant was removed and centrifuged 60 min at 105,000 *g*. The pellet was resuspended in the homogenizing buffer with a glass-glass Potter Elvejhem homogenizer and used for assays of ACAT and cholesterol-7α-hydroxylase.

Assay of lecithin:cholesterol acyltransferase

LCAT was assayed in portions of Hep-G2 cell culture medium with a synthetic substrate. Samples were assayed

directly or after concentration two- to threefold in a Centricon 10 microconcentrator (Amicon Corp, Danvers, MA). No activity could be detected after concentration of the cell culture media in an Amicon pressure cell.

Synthetic substrates for LCAT were prepared by two methods. French press liposomes containing cholesterol and phospholipid (1:8 wt/wt) were made according to a previously published procedure (27). The liposomes were activated for LCAT activity by the addition of pure human apoA-I. A standard assay contained 12.5 μg of apoA-I, 12.5 μg of cholesterol (at a specific activity of 1.5×10^5 cpm/μg), and human serum albumin at a final concentration of 2.5% in a total volume of 0.5 ml. Aliquots (0.25 ml) of culture media were added to 0.25 ml of the assay medium and incubated at 37°C for up to 90 min. Under these conditions, the production of cholesteryl ester was linear. The reaction was stopped by the addition of 0.5 ml of methanol and 0.5 ml of $CHCl_3$. Portions of the bottom phase were separated by thin-layer chromatography. The cholesteryl ester region was scraped and the radioactivity was determined by liquid scintillation spectrometry.

LCAT was also assayed with cholate liposomes prepared as described by Chen and Albers (28). This assay contained 2.9 μg of free cholesterol (at a specific activity of 1.5×10^5 cpm/μg), 22 μg of apoA-I, and human serum albumin at a final concentration of 0.5%. Mercaptoethanol (5 mM final concentration) was added both to the assays with French press liposomes and to those with cholate liposomes immediately before incubation. Media samples (0.25 ml) were added to the cholate liposome assay medium and the remaining protocol was identical to that described above for French press liposomes. All determinations were carried out in the presence and absence of apoA-I. Under the conditions of this assay, production of cholesteryl ester was linear for at least 90 min.

Cellular enzyme assays

HMG-CoA reductase was assayed in cell homogenates essentially as described by Cohen et al. (18) for Hep-G2 cells except that the product was extracted and separated by thin-layer chromatography as described previously (29). Enzyme activity was assayed routinely in cell homogenates because assay in the microsomal fraction gave tenfold lower values, suggesting that the enzyme may have been solubilized during membrane preparation.

ACAT activity and triglyceride synthesis were assayed in the microsomal fraction as described previously (30) with [^{14}C]oleoyl-CoA and endogenous cholesterol or acylglycerols as substrates. The radiolabeled cholesteryl esters and triglycerides were separated by thin-layer chromatography on Silica Gel H (31). The cholesteryl esters and triglycerides were scraped from the plates and radioactivity was determined by liquid scintillation spectrometry.

ACAT activity was also assayed with exogenous [^{3}H]cholesterol-phospholipid liposomes prepared by the French pressure cell method (27). The incubation mixture contained, in addition to the liposomes (5 μg of cholesterol), human serum albumin (2.5% final concentration) and 100–150 μg of microsomal protein in a final volume of 0.2 ml of buffer containing 0.25 M sucrose, 0.5 mM EDTA, and 0.05 M Tris-HCl, pH 8.0. The mixture was incubated for 60 min at 37°C with shaking and the reaction was initiated by addition of 5 nmol of unlabeled oleoyl coenzyme A. The reaction was stopped at 5 min by the addition of chloroform–methanol 2:1 (v/v). The product was separated as described above. Cholesteryl ester production was linear over 15 min. Longer preincubation times before initiation of the assay resulted in apparent loss of activity. In every case a substrate blank that contained the incubation mixture but no microsomes was run in parallel and this value (about 10% of the total radioactivity migrating as cholesteryl ester) was subtracted from the values obtained for microsomes.

Cholesterol-7α-hydroxylase activity was estimated based on the method of Nicolau et al. (32), by measuring conversion of exogenous [^{3}H]cholesterol to 7α-hydroxycholesterol. Briefly, 200–600 μg of microsomal protein was incubated with shaking with 5 μg of cholesterol contained in [^{3}H]cholesterol-phosphatidylcholine liposomes prepared by the French pressure cell method (27) in a mixture containing 2.5% human serum albumin, glucose-6-phosphate, NADP, and dithiothreitol in homogenizing buffer for 60 min at 37°C. The assay was initiated by addition of 2 units of glucose-6-phosphate dehydrogenase. After 30 min, the reaction was terminated by addition of 5 ml of chloroform–methanol 2:1 (v/v), and the product was separated as described previously (29). Authentic 7α-hydroxycholesterol was plated as a reference standard. In all cases, substrate blank incubations were run simultaneously, in which microsomes were omitted to assess nonenzymatic conversion of cholesterol to 7α-hydroxycholesterol. These values were routinely about 20% of those obtained in the complete incubations. Values for hydroxylase activity were corrected by subtracting the nonenzymatic conversion values. Levels of enzyme activity were calculated from the specific radioactivity of the total free cholesterol in the system.

Chemical determinations

All protein estimations were carried out according to the method of Lowry et al. (33) with bovine serum albumin as standard. Free cholesterol and cholesteryl ester masses of media and cell samples were determined with a fluorimetric assay with cholesterol oxidase and cholesteryl esterase as previously described (34).

RESULTS

Effect of culture conditions on Hep-G2 cellular cholesterol content and on cholesterol accumulation in the media

Incubation of Hep-G2 cells for 10 hr under the four culture conditions described in Methods had a significant effect on the mass of cellular cholesterol only with

mevalonolactone supplementation (**Table 1**). Esterified cholesterol increased about twofold with little change in free cholesterol content. Gastric intubation of mevalonate in vivo has been shown to increase hepatic cholesterol content (19), and incubation with mevalonate has been shown to increase intracellular cholesterol content in cultured rat hepatocytes (35, 36).

Incubation with mevalonolactone also resulted in increased cholesterol content in the medium (Table 1). As in the cells, there was little change in the free cholesterol but the esterified cholesterol increased about twofold. This change in medium cholesterol content is similar to that which has been observed for medium VLDL from rat hepatocytes in primary culture incubated with mevalonate (37).

The presence of free fatty acids in the medium of a perfused liver (38, 39) or rat hepatocytes in culture (40, 41) stimulates triglyceride synthesis and VLDL secretion. The addition of 0.3 mM linoleate to Hep-G2 cell culture media increased the mass of accumulated cholesterol in the medium (Table 1), but the proportion of esterified cholesterol was not changed significantly.

Maintenance of the cells in the hormone medium had no statistically significant effect either on the total amount of cholesterol or on the proportion of esterified cholesterol that accumulated in the medium.

Apolipoprotein contents of Hep-G2 cells and their accumulation in the culture media

Inclusion of 0.3 mM linoleate bound to human serum albumin in the medium of Hep-G2 cells significantly increased the masses of apolipoproteins B, E, and A-I when compared with medium from cells incubated in MEM alone (**Table 2**).

Addition of physiological concentrations of the hormones had a similar effect. There appeared to be little change in the concentrations of apoA-II under the different culture conditions. Apolipoprotein D was present in the hormone medium but was undetectable in the media under the other three culture conditions (Table 2).

Despite the changes in the contents of free and esterified cholesterol in the medium and in the cells observed in the presence of 10 mM mevalonate, there were no significant effects on any of the apoprotein levels measured (**Table 3**).

Accumulation of lecithin:cholesterol acyltransferase in the culture media of Hep-G2 cells

LCAT activity was detected in the medium of Hep-G2 cells under all conditions tested (**Table 4**).

The presence of linoleate or hormones in the medium had no significant effect on the level of enzyme activity measured by using an exogenous radiolabeled substrate. Incubation of the cells in the presence of mevalonate, which caused an increase in the amounts of esterified cholesterol both in the cells and in the medium, had no effect on the amount of active LCAT that accumulated over a 10-hr period. Assays were carried out with liposomes containing cholate or with those made in the French pressure cell. The latter gave about 75% of the activity obtained with those made by the detergent method. No LCAT activity was detected in incubations without apoA-I.

Intracellular enzyme activities of Hep-G2 cells associated with cholesterol metabolism

Under all culture conditions investigated, measurable levels of ACAT, HMG-CoA reductase, and cholesterol-7α-hydroxylase activities were detected in Hep-G2 cells.

Incubation of Hep-G2 cells in the presence of 10 mM

TABLE 1. Effect of culture condition on the accumulation of cholesterol in Hep-G2 cells and their media after 10 hr

Culture Medium	Free Cholesterol	Ester Cholesterol	% as Ester
	μg cholesterol · mg cell protein^{-1}		
A. Medium			
MEM[a]	1.00	1.00	11.8
Mevalonolactone, 10 mM (n = 5)	1.29 ± 0.04	2.47 ± 0.88*	20.4
Linoleate, 0.3 mM (n = 3)	1.97 ± 0.30*	2.52 ± 0.53*	12.7
Hormone mix (n = 3)	1.76 ± 0.43**	1.70 ± 0.47	11.5
B. Cells			
MEM[a]	1.00	1.00	36.9
Mevalonolactone, 10 mM (n = 6)	1.09 ± 0.16	1.59 ± 0.57*	46.2
Linoleate, 0.3 mM (n = 6)	0.90 ± 0.10	0.79 ± 0.17	31.1
Hormone mix (n = 6)	0.98 ± 0.13	0.53 ± 0.13	24.1

All values are ± SEM; the number of experiments, n, is given in parentheses. * Statistically significantly different from MEM values at $P < 0.005$, unpaired Student's t-test. **Statistically significantly different from MEM values at $P < 0.05$, unpaired Student's t-test.

[a] All values for free and esterified cholesterol have been normalized to the MEM or basal value as 1.00. The values for MEM accumulation (μg cholesterol · mg cell protein^{-1}) were: free cholesterol, medium = 1.27 ± 0.1 (n = 5), cells = 13.3 ± 1.9 (n = 7); esterified cholesterol, medium = 0.17 ± 0.03 (n = 5), cells = 7.9 ± 3.1 (n = 7).

TABLE 2. Mass of apolipoproteins that accumulated in the medium of Hep-G2 cells cultured under a variety of conditions for 10 hr

Culture Medium	Apolipoprotein B	E	A-I	A-II	D
	μg apoprotein · mg cell protein^{-1}				
MEM[a]	1.00	1.00	1.00	1.00	0
Mevalonolactone, 10 mM	1.65 ± 0.27 (n = 5)	1.44 ± 0.38 (n = 5)	1.20 ± 0.12 (n = 5)	0.45 ± 0.20 (n = 3)	0
Linoleate, 0.3 mM	2.84 ± 0.18* (n = 6)	1.81 ± 0.12* (n = 6)	2.24 ± 0.16* (n = 6)	0.74 ± 0.13 (n = 3)	0
Hormone mix	2.12 ± 0.21* (n = 7)	1.53 ± 0.10** (n = 6)	1.64 ± 0.29** (n = 7)	0.97 ± 0.20 (n = 3)	0.29 ± 0.05[a] (n = 4)

All values are ± SEM; the number of experiments, n, is given in parentheses. *Statistically significantly different from MEM values at $P < 0.001$ by Student's unpaired t-test. **Statistically significantly different from MEM value at $P < 0.05$ by Student's unpaired t-test.

[a]All values for the apoproteins have been normalized to the MEM or basal value as 1.00. The values for MEM accumulation in μg apoprotein · mg cell protein^{-1} were: apoB, 1.34 ± 0.10 (n = 7), apoE, 0.48 ± 0.11 (n = 6); apoA-I, 1.99 ± 0.26 (n = 7); apoA-II, 1.43 ± 0.37 (n = 3); apoD, no immunologically detectable apoD accumulated in the medium of cells cultured in MEM; the values given for apoD are the actual values measured.

mevalonolactone increased ACAT activity (**Table 5**). Cell culture in the presence of the mixture of hormones resulted in a small but significant decrease in activity while culture with linoleate had little effect.

HMG-CoA reductase was detectable in these cells at levels comparable to those reported previously (18). As reported previously (18), incubation in the presence of mevalonate significantly suppressed reductase activity (Table 5). Neither the addition of linoleate nor hormones at the concentrations used in these studies, and 10 hr after initiation of the experiment, had any effect on reductase activity.

The activity of cholesterol-7α-hydroxylase was measured in a crude microsomal fraction of Hep-G2 cells using a radiolabeled cholesterol-phospholipid liposome assay. The level of activity detected in these experiments, about 60 pmol of 7α-hydroxycholesterol · hr^{-1} · mg of protein^{-1}, appeared to be of the same order of magnitude as the estimated rate of bile acid synthesis and secretion by these cells reported by Everson and Polakoff (15). The activity was not significantly affected by the different culture conditions (Table 5).

The rates of triglyceride synthesis as estimated in a crude microsomal fraction of Hep-G2 cells using endogenous glyceride substrates and exogenous radiolabeled oleoyl CoA did not change under any of the culture conditions (Table 5), including culture in 0.3 mM linoleate. The values for Hep-G2 cell preparations tended to be higher than those reported previously for normal human liver assayed under similar conditions (30).

DISCUSSION

The human hepatoma cell line, Hep-G2, has been shown by a number of investigators to express many func-

TABLE 3. Mass of apolipoproteins that accumulated in Hep-G2 cells cultured under a variety of conditions for 10 hr

Culture Medium	Apolipoprotein B	E	A-I	A-II	D
	μg apoprotein · mg cell protein^{-1}				
MEM[a]	1.00	1.00	1.00	1.00	1.00
Mevalonolactone, 10 mM	0.88 ± 0.09 (n = 4)	1.08 ± 0.10 (n = 4)	1.45 ± 0.22 (n = 4)	1.59 ± 0.72 (n = 3)	1.13 ± 0.21 (n = 4)
Linoleate, 0.3 mM	0.98 ± 0.02 (n = 3)	1.19 ± 0.03* (n = 4)	1.59 ± 0.22 (n = 3)	1.29 ± 0.05 (n = 3)	1.32 ± 0.13 (n = 3)
Hormone mix	1.24 ± 0.09*** (n = 5)	1.40 ± 0.12** (n = 4)	1.45 ± 0.28 (n = 5)	1.67 ± 0.77 (n = 3)	1.16 ± 0.18 (n = 5)

All values are ± SEM. The number of experiments, n, is given in parentheses. *Statistically significantly different from MEM values at $P < 0.001$ by Student's unpaired t-test. **Significantly different from MEM values at $P < 0.02$ by unpaired Student's t-test. ***Significantly different from MEM values at $P < 0.05$ by unpaired Student's t-test.

[a]All values for the apoproteins have been normalized to the MEM or basal value as 1.00. The values for MEM expressed as μg apoprotein · mg cell protein^{-1} were: apoB, 6.42 ± 1.47; apoE, 1.19 ± 0.22; apoA-I, 0.23 ± 0.03; apoA-II, 0.41 ± 0.07; apoD, 1.00 ± 0.23.

TABLE 4. Accumulation of lecithin:cholesterol acyltransferase activity in medium of Hep-G2 cells cultured under a variety of conditions for 10 hr

Culture Medium	LCAT Activity
	pmol cholesteryl ester · h^{-1} · mg cell protein^{-1}
MEM[a]	1.00
Mevalonolactone, 10 mM	0.82 ± 0.30 (n = 4)
Linoleate, 0.3 mM	0.96 ± 0.27 (n = 4)
Hormone mix	0.82 ± 0.25 (n = 4)

All values have been normalized to the MEM or basal value as 1.00. The values for LCAT activity in MEM ranged from 6.67 to 19.7 pmol cholesteryl ester · hr^{-1} · mg cell protein^{-1}, mean = 12.3 ± 5.5. All values are ± SEM.

tions attributed to normal human hepatocytes. The present research extends this list to include the major enzymes of intra- and extracellular cholesterol metabolism. Thus, these cells are a potentially useful in vitro model for the study of regulation of human hepatic cholesterol and lipoprotein metabolism.

Changes in availability of endogenous cholesterol induced by exposure to mevalonate are known to correlate in rat liver (19, 31, 35, 42) and in rat hepatocytes in primary culture (35, 36) with changes in the activities of HMG-CoA reductase and ACAT. Similar regulatory responses were operative in Hep-G2 cells. In the presence of 10 mM mevalonate, ACAT activity increased about 1.3-fold. This change was paralleled by an increase in intracellular cholesteryl ester mass with little change in free cholesterol mass. Levels of ACAT activity measured in these cells were comparable to those in human livers (30, 43).

In agreement with a previous report (18), HMG-CoA reductase activity under these conditions was inhibited, suggesting that the mechanisms for regulation of reductase by elevated levels of intracellular mevalonate-derived products are present in this hepatoma cell line. Reductase values in this study were obtained using whole cell homogenates and were similar to levels previously reported for Hep-G2 cells (18) and for normal human liver (44, 45). As reported by Cohen et al. (18), we also observed that reductase assays carried out using a crude microsomal fraction from Hep-G2 cells resulted in very low activities. This suggested that either there is altered compartmentation of the enzyme or that the Hep-G2 cell enzyme was especially sensitive to solubilization and/or inactivation during preparation of the microsomal fraction.

Hep-G2 cells also had an active cholesterol-7α-hydroxylase system. The activity tended to be higher in the hormone-supplemented medium (which maintains cytochrome P450 levels and functions in primary hepatocyte culture, ref. 21); however, this did not reach statistical significance. Although additions of mevalonate to Hep-G2 cells increased cellular cholesterol mass, there was no observable change in cholesterol-7α-hydroxylase activity under the conditions of the assay. Davis et al. (46), using isolated rat hepatocytes in primary culture, showed that exposure to mevalonolactone resulted in increased synthesis of bile acids which they attributed to increased

TABLE 5. Intracellular enzyme activities in Hep-G2 cells after culture under a variety of conditions for 10 hr

	Enzyme Activity				
	ACAT				
Culture Medium	[^{14}C]Oleoyl CoA Assay	[^{3}H]Cholesterol Assay	Triglyceride Synthesis	HMG-CoA Reductase	Cholesteryl-7α Hydroxylase
	pmol product · min^{-1} · mg protein^{-1}				
MEM[a]	1.00	1.00	1.00	1.00	1.00
Mevalonolactone, 10 mM	1.34 ± 0.10** (n = 6)	1.22 ± 0.04** (n = 3)	1.20 ± 0.28 (n = 5)	0.24 ± 0.04* (n = 4)	1.12 ± 0.16 (n = 5)
Linoleate, 0.3 mM	1.10 ± 0.13 (n = 6)	0.94 ± 0.10 (n = 3)	1.08 ± 0.08 (n = 5)	1.14 ± 0.31 (n = 3)	1.16 ± 0.09 (n = 5)
Hormone mix	0.86 ± 0.07** (n = 6)	0.91 ± 0.06 (n = 3)	1.11 ± 0.09 (n = 5)	1.07 ± 0.25 (n = 3)	1.25 ± 0.18 (n = 5)

All values are ± SEM. The number of experiments, n, is in parentheses. *Statistically significantly different from MEM value at $P < 0.001$ by unpaired Student's unpaired *t*-test. **Significantly different from MEM value at $P < 0.01$ by Student's unpaired *t*-test. ***Significantly different from MEM value at $P < 0.02$ by Student's unpaired *t*-test.

[a]All values are normalized to the MEM or basal value as 1.00. Activities for cellular enzyme activities from cells cultured in MEM were: ACAT, 17.4 ± 3.3 pmol cholesteryl oleate · min^{-1} · mg microsomal protein^{-1} assayed with [^{14}C]oleoyl CoA and endogenous cholesterol, and 254.1 ± 105.0 pmol cholesteryl ester · min^{-1} · mg microsomal protein^{-1} assayed with [^{3}H]cholesterol; triglyceride synthesis, 340 ± 20 pmol triolein · min^{-1} · mg microsomal protein^{-1}; HMG-CoA reductase, 42.2 ± 14.3 pmol mevalonate · min^{-1} · mg homogenate protein^{-1}; cholesterol-7α-hydroxylase, 1.72 ± 0.40 pmol 7α-hydroxycholesterol · min^{-1} · mg microsomal protein^{-1}.

cholesterol availability; however, effects on cholesterol-7α-hydroxylase activity were not assessed. Whether incubation of Hep-G2 cells in mevalonate-containing media increases their bile acid synthesis and secretion has not been reported.

The activity of cholesterol-7α-hydroxylase from Hep-G2 cells was about 2% of that estimated for normal human liver (47) and about 6% of that reported for human liver samples from individuals with cholesterol gallstones (48, 49); however, the activity appeared to be of the same order of magnitude as the estimated rates of secretion of bile acids by these cells (15).

The source of the increased cholesteryl ester mass in the culture medium of cells incubated in the presence of 10 mM mevalonolactone is unclear, but it may be the result of the increased ACAT activity since the amount of LCAT activity that accumulated in the medium under this condition did not differ from baseline values. There were no significant changes in media apoprotein levels under these culture conditions, including that of apoA-I which is a cofactor for LCAT activity (50). All LCAT assays in this study were carried out in the presence and absence of human apoA-I. Assay of LCAT without addition of serum apoA-I gave background values demonstrating that LCAT secreted from these cells, as from other sources, requires the presence of a protein cofactor for activity. The amount of LCAT activity that accumulated in the culture medium of Hep-G2 cells was about 10% of the level reported for rabbit liver perfusate (51). ACAT activity in these cells was comparable to values obtained in samples of normal human liver (30, 43).

Culture in mevalonate had no effect on the accumulation of apolipoprotein masses in the medium relative to baseline values despite changes in medium cholesterol content. Little data regarding effects of the presence of mevalonate in culture media on apolipoprotein levels appears to be available in other systems.

Addition of exogenous fatty acids to Hep-G2 cell culture medium had no significant effect on the activity of any of the enzymes measured in this study. Previous research (20, 38) has shown that increased triglyceride synthesis and VLDL secretion induced by increased fatty acid availability can stimulate HMG-CoA reductase activity. The degree of stimulation is dependent on the amount of exogenous fatty acid available. The lack of such a change in reductase activity under the conditions of this study suggests that any increased intracellular demand for cholesterol for lipoprotein synthesis and secretion was not great enough to stimulate the enzyme activity.

Although the activity of the microsomal enzyme complex responsible for triglyceride synthesis was unchanged as measured in vitro after maintenance of the cells in 0.3 mM linoleate, it is possible that the activities of these enzymes in the intact cell were relatively increased due to increased intracellular fatty acid availability. Evidence in the rat system suggests that rates of triglyceride synthesis, accumulation, and secretion are regulated by intracellular factors that determine fatty acyl CoA availability to these enzymes (52–54).

Incubation with 0.3 mM linoleate, however, did change several of the apoprotein levels in the media. Apolipoproteins B,E and A-I were all increased in mass. The data for apoB support the results of Rash, Rothblat, and Sparks (3) who demonstrated that the addition of oleic acid to Hep-G2 cell culture medium caused an increase in the incorporation of radiolabel into secreted apoB compared to incubations containing albumin alone. Data from studies of rat hepatocytes in culture, however, showed little change in the masses of apoB,E and A-I after 16 hr exposure to oleate (40).

It is of interest to compare the level of accumulation of apoB in Hep-G2 cell culture medium with measurements of apoB secretion by human liver in vivo. Turner et al. (55) determined that the splanchnic secretion rate of apoB (S_f 100–400) was in the range of 0.46–1.68 $\mu g \cdot hr^{-1} \cdot mg$ of hepatocyte protein^{-1}. (This calculation is extrapolated from the data of Turner et al. (55) and assumes that hepatocyte protein equals about 15% of liver wet weight (56).) For Hep-G2 cells incubated in the presence of 0.3 mM linoleate, which approximates the in vivo fatty acid concentration of plasma (20), 0.38 μg of apoB $\cdot hr^{-1} \cdot mg$ of cell protein^{-1} accumulated in the media. Since this number represents accumulation and not secretion, it is a minimal value. Thus, this suggests that Hep-G2 cells are quite active in apoB production compared to normal human liver. A recent publication concerning human hepatocytes in primary culture reported the accumulation of apoB in the culture medium (57). Much of this apoB was recovered in the VLDL density range.

Hep-G2 cells have an active LDL receptor (9–12), thus it is possible that a portion of the apoB secreted is reinternalized via this system. In the present study, of the total immunologically detectable apoB in the cell culture system, 70–80% was recovered in the cellular fraction. Similar results were obtained for apoE. Whether this represents apoproteins associated with the secretory or with the endocytotic pathway is at present unknown. However, for apoA-I and apoA-II, only about 20% of the total masses were recovered in the cellular fraction.

The hormones in the medium used in this investigation represent a mixture at physiological concentrations; they were selected for their ability to maintain cytochrome P450 content in primary hepatocytes (21). The activity of this enzyme complex is considered to be a sensitive index of hepatocyte function. This combination of hormones added to the media of Hep-G2 cells had little effect on the activities of the enzymes measured. However, the levels of apoproteins that accumulated in the medium, specifically apoB,E, and A-I, were all significantly increased and apoB and E masses were increased in the cells. Although

apoD was detected under all conditions in the cellular fraction, it was measurable in the culture medium only in the presence of the hormone mixture.

In summary, Hep-G2 cells express the major regulatory enzymes of hepatic, plasma, and biliary cholesterol metabolism. These activities respond, at least qualitatively, to physiological alterations in a manner consistent with what is known about human cholesterol metabolism in vivo and by extrapolation from animal models. Although there may be quantitative differences in the expression of these functions, nevertheless, the Hep-G2 cell model system may provide useful insights into potential mechanisms of regulation of human hepatic cholesterol, lipoprotein, and biliary metabolism.

The Hep-G2 cell culture was obtained from Dr. John Watson and originally from Dr. Barbara Knowles and Dr. David Aden. We would like to acknowledge Mary Fisher, Satya Krishnan, Mercidita Del Rosario, and Laurie Shigekuni for excellent technical assistance. We are indebted to Dr. Christopher Fielding for discussion of the manuscript and to Dr. Richard Havel for his interest and encouragement in making this work possible. We also wish to thank Ms. Maggie Joe for assistance in preparation of the manuscript. This work was supported by grants HL 05360 and Arteriosclerosis SCOR HL 14237 from the National Institutes of Health. Dr. Fielding is an Established Investigator of the American Heart Association.

Manuscript received 9 October 1985.

REFERENCES

1. Knowles, B. B., C. C. Howe, and D. P. Aden. 1980. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science.* **209:** 497–499.
2. Zannis, V. I., J. L. Breslow, T. R. San Giacomo, D. P. Aden, and B. B. Knowles. 1981. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* **20:** 7089–7096.
3. Rash, J. M., G. H. Rothblat, and C. E. Sparks. 1981. Lipoprotein apolipoprotein synthesis by human hepatoma cells in culture. *Biochim. Biophys. Acta.* **666:** 294–298.
4. Gordon, J. L., H. F. Sims, S. R. Lentz, C. Edelstein, A., Scanu, and A. W. Strauss. 1983. Proteolytic processing of human preproapolipoprotein A-I. A proposed defect in the conversion of pro A-I to A-I in Tangier's disease. *J. Biol. Chem.* **258:** 4037–4044.
5. Gordon, J. L., C. L. Bisgaier, H. F. Sims, O. P. Sachdev, R. M. Glickman, and A. W. Strauss. 1984. Biosynthesis of human preapolipoprotein A-IV. *J. Biol. Chem.* **259:** 468–474.
6. Tam, S. P., T. K. Archer, and R. G. Deeley. 1985. Effects of estrogen on apolipoprotein secretion by the human hepatocarcinoma cell line, Hep-G2. *J. Biol. Chem.* **260:** 1670–1675.
7. Kane, J. P. 1983. Apolipoprotein B: structural and metabolic heterogeneity. *Annu. Rev. Physiol.* **45:** 637–650.
8. Ciechanover, A., A. L. Schwartz, and H. F. Lodish. 1983. The asialoglycoprotein receptor internalizes and recycles independently of the transferrin and insulin receptors. *Cell.* **32:** 267–275.
9. Havekes, L., V. van Hinsbergh, H. J. Kempen, and J. Emeis. 1983. The metabolism in vitro of human low density lipoprotein by the human hepatoma cell line Hep-G2. *Biochem. J.* **214:** 951–958.
10. Wu, G. Y., C. H. Wu, V. A. Rifici, and R. J. Stockert. 1984. Activity and regulation of low density lipoprotein receptors in a human hepatoblastoma cell line. *Hepatology.* **4:** 1190–1194.
11. Leichtner, A. M., M. Krieger, and A. L. Schwartz. 1984. Regulation of low density lipoprotein receptor function in a human hepatoma cell line. *Hepatology.* **4:** 897–901.
12. Dashti, N., G. Wolfbauer, E. Koren, B. Knowles, and P. Alaupovic. 1984. Catabolism of human low density lipoproteins by human hepatoma cell line Hep-G2. *Biochim. Biophys. Acta.* **794:** 373–384.
13. Hoeg, J. M., S. J. Demosky, Jr., S. B. Edge, R. E. Gregg, J. C. Osborne, Jr., and H. B. Brewer, Jr. 1985. Characterization of a human hepatic receptor for high density lipoproteins. *Arteriosclerosis.* **5:** 228–237.
14. Schwartz, A. L., S. E. Fridovich, B. B. Knowles, and H. F. Lodish. 1981. Characterization of the asialoglycoprotein receptor in a continuous hepatoma cell line. *J. Biol. Chem.* **256:** 8878–8881.
15. Everson, G. T., and M. A. Polakoff. 1985. Hep-G2: a cell line exhibiting defects in bile acid synthesis. *Hepatology.* **5:** 302.
16. Geuze, H. J., J. W. Slot, G. J. A. M. Strous, and A. F. Schwartz. 1983. The pathway of the asialoglycoprotein-ligand during receptor-mediated endocytosis: a morphological study with colloidal gold/ligand in the human hepatoma cell line, Hep-G2. *Eur. J. Cell. Biol.* **32:** 38–44.
17. Simon, D., D. P. Aden, and B. B. Knowles. 1982. Chromosomes of human hepatoma cell lines. *Int. J. Cancer* **30:** 27–33.
18. Cohen, L. H., M. Griffioen, L. Havekes, D. Schouten, V. Van Hinsbergh, and H. J. Kempen. 1984. Effects of compactin, mevalonate and low-density lipoprotein on 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and low-density lipoprotein receptor activity in the human hepatoma cell line Hep-G2. *Biochem. J.* **222:** 35–39.
19. Gould, R. G. 1977. Some aspects of the control of hepatic cholesterol biosynthesis. *In* Cholesterol Metabolism and Lipolytic Enzymes. J. Polonovski, editor. Masson, NY. 13–38.
20. Heimberg, M., M. G. Wilcox, G. D. Dunn, W. F. Woodside, K. J. Breen, and G. Soler-Argilaga. 1974. Studies on the regulation of secretion of the very low density lipoprotein and on ketogenesis by the liver. *In* Regulation of Hepatic Metabolism. F. Lundquist and N. Tygstrup, editors. Munksgaard, Copenhagen. 110–143.
21. Decad, G. M., D. P. H. Hsieh, and J. L. Byard. 1977. Maintenance of cytochrome P-450 and metabolism of aflatoxin B_1 in primary hepatocyte cultures. *Biochem. Biophys. Res. Commun.* **78:** 279–287.
22. Fielding, C. J., J. Frohlich, K. Moser, and P. E. Fielding. 1982. Promotion of sterol efflux and net transport by apolipoprotein E in lecithin:cholesterol acyltransferase deficiency. *Metabolism.* **31:** 1023–1028.
23. Shore, B., and V. Shore. 1969. Isolation and characterization of polypeptides of human serum lipoproteins. *Biochemistry.* **8:** 4510–4516.
24. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
25. Laemmli, U. K. 1970. Cleavage of structural proteins

during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
26. Davis, B. J. 1964. Disc electrophoresis method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121:** 404–427.
27. Fielding, P. E., and C. J. Fielding. 1980. A cholesteryl ester transfer complex in human plasma. *Proc. Natl. Acad. Sci. USA.* **77:** 3327–3330.
28. Chen, C-H., and J. J. Albers. 1982. Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin:cholesterol acyltransferase activity. *J. Lipid Res.* **23:** 680–691.
29. Erickson, S. K., A. D. Cooper, S. M. Matsui, and R. G. Gould. 1977. 7-Ketocholesterol. Its effects on hepatic cholesterogenesis and its hepatic metabolism in vivo and in vitro. *J. Biol. Chem.* **252:** 5186–5193.
30. Erickson, S. K., and A. D. Cooper. 1980. Acyl coenzyme A:cholesterol acyltransferase in human liver. In vitro detection and some characteristics of the enzyme. *Metabolism.* **29:** 991–996.
31. Erickson, S. K., M. A. Shrewsbury, C. Brooks, and D. J. Meyer. 1980. Rat liver acyl-coenzyme A:cholesterol acyltransferase: its regulation in vivo and some of its properties in vitro. *J. Lipid Res.* **21:** 930–941.
32. Nicolau, G., S. Shefer, G. Salen, and E. H. Mosbach. 1974. Determination of hepatic cholesterol 7α-hydroxylase activity in man. *J. Lipid Res.* **15:** 146–151.
33. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
34. Heider, J. G., and R. L. Boyett. 1978. The picomole determination of free and total cholesterol in cells in culture. *J. Lipid Res.* **19:** 514–518.
35. Edwards, P. A., G. Popjak, A. M. Fogelman, and J. Edmond. 1977. Control of 3-hydroxy-3-methylglutaryl coenzyme A reductase by endogenously synthesized sterols in vitro and in vivo. *J. Biol. Chem.* **252:** 1057–1063.
36. Drevon, C. A., D. B. Weinstein, and D. Steinberg. 1980. Regulation of cholesterol esterification and biosynthesis in monolayer cultures of normal adult rat hepatocytes. *J. Biol. Chem.* **255:** 9128–9137.
37. Drevon, C. A., S. C. Engelhorn, and D. Steinberg. 1980. Secretion of very low density lipoproteins enriched in cholesteryl esters by cultured rat hepatocytes during stimulation of intracellular cholesterol esterification. *J. Lipid Res.* **21:** 1065–1071.
38. Kohout, M., B. Kohoutova, and M. Heimberg. 1971. The regulation of hepatic triglyceride metabolism by free fatty acids. *J. Biol. Chem.* **246:** 5067–5074.
39. Schonfeld, G., and B. Pfleger. 1971. Utilization of exogenous free fatty acids for the production of very low density lipoprotein triglyceride by livers of carbohydrate-fed rats. *J. Lipid Res.* **12:** 614–621.
40. Patsch, W., T. Tamai, and G. Schonfeld. 1983. Effect of fatty acids on lipid and apoprotein secretion and association in hepatocyte cultures. *J. Clin. Invest.* **72:** 371–378.
41. Davis, R. A., and Boogaerts, J. R. 1982. Intrahepatic assembly of very low density lipoproteins. Effects of fatty acids on triacylglycerol and apolipoprotein synthesis. *J. Biol. Chem.* **257:** 10908–10913.
42. Erickson, S. K., M. A. Shrewsbury, R. G. Gould, and A. D. Cooper. 1980. Studies on the mechanisms of the rapid modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in intact liver by mevalonolactone and 25-hydroxycholesterol. *Biochim. Biophys. Acta.* **620:** 70–79.
43. Balasubramaniam, S., K. A. Mitropoulos, N. B. Myant, M. Mancini, and A. Postiglione. 1979. Acyl coenzyme A-cholesterol acyltransferase activity in human liver. *Clin. Sci.* **56:** 373–375.
44. Ahlberg, J., B. Angelin, I. Björkhem, K. Einarsson, and B. Leijd. 1979. Hepatic cholesterol metabolism in normo- and hyperlipidemic patients with cholesterol gallstones. *J. Lipid Res.* **20:** 107–115.
45. Nicolau, G., S. Shefer, G. Salen, and E. H. Mosbach. 1974. Determination of hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity in man. *J. Lipid Res.* **15:** 94–98.
46. Davis, R. A., P. M. Hyde, J-C. W. Kuan, M. Malone-McNeal, and J. Archambault-Schexnayder. 1983. Bile acid secretion by cultured rat hepatocytes. *J. Biol. Chem.* **258:** 3661–3667.
47. Erickson, S. K., and B. Bösterling. 1981. Cholesterol-7α-hydroxylase from human liver: partial purification and reconstitution into defined phospholipid–cholesterol vesicles. *J. Lipid Res.* **22:** 872–876.
48. Carulli, N., M. Ponz de Leon, F. Zironi, R. Iori, and P. Loria. 1980. Bile acid feeding and hepatic sterol metabolism: effect of deoxycholic acid. *Gastroenterology.* **79:** 637–641.
49. Carulli, N., M. Ponz de Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short-term feeding of chenodeoxycholic and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
50. Fielding, C. J., and P. E. Fielding. 1971. Purification and substrate specificity of lecithin:cholesterol acyltransferase from human plasma. *FEBS Lett.* **15:** 355–358.
51. De Parscau, L., and P. E. Fielding. 1984. Lecithin:cholesterol acyltransferase and cholesteryl ester transfer activity from the isolated perfused rabbit liver. *J. Lipid Res.* **25:** 721–728.
52. Ockner, R. K., N. Lysenko, J. A. Manning, S. E. Monroe, and D. A. Burnett. 1980. Sex steroid modulation of fatty acid utilization and fatty acid binding protein concentration in rat liver. *J. Clin. Invest.* **65:** 1013–1023.
53. Pikkukangas, A. H., R. A. Vaananen, M. J. Savolainen, and I. E. Hassinen. 1982. Precursor supply and hepatic enzyme activities as regulators of triacylglycerol synthesis in isolated hepatocytes and perfused liver. *Arch. Biochem. Biophys.* **217:** 216–225.
54. Brindly, D. N. 1985. Metabolism of triacylglycerols. *In* Biochemistry of Lipids in Membranes. D. E. Vance and J. E. Vance, editors. Benjamin/Cummings Publication Co., Menlo Park, CA. 213–241.
55. Turner, P. R., N. E. Miller, C. Cortese, W. Hazzard, J. Coltart, and B. Lewis. 1981. Splanchnic metabolism of plasma apolipoprotein B. *J. Clin. Invest.* **67:** 1678–1686.
56. Jones, A. L. 1982. Anatomy of the normal liver. *In* Hepatology-A Textbook of Liver Disease. D. Zakim and T. D. Boyer, editors. W. B. Saunders Co., Philadelphia, PA. 3–31.
57. Kosykh, V. A., S. N. Preobrazhensky, I. V. Fuki, O. E. Zaikina, V. P. Tsibulsky, V. S. Repin, and V. N. Smirnov. 1985. Cholesterol can stimulate secretion of apolipoprotein B by cultured human hepatocytes. *Biochim. Biophys. Acta.* **836:** 385–389.