Identification and Partial Characterization of Discrete Apolipoprotein B Containing Lipoprotein Particles Produced by Human Hepatoma Cell Line HepG2†

Nassrin Dashti, Peter Alaupovic,* Carolyn Knight-Gibson, and Eugen Koren

Lipoprotein and Atherosclerosis Research Program, Oklahoma Medical Research Foundation, and the Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104

Received September 19, 1986; Revised Manuscript Received March 9, 1987

ABSTRACT: The purpose of this study was to test the use of human hepatocarcinoma HepG2 cells as a model for studying the formation and secretion of human hepatic lipoproteins. To this end, we determined the rate of accumulation and percent composition of neutral lipids and apolipoproteins in the culture medium of HepG2 cells and isolated and partially characterized the apolipoprotein B (ApoB) containing lipoprotein particles. The rates of accumulation in the medium of HepG2 cells, grown in minimum essential medium during a 24-h incubation, of triglycerides, cholesterol, and cholesterol esters expressed as $\mu g/(g$ of cell protein·h) were 373 ± 55, 167 ± 14, and 79 ± 10, respectively; the secretion rates for apolipoproteins B, A-I, E, A-II, and C-III were 372 ± 36, 149 ± 14, 104 ± 13, 48 ± 4, and 13 ± 1 $\mu g/(g$ of cell protein·h), respectively. The major portion of ApoB was present in very low density lipoproteins (VLDL) and low-density lipoproteins (LDL) (84%), with the remainder occuring in high-density lipoproteins (HDL) (16%). Approximately 10–13% of ApoA-I and ApoA-II were present in VLDL and LDL, while 60% of ApoE occurred in HDL and 40% in VLDL and LDL. To separate ApoB-containing lipoproteins, secreted lipoproteins were fractionated by either sequential immunoprecipitation or immunoaffinity chromatography with antibodies to ApoB and ApoE. Results showed that 60–70% of ApoB occurred in the culture medium as lipoprotein B (LP-B) and 30–40% as lipoprotein B:E (LP-B:E). Both ApoB-containing lipoproteins represent polydisperse systems of spherical particles ranging in size from 100 to 350 Å for LP-B and from 200 to 500 Å for LP-B:E. LP-B particles were identified in VLDL, LDL, and HDL, while LP-B:E particles were only present in VLDL and LDL. The major neutral lipid of both ApoB-containing lipoproteins was triglyceride (50–70% of the total neutral lipid content); cholesterol and cholesterol esters were present in equal amounts. The LP-B:E particles contained 70–90% ApoB and 10–30% ApoE. The ApoB was identified in both types of particles as B-100. A time study on the accumulation of ApoB-containing lipoproteins showed that LP-B particles were secreted independently of LP-B:E particles.

The human plasma lipoproteins consist of particles directly secreted by liver and intestine and particles generated within the vascular compartment through enzymic and nonenzymic conversions and interactions of nascent lipoproteins (Osborne & Brewer, 1977; Bisgaier & Glickman, 1983). Due to this remarkable heterogeneity of lipoprotein particles and the lack of an adequate experimental model, the chemical nature of lipoproteins secreted by human liver and intestine remains largely unknown. However, recent studies on the identification and characterization of a human hepatocellular carcinoma cell line, HepG2, have shown that these cells retain many of the normal biochemical functions of liver parenchymal cells (Knowles et al., 1980; Schwartz, et al., 1981; Morris et al., 1982) and, thus, represent a potentially important model for studying the chemical nature and metabolism of human hepatic lipoproteins. Indeed, HepG2 cells have already been shown to secrete most of the plasma apolipoproteins (A-I, A-II, A-IV, B, C-I, C-II, C-III, and E) as integral components of lipoprotein particles (Zannis et al., 1981; Rash et al., 1981; Gordon et al., 1984; Wolfbauer & Dashti, 1983) and have successfully been used for establishing the regulatory role of lipids (Rash et al., 1981; Wolfbauer & Dashti, 1983) and hormones (Wolfbauer & Dahti, 1983; Tam et al., 1985) in the synthesis and secretion of apolipoproteins and lipoproteins and for studying the removal of various lipoprotein density classes through receptor-mediated processes (Havekes et al., 1983; Dashti et al., 1984, 1985; Illingworth et al., 1984; Hoeg et al., 1985). It has also been reported that HepG2 cells synthesize lecithin:cholesterol acyltransferase (EC 2.3.1.43, LCAT), an enzyme catalyzing the esterification of lipoprotein cholesterol, but that its secretion into culture medium may be partially or totally impaired (Koren et al., 1985).

However, since the distribution and localization of apolipoproteins on individual lipoproteins has not been established, we have undertaken a study on the identification, isolation, and characterization of lipoprotein particles secreted by and accumulated in the culture medium of HepG2 cells. This investigation has been based on the concept that discrete, polydisperse lipoprotein families or particles characterized by their specific apolipoprotein composition rather than density properties represent the fundamental physical–chemical and metabolic entities of the plasma lipoprotein system (Alaupovic, 1972).

In this paper, we present data on the accumulation rates and percent composition of neutral lipids and apolipoproteins in the HepG2 cell culture medium and report on the isolation

† This work has been supported in part by Grant HL 23181 from the U.S. Public Health Service and by the resources of the Oklahoma Medical Research Foundation.

* Correspondence should be addressed to this author at the Lipoprotein and Atherosclerosis Research Program, Oklahoma Medical Research Foundation.

0006-2960/87/0426-4837$01.50/0

and partial characterization of ApoB-containing lipoproteins.[1] Results show the presence in culture medium of two distinct ApoB-containing lipoprotein particles identified after separation by immunoprecipitation or immunoaffinity chromatography as lipoprotein B (LP-B) and lipoprotein B:E (LP-B:E).

Materials and Methods

Materials. Modified minimum essential medium was obtained from Grand Island Biological Co. (Grand Island, NY) and fetal calf serum from Hazleton Dutchland, Inc. (Denver, PA). Sodium pyruvate, L-glutamine, and minimum essential medium vitamin solution were from KC Biological, Inc. (Lenexa, KS). Microtiter plates (96-2311 Serocluster "U" vinyl) were purchased from Costar (Cambridge, MA). Bovine and human serum albumin, pristane (2,6,10,14-tetramethylpentadecane), Tween, and 2,2′-azinobis(3-ethylbenzothiazolinesulfonate) (ABTS) were obtained from Sigma (St. Louis, MO). Horseradish peroxidase labeled antibodies to mouse IgG and IgM were obtained from Kirkegaard and Perry's Laboratories (Gaithersburg, MD) and peroxidase substrates TTF_1 and TTF_2 from Servia (Issy-les-Moulineaux, France). Monospecific antisera to mouse IgG_1, IgG_2A, and IgM were purchased from Meloy (Springfield, VA), and mouse immunoglobulin subtype identification kit was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Affi-Gel 10 (*N*-hydroxysuccinimide ester derivative of cross-linked agarose) was obtained from Bio-Rad Laboratories (Richmond, CA) and Sephadex G-25 from Pharmacia (Piscataway, NJ).

Cell Culture. Human hepatocellular carcinoma cell line HepG2 was kindly provided by Dr. Barbara Knowles, The Wistar Institute, Philadelphia, PA. Cells were maintained in a supplemented minimum essential medium containing 10% (v/v) fetal calf serum under conditions described previously (Dashti et al., 1984). Four to five days prior to each experiment, cells were seeded in 100-mm-diameter dishes (15–20 plates per experiment) in 15 mL of minimum essential medium containing 10% fetal calf serum. The maintenance medium was removed, the monolayers were washed twice with 15 mL of phosphate-buffered saline (pH 7.4) to remove traces of fetal calf serum, and 15 mL of serum-free medium was added. After a 19–24-h incubation, unless otherwise stated, culture medium was collected and centrifuged at 2000 rpm for 20 min to remove cells and debris. The supernatant fraction was concentrated approximately 10–15-fold with sucrose placed outside the dialysis bag (M_r 5000 cutoff) as described previously (McConathy et al., 1985). This concentration procedure had no effect on the lipoprotein composition. The monolayers were washed twice with phosphate-buffered saline and scraped off the plate after addition of 2 × 2 mL of phosphate-buffered saline.

Isolation of Lipoprotein Density Classes. Blood samples were obtained from asymptomatic, normolipidemic subjects who had fasted overnight (12–14 h). Blood was drawn by antecubial venipuncture into vacutainer tubes containing Na_2EDTA (1.5 mg/mL), and the plasma samples were collected by low-speed centrifugation. Plasma samples were used for the quantitative determination of lipids and apolipoproteins and for the isolation of lipoprotein density classes.

The HepG2 cell culture medium was used as collected for the isolation of lipoprotein density classes. Very low density and intermediate-density lipoproteins (VLDL + IDL, $d <$ 1.019 g/mL), low-density lipoproeins (LDL, d = 1.019–1.063 g/mL), and high-density lipoproteins (HDL, d = 1.063–1.21 g/mL) were isolated as previously described (Alaupovic et al., 1972). However, the quantitative determination of neutral lipids and apolipoproteins was carried out on the supernatant ($d <$ 1.063 g/mL) and infranatant ($d >$ 1.063 g/mL) fractions of culture medium ultracentrifuged at d = 1.063 g/mL.

Immunoprecipitation of ApoB- and ApoE-Containing Lipoproteins. The immunoprecipitation of ApoB- and ApoE-containing lipoproteins from the culture medium of HepG2 cells was carried out by a modification of a previously described procedure (Lee & Alaupovic, 1974). The cell medium was concentrated by the sucrose treatment to one-tenth of its original volume and apolipoproteins A-I, A-II, B, C-III, and E were quantified by electroimmunoassay. One milliliter of concentrated medium was mixed with an equivalent amount of a polyclonal antiserum to human ApoB (IgG fraction) determined by a microdilution technique. The reaction mixture was incubated for 2 h at 4 °C, and the precipitated antigen–antibody complex was collected by low-speed centrifugation for 10 min. The supernatant fraction was tested

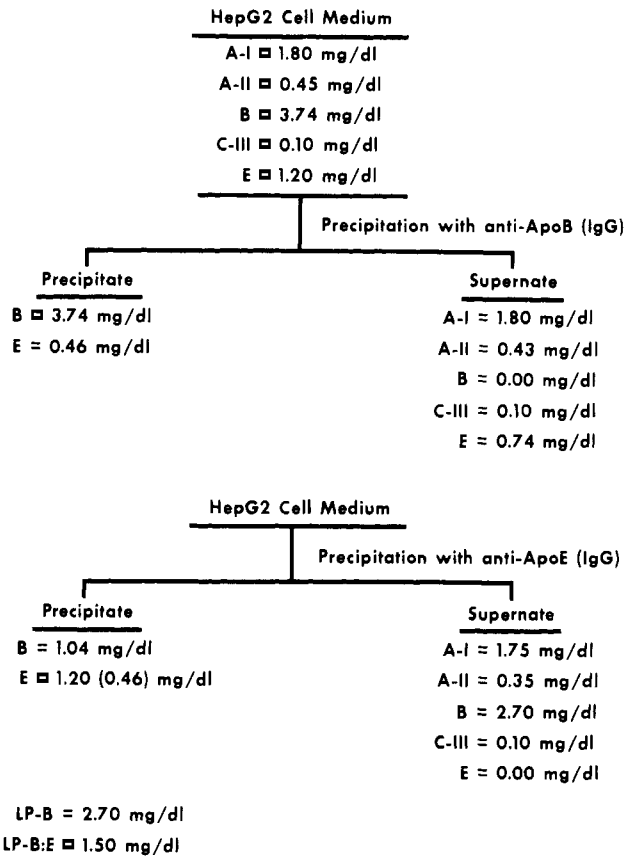

FIGURE 1: Immunoprecipitation of ApoB-containing lipoprotein particles from culture medium of HepG2 cells by antibodies to ApoB and ApoE. After 5 days in culture, the maintenance medium was removed and cells were washed twice with phosphate-buffered saline. Serum-free medium was added and cells were incubated for an additional 24 h. The culture medium was concentrated 10–15-fold prior to immunoprecipitation with antibodies to apolipoproteins B and E.

[1] Abbreviations: Apo, apolipoprotein; VLDL, very low density lipoproteins ($d <$ 1.006 g/mL); IDL, intermediate-density lipoproteins (d = 1.006–1.019 g/mL); LDL, low-density lipoproteins (d = 1.019–1.063 g/mL); HDL, high-density lipoproteins (d = 1.063–1.21 g/mL); LP-B, lipoprotein particles that contain ApoB as the sole protein constituents; LP-B:E, lipoprotein particles that contain ApoB and ApoE as protein constituents; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; BSA, bovine serum albumin; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; ABTS, 2,2′-azinobis(3-ethylbenzothiazolinesulfonate); LCAT, lecithin:cholesterol acyltransferase; IgG, immunoglobulin G; SDS, sodium dodecyl sulfate.

for the presence of ApoB by double-diffusion analysis and electroimmunoassay. If ApoB was still detectable, the supernatant fraction was treated with an additional volume of antiserum until free of ApoB. The unbound apolipoproteins A-I, A-II, C-III, and E present in the supernatant fraction were then quantified by electroimmunoassay. A separate 1-mL aliquot of concentrated culture medium was treated with an equivalent amount of a polyclonal antiserum to ApoE (IgG fraction) in the same manner as described for the immunoprecipitation of ApoB-containing lipoproteins. As illustrated in an experiment in Figure 1, differences between the concentrations of apolipoproteins in the culture medium and supernatant fractions were taken as the amounts of apolipoproteins complexed with ApoB and ApoE, respectively. To test for the possible presence of soluble ApoB or ApoE antigen–antibody complexes, the pH of supernatant fractions was adjusted to 3.2 with 0.2 M HCl and the solution density was raised to 1.063 g/mL by the addition of solid NaCl. After centrifugation in the Ti-60 rotor at 105000*g* for 36 h, the supernatant fraction was removed and concentrated to one-tenth of its initial volume. The double-diffusion analysis and electroimmunoassay of the dialyzed supernatant fractions gave negative reactions with antibodies to ApoB or ApoE. However, when applied to the ApoB and ApoE antigen–antibody precipitates, this procedure resulted in positive identification of ApoB and ApoE, respectively. To exclude the possible coprecipitation by antibodies to ApoB of ApoE not bound to ApoB, LP-B particles isolated by immunoaffinity chromatography were mixed with an ApoB-free plasma fraction containing ApoE and ApoC-III. Addition of an anti-ApoB serum (IgG fraction) to this mixture resulted in the precipitation of Lp-B without traces of ApoE or ApoC-III.

Production and Characterization of *Monoclonal Antibodies.* Two young adult female Balb/c mice were immunized with LDL and ApoE, respectively. Each antigen solution was mixed with an equal volume of complete Freund's adjuvant and vortexed vigorously until a thick foamy suspension was formed. Antigens (50 μg of protein per mouse) were injected subcutaneously into the back of the animal. Two weeks later, 10 μg of antigen mixed with incomplete Freund's adjuvant was injected intraperitoneally. This was repeated until the antibody titer of 1:100 000 was detected by an enzyme immunoassay described below. Two days before splenectomy, 10 μg of antigen (adjuvant free) was injected intravenously. To obtain the desired titer, LDL had to be injected 4 times during a 6-week period and ApoE 7 times during 12 weeks. Fusion of spleen cells with mouse myeloma cells (Sp2/0), growth in the selective media, cloning, and production of monoclonal antibodies in ascites were preformed according to the protocol described by Galfrē and Milstein (1981).

Specificity of monoclonal antibodies was examined by an enzyme immunoassay against apolipoproteins as follows. Microtiter plates were coated with apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E (Alaupovic et al., 1972; McConathy & Alaupovic, 1976; Curry et al., 1976b) (1 μg/well) overnight at room temperature. After blocking with 1% bovine serum albumin (BSA), hybridoma supernates (50 μL/well) containing monoclonal antibodies were incubated with antigen-precoated plates for 3 h at room temperature. The plates were then washed 3 times with phosphate-buffered saline (PBS) and incubated with peroxidase-labeled goat antibody to mouse immunoglobulins (IgG + M) for 2 h. After five washes with PBS, peroxidase substrate was added (50 μL/well) and the optical density was read at 405 nm using Dynatech's micro-ELISA reader MR 580 (Dynatech, Torrance, CA). In these experiments, two types of controls were always used. Each of the antibody-containing supernates was incubated with plates only coated with BSA, and each of the antigen-coated plates was incubated with spent hybridoma supernate containing no antibodies. The nonspecific background was always determined by the use of these controls. Optical density reading was not considered significant unless it was at least twice as high as the corresponding background.

Binding of monoclonal antibodies to chylomicrons, VLDL, LDL, and HDL was determined by using the same assay. Concentrations of ApoB and ApoE in these lipoproteins were adjusted to the same level before pipetting into the microtiter plates. One microgram of the respective apolipoprotein was always added per well.

Monoclonality of antibodies was determined by the use of Boehringer's subtype classification kit. An antibody was considered monoclonal only if one type of heavy immunoglobulin chain and one type of light immunoglobulin chain were found and if identical results were obtained with three consecutive subclones. A monoclonal antibody to ApoB (IgG_1, κ) and a monoclonal antibody to ApoE (IgG_1, κ) were selected on the basis of their specificity and binding capacity to all major lipoprotein density classes.

Isolation of ApoB- and ApoE-Containing Lipoproteins by Immunoaffinity Chromatography. Purified monoclonal antibodies to ApoB and ApoE were coupled to the cross-linked agarose activated with *N*-hydroxysuccinimide (Affi-Gel 10). After exhaustive washing of Affi-Gel with cold, double-distilled, deionized water, the antibody solutions were added to the gel slurry (10 mg of protein/mL of gel) and the mixture was gently shaken for 2 h at 20 °C. The gel was allowed to settle, and the supernatant liquid was carefully removed. To block the remaining active sites, the gel was incubated with 0.1 M Tris-HCl buffer containing 0.15 M NaCl, pH 7.4, for 18 h at 4 °C. The gel was then washed with 3 volumes of 1.0 M NaCl and 3 volumes of 3 M NaSCN and eventually reequilibrated with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 mg/mL EDTA. The binding capacity of the anti-ApoB immunosorber was 0.144 mg of ApoB/mL of gel and that of the anti-ApoE immunosorber was 0.010 mg of ApoE/mL of gel.

Columns (1.2 × 49 cm, K-50 Pharmacia) for the immunoaffinity chromatography were first packed with 25 mL of Sephadex G-25 followed by 10 mL of antibody-coupled Affi-Gel and another protective layer of 5 mL of Sephadex G-25 (McConathy et al., 1985). The purpose of including layers of Sephadex G-25 was to minimize the exposure time between the lipoprotein and dissociating agent. The Sephadex G-25 layers were 3 times the bed volume of the antibody-coupled gel and served as a desalting column for separating lipoproteins from the dissociating agent. The HepG2 cell culture medium (40–80 mL) was applied to the column as collected and allowed to pass through the column at a flow rate of 30 mL/h. The unretained fraction was eluted with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 mg/mL EDTA. After the absorbance at 280 nm returned to base line, the column was washed with 150 mL of the elution buffer and the retained fraction was eluted with 5 mL of 3 M NaSCN, pH 7.4. The bottom layer of Sephadex G-25 allowed an immediate separation of retained lipoproteins from the dissociating agent (McConathy et al., 1985) which resulted in two distinct peaks at 280 nm; the first peak contained the retained lipoproteins, and the second peak consisted of NaSCN. Column chromatography monitored by measuring absorption at 280 nm was performed at room temperature and

Table I: Percent Composition of Neutral Lipids and Apolipoproteins in the Culture Medium of HepG2 Cells and Normolipidemic Human Plasma

	neutral lipids (%)			apolipoproteins (%)				
sample	cholesterol	cholesterol esters	triglycerides	A-I	A-II	B	C-III	E
HepG2 cell culture medium[a]	28.6 ± 1.4[b] (n = 13)[c]	13.3 ± 0.9 (n = 13)	58.1 ± 1.9 (n = 13)	22.5 ± 1.4 (n = 15)	7.6 ± 0.8 (n = 15)	54.2 ± 1.8 (n = 15)	1.0 ± 0.3 (n = 15)	14.7 ± 1.0 (n = 15)
human plasma	12.9 (n = 191)[d]	62.9 (n = 191)	24.1 (n = 191)	42.2 (n = 198)	22.1 (n = 198)	29.7 (n = 198)	2.4 (n = 198)	3.3 (n = 198)

[a] The maintenance medium was removed, and cells were washed twice with phosphate-buffered saline and incubated with serum-free medium for 24 h. An aliquot of culture medium was lyophilized for determination of neutral lipids. A separate aliquot was concentrated 10–15-fold with sucrose for measurement of apolipoproteins. [b] Mean ± SE. [c] Number of separate experiments. [d] Number of subjects.

lasted 4–6 h. Both the anti-ApoB and anti-ApoE immunosorbers were run under identical experimental conditions. The structural integrity of ApoB-containing lipoprotein particles isolated by immunoaffinity chromatography has been verified and documented in a previous study from this laboratory (McConathy et al., 1985). The recovery of LP-B recycled through the anti-ApoE immunosorber was 95–98%, based on the amount of ApoB applied to the immunosorber.

Micro-ELISA Procedure for Assay of ApoB- and ApoE-Containing Lipoprotein Particles. LP-B and LP-B:E particles secreted into the culture medium at various time intervals were assayed by a micro-ELISA procedure. Microtiter plates were coated with an affinity-purified monoclonal antibody to ApoB ("pan B") shown to bind equally to all apolipoprotein and lipoprotein forms of human plasma ApoB (Koren et al., 1986a,b); the coating (2 μg/well) was done in a humidified chamber overnight at room temperature. After the coated microtiter plates were blocked with 1% bovine serum albumin for 1 h, they were incubated with HepG2 cell culture medium (50 μL/well) for 3 h at room temperature. Samples of the culture medium were removed from cell culture dishes after 2, 4, 6, 8, 10, 12, and 24 h of incubation. After incubation with the culture medium, microtiter plates were washed with 1% bovine serum albumin 3 times and incubated (50 μL/well for 2 h at room temperature) with peroxidase-coupled polyclonal antibodies to ApoB and ApoE, respectively, as described by Avrameas (1969). After washing, the microtiter plates were incubated (50 μL/well for 30 min at room temperature) with peroxidase substrate and the color developed was measured by a micro-ELISA reader (MR 850, Dynatech, Torrance, CA). Microtiter plates without the coating antibody and plates that had not been incubated with the culture medium served as corresponding controls. All other steps of the assays were identical. This "sandwich" micro-ELISA procedure was used to determine all ApoB-containing lipoproteins (monoclonal "pan B"–peroxidase-coupled polyclonal anti-ApoB "sandwich") and lipoproteins containing ApoB and ApoE (monoclonal "pan B"–peroxidase-coupled polyclonal anti-ApoE "sandwich"). The lowest quantities of ApoB and ApoE measurable in a 50-μL sample applied per well were 0.1 and 0.25 ng, respectively. Thus, as little as 2 ng of ApoB and 5 ng of ApoE per one milliliter of the cell culture supernatant fraction could be measured by this assay, the sensitivity of which is approximately 1000-fold higher than that of corresponding electroimmunoassays. The ApoB concentration of LP-B was determined by subtracting the measured concentration of ApoB in LP-B:E from the total ApoB.

Immunological Methods and Assays. Procedures for the preparation of rabbit polyclonal antisera to human apolipoproteins A-I and A-II (Curry et al., 1976a) and sheep polyclonal antisera to human aplipoproteins B, C-I, C-II, C-III, D, and E (Curry et al., 1976b, 1978, 1981, 1980, 1977) were previously described. Specificity of antisera was tested by double-diffusion analysis, cross-immunoelectrophoresis (Alaupovic, 1984a), and electroimmunoassay. On the basis of these tests, all antisera were found to be monospecific.

Double-diffusion analyses of lipoprotein particles were performed in 1% agar (Special Agar Noble, Difco Laboratories, Detroit, MI) as previously described (Alaupovic et al., 1972).

The quantitative determination of apolipoproteins A-I, A-II, B, C-III, and E was carried out by electroimmunoassays developed in this laboratory (Curry et al., 1976a,b, 1978, 1980).

Gradient SDS–Polyacrylamide Gel Electrophoresis. Linear gradient (2.5–10%) polyacrylamide slab gels (140 × 130 × 1.5 mm) were prepared by using a gradient gel former (LKB 2001-500, LKB, Bromma, Sweden). Lipoprotein samples (150 μL/well) were electrophoresed as previously described (Lee et al., 1984). Silver staining of gel slabs was done by using Bio-Rad's staining kit according to the producer's instructions.

Lipid and Protein Analyses. Triglycerides, cholesterol, and cholesterol esters were quantified by gas–liquid chromatography as described by Kuksis et al. (1975) using cholesterol butyrate as internal standard. Cell protein was determined by the method of Lowry et al. (1951).

Electron Microscopy. Electron microscopy of negatively stained lipoprotein particles was carried out as previously described (McConathy et al., 1985) with a JEOL-100 CX (JEOL, Tokyo, Japan) electron microscope.

Measurement of Lipolytic Activities. The determination of lipoprotein lipase activities in culture medium of HepG2 cells was performed according to the procedure described by Wang et al. (1981). Samples used for measuring lipolytic activities were taken after 2, 4, 7, and 24 h of incubation.

Results

Neutral Lipid and Apolipoprotein Composition of Culture Medium. After a 24-h incubation of HepG2 cells in a serum-free medium, triglycerides were found to be the major and cholesterol and cholesterol esters the minor neutral lipids of the culture medium. In a series of 13 experiments, the rates of accumulation of triglycerides, cholesterol, and cholesterol esters in the medium were 373.7 ± 55.4, 166.7 ± 14.3, and 79.5 ± 9.7 (mean ± SE) μg/(g of cell protein·h), respectively. In contrast to the normolipidemic human plasma, triglycerides accounted for almost 60% and cholesterol for 30% of the total neutral lipid content of HepG2 cell medium (Table I). Whereas in human plasma cholesterol esters accounted for more than 60%, in the culture medium their contribution barely exceeded 10% of the total neutral lipids. This difference in the cholesterol/cholesterol ester ratio between plasma and culture medium reflects most probably the impaired secretion of LCAT into the culture medium (Koren et al., 1985). Phospholipid analysis was not carried out due to the insufficient amount of medium lipoproteins available for lipid and apolipoprotein measurements.

Table II: Distribution of Neutral Lipids and Apolipoproteins between "Low-Density" and "High-Density" Lipoproteins in the Culture Medium of HepG2 Cells[a]

density class	neutral lipids (%)			apolipoproteins (%)			
	cholesterol	cholesterol esters	triglycerides	A-I	A-II	B	E
d <1.063 g/mL ("low-density" lipoproteins; n =5)	68.9 ± 5.5[b]	71.8 ± 7.5	85.4 ± 5.3	9.9 ± 3.3	13.2 ± 5.8	84.3 ± 5.0	41.6 ± 6.3
d >1.063 g/mL ("high-density" lipoproteins; n = 5)	31.1 ± 5.5	28.2 ± 7.5	14.5 ± 5.3	90.0 ± 3.3	86.7 ± 5.8	15.7 ± 5.0	58.4 ± 6.3

[a] Cells were processed and the culture medium was collected as described in Table I. However, the culture medium was not concentrated but was used as collected for the isolation of density classes. [b] Mean ± SE.

Apolipoprotein B was the major apolipoprotein secreted into the culture medium followed, in the order of decreasing concentrations, by apolipoproteins A-I, E, A-II, and C-III. The rates of accumulation of apolipoproteins B, A-I, E, A-II, and C-III in the culture medium were 372.2 ± 35.7, 149.4 ± 13.6, 104.2 ± 13.0, 48.5 ± 4.3, and 12.7 ± 1.3 (mean ± SE) μg/(g of cell protein·h), respectively. When data were expressed on a molar basis, the ratios of apolipoproteins A-I:A-II:B:C-III:E in the culture medium were 7.4:3.8:1:1.8:4.2. Although apolipoproteins C-I, C-II, and D were detectable in some culture media, in most experiments the concentrations of these apolipoproteins were too small to be quantified accurately by the electroimmunoassay. The weight percent apolipoprotein composition of HepG2 cell culture medium differed considerably from that of normal plasma (Table I). As a major apolipoprotein in the culture medium, ApoB accounted for over 50% of the total apolipoprotein content. In plasma, however, ApoA-I was the major apolipoprotein, representing 42% of the total apolipoprotein content. The weight ratio of apolipoproteins A-I:A-II in the culture medium was 3:1, but in the plasma it was 2:1. Another remarkable difference was the percent content of ApoE which, in culture medium, was 4.5 times higher than in plasma and twice as high as the percent content of ApoA-II. On the other hand, the percent content of ApoC-III and other ApoC peptides was at least 2.5 times higher in plasma than in culture medium. These differences could be explained by the intestinal contribution of apolipoproteins, especially ApoA-I and ApoA-II, different residence times of apolipoproteins in the plasma compartment, and experimental conditions used for culturing HepG2 cells in this study. Alternatively, these variations might be due to possible differences in the apolipoprotein synthetic rates by the transformed HepG2 cells when compared with normal human liver.

To avoid a prolonged exposure of lipoproteins to high gravitational forces, they were separated into "low-density" and "high-density" lipoproteins by a single ultracentrifugation at the density 1.063 g/mL (Table II). Similar to the cholesterol and triglyceride distributions in normolipidemic plasma (Wahl et al., 1981; Carlson & Holmquist, 1982), 70–85% of neutral lipids in the culture medium were present in lipoproteins with d <1.063 g/mL, and the remainder in lipoproteins with d >1.063 g/mL. As expected, the major portion of ApoB was present in the d <1.063 g/mL fraction and major portions of ApoA-I and Apo-II were present in the d >1.063 g/mL fraction. However, in contrast to normolipidemic plasma (Curry et al., 1976a, 1978), higher percentages of ApoA-I and ApoA-II occurred in "low-density" lipoproteins (d <1.063 g/mL) and a higher percentage of ApoB in "high-density" lipoproteins (d >1.063 g/mL) of HepG2 cell medium. Relatively more ApoE was present in the d >1.063 g/mL fraction of culture medium (60%) than in the same density region of plasma (40%) (Curry et al., 1976b).

Double-diffusion analysis of human plasma and culture medium of HepG2 cells with antisera to apolipoproteins A-I, A-II, B, and E showed reactions of complete identity for all four major apolipoproteins (Figure 2).

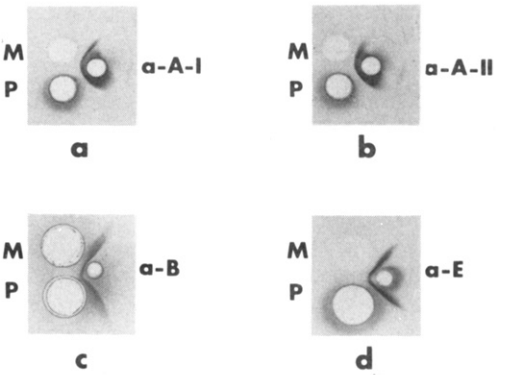

FIGURE 2: Double-diffusion analyses of HepG2 cell culture medium (M) and human plasma (P) with antisera to apolipoproteins A-I (pattern a), A-II (pattern b), B (pattern c), and E (pattern d).

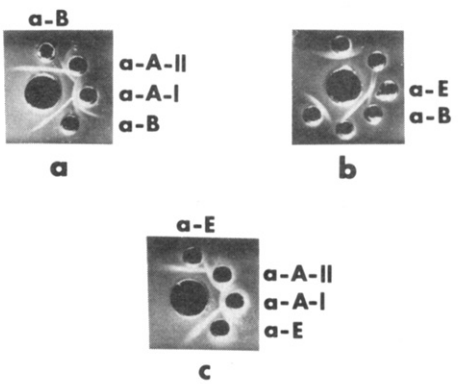

FIGURE 3: Double-diffusion analysis of HepG2 cell culture medium with antisera to apolipoproteins A-I, A-II, and B (pattern a), antisera to apolipoproteins B and E (pattern b), and antisera to apolipoproteins A-I, A-II, and E (pattern c).

Double-Diffusion Analysis of Lipoproteins in Culture Medium. As a first step in determining the distribution of apolipoproteins on lipoprotein particles, the culture medium was tested by double-diffusion analyses with polyclonal antisera to major apolipoproteins. The nonidentity reaction between ApoB and both ApoA-I and ApoA-II (Figure 3, pattern a) indicated that ApoB did not reside on the same lipoprotein particles with either ApoA-I or ApoA-II. On the other hand, testing of the culture medium with antisera to ApoB and ApoE (Figure 3, pattern b) revealed a rather complex configuration of precipitin lines, suggesting the possible presence of three types of lipoprotein particles including lipoproteins that only contained ApoB (LP-B), lipoproteins that only contained ApoE (LP-E), and lipoproteins that contained both ApoB and ApoE (LP-B:E). ApoE gave an apparent reaction of partial identity with ApoA-I and a nonidentity reation with ApoA-II (Figure 3, pattern c); these patterns indicated that at least a portion of ApoE may have been associated with ApoA-I (LP-A-I:E). Precipitin lines of ApoA-I and ApoA-II showed a typical reaction of partial identity, clearly indicating the presence of lipoprotein particles containing both apoliproteins (LP-A-I:

Table III: Apolipoprotein Composition of ApoB-Containing Lipoprotein Particles Isolated by Immunoprecipitation from HepG2 Cell Culture Medium

lipoprotein particles	experiment	apolipoproteins (%) B	E
LP-B:E	1	69.3	30.6
LP-B	1	100.0	ND[a]
LP-B:E	2	69.8	30.2
LP-B	2	100.0	ND
LP-B:E	3	85.0	15.0
LP-B	3	100.0	ND

[a] ND, not detectable.

A-II) and lipoprotein particles only containing ApoA-I (LP-A-I). Thus, the results of these experiments suggest that there is a clear delineation in the culture medium of HepG2 cells between ApoB- and ApoA-containing lipoprotein particles, that ApoA-I and ApoA-II occur as two subpopulations of lipoprotein particles, and that ApoE forms associations with both the ApoB- and ApoA-containing lipoproteins. To further test some of these conclusions and to identify lipoprotein particles belonging to the ApoB group, lipoproteins accumulating in the culture medium were fractionated by immunoprecipitation and immunoaffinity chromatography. A study on the lipoprotein particles of the ApoA group will be described in a separate report.

Immunoprecipitation of ApoB-Containing Lipoproteins. To further test the suggestion based on double-diffusion analysis that ApoB occurs mainly, if not exclusively, as LP-B and LP-B:E particles, the aliquots of culture medium were treated separately with polyclonal antibodies (IgG fraction) to apolipoproteins B and E. In a typical experiment shown in Figure 1, treatment of culture medium with anti-ApoB resulted in a complete precipitation of ApoB but only a partial coprecipitation (38%) of ApoE. Apolipoproteins A-I, A-II, and C-III were quantitatively recovered in the supernatant fraction. This result suggested that, of all apolipoproteins present in the culture medium, only ApoE was associated with ApoB. To establish the proportion of ApoB associated with ApoE, another aliquot of culture medium was treated with antibodies to ApoE (Figure 1). Analysis of apolipoproteins in the supernate revealed that while approximately 28% of total ApoB was associated with ApoE in LP-B:E, 72% was present as LP-B. This conclusion was supported by the previous immunoprecipitation experiment which showed that only ApoE coprecipitated partially with ApoB and by double-diffusion analysis of the supernatant fraction showing a nonidentity reaction between ApoB and apolipoproteins A-I, A-II, and C-III. Several experiments showed (Table III, experiments 1 and 2) that ApoB accounted for 70% and ApoE for 30% of the total apolipoprotein content of LP-B:E particles. However, in some experiments (Table III, experiment 3) the ApoB content was as high as 85%. Double-diffusion analyses of major lipoprotein density classes indicated that LP-B particles occurred in VLDL, LDL, and HDL, while LP-B:E particles were only present in VLDL and LDL (Figure 4). In contrast, LP-E particles were present mainly in HDL. Immunoprecipitation experiments corroborated and expanded the results of immunodiffusion analyses by demonstrating the proportion of LP-B and LP-B:E particles in the culture medium and by establishing the ApoB and ApoE composition of LP-B:E.

Isolation of ApoB-Containing Lipoproteins by Immunoaffinity Chromatography. Although immunoprecipitation was shown to be a useful procedure for identifying and quantifying various types of lipoprotein particles, immunoaffinity chromatography was a more suitable method for

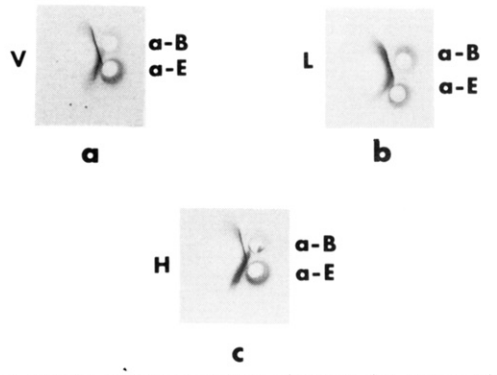

FIGURE 4: Double-diffusion analysis of VLDL (V, pattern a), LDL (L, pattern b), and HDL (H, pattern c) isolated from HepG2 cell culture medium by sequential ultracentrifugation with antisera to apolipoproteins B and E.

Table IV: Reactivity of Monoclonal Antibodies to ApoB (D_6) and ApoE (EfD_3) with Human Plasma Apolipoproteins and Lipoproteins

apolipoproteins and lipoproteins	optical density (405 nm)[a] anti-ApoB antibody (D_6)	anti-ApoE antibody (EfD_3)
ApoB	0.194[b]	NS[c]
ApoE	NS	0.864
chylomicrons	1.250	ND[d]
VLDL	1.014	1.004
LDL_1	1.020	ND
LDI_2	1.270	0.985
HDL	NS	0.898

[a] Peroxidase color reagent [2,2′-azinobis(3-ethylbenzothiazoline-sulfonate)] was oxidized by the oxygen generated from H_2O_2 upon incubation with peroxidase. The intensity of the green color developed by this reaction was measured at 405 nm with micro-ELISA reader MR 580. [b] Mean optical density at 405 nm (n = 3). [c] NS indicates that the optical density readings are less than 0.100; these readings are considered insignificant. [d] ND, not determined.

their isolation and purification. To obtain sufficient amounts of lipoprotein particles for their further characterization, we produced monoclonal antibodies to ApoB and ApoE and prepared corresponding immunosorbers.

The monoclonal antibody to ApoB described earlier as "pan B" antibody (Koren et al., 1986a,b) was used in the present study. This antibody was shown to bind to water-soluble, denaturant-free ApoB (Lee et al., 1981) and no other apolipoprotein or albumin. A comparable binding to chylomicrons, VLDL, and LDL (Table IV) indicated an equal capacity of this antibody to react with all ApoB-containing lipoproteins. The monoclonal antibody to ApoE only reacted with ApoE and showed a comparable binding capacity for all major lipoprotein density classes (Table IV). Coupled to Affi-Gel 10, these antibodies allowed complete removal of ApoB- and ApoE-containing lipoproteins, respectively. This applied equally to whole plasma, VLDL, LDL, and HDL. Apolipoproteins A-I, A-II, C-I, C-II, C-III, and D gave insignificant optical density readings (less than 0.100) with both antibody D_6 and antibody EfD_3.

The first step in the separation and isolation of LP-B and LP-B:E particles consisted in applying the HepG2 cell culture medium to the immunosorber with monoclonal antibodies to ApoB in order to remove all ApoB-containing lipoproteins. The fractionation of lipoprotein particles was monitored by double-diffusion analyses and electroimmunoassay. The unretained fraction contained apolipoproteins A-I and A-II and a part of ApoE not associated with the ApoB-containing lipoproteins. The retained fraction containing ApoB and ApoE was then applied to the immunosorber with monoclonal antibodies to ApoE. A single passage over the column was

Table V: Neutral Lipid and Apolipoprotein Composition of ApoB-Containing Lipoproteins Isolated from HepG2 Cell Medium by Immunoaffinity Chromatography

lipoprotein particles	experiment	apolipoproteins (%)		neutral lipids (%)		
		B	E	triglycerides	cholesterol	cholesterol esters
LP-B:E	1	70.5[a]	29.5	65.2	19.2	15.6
LP-B:E	2	80.0	20.0		NA[b]	
LP-B:E	3	80.0	20.0	69.3	13.1	17.6
LP-B:E	4	90.0	10.0	56.4	26.5	17.1
LP-B:E	5	NA	NA	63.2	17.6	19.2
LP-B:E	6	NA	NA	62.2	16.2	21.5
LP-B	1	100	ND[c]	71.1	13.6	15.3
LP-B	2	100	ND		NA	
LP-B	3	100	ND	68.4	12.1	19.5
LP-B	4	100	ND	71.1	14.3	14.6

[a] The concentrations of ApoB and ApoE in the culture medium were in the range of 0.24–0.47 and 0.07–0.15 mg/100 mL, respectively. [b] NA, not analyzed due to insufficient amount of sample. [c] ND, not detectable.

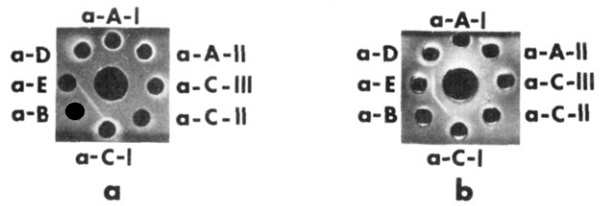

FIGURE 5: Double-diffusion analysis of LP-B (pattern a) and LP-B:E (pattern b) particles with antisera to apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E.

sufficient to retain quantitatively LP-B:E particles. Thus, the retained fraction contained LP-B:E, and the unretained fraction consisted of LP-B particles.

Analysis of LP-B by double immunodiffusion only showed a positive reaction with antibodies to ApoB (Figure 5, pattern a). This was confirmed by electroimmunoassay, which gave negative reactions with antisera to apolipoproteins A-I, A-II, and E but a positive reaction with anti-ApoB serum. The gradient SDS–polyacrylamide gel electrophoresis of LP-B (data not shown) only showed a single band typical of ApoB-100 which is the only form of ApoB secreted by HepG2 cells (Dashti et al., 1986). The neutral lipid composition of LP-B particles (Table V) was characterized by high content of triglycerides (70%) and almost equal percentages of cholesterol (12–14%) and cholesterol esters (14–19%). Electron microscopy of LP-B revealed spherical particles ranging in size from 100 to 350 Å (Figure 6, pattern a). Analysis of the LP-B size distribution showed that 40% of particles had diameters between 100 and 200 Å, 50% had diameters between 200 and 300 Å, and 10% of all particles had an average diameter of 350 Å. These results agree with the density distribution of LP-B particles which was shown to span from VLDL to HDL regions (Figure 4).

On double diffusion, LP-B:E particles gave a reaction of complete identity when tested with antisera to ApoB and ApoE (Figure 5, pattern b). The protein moiety of LP-B:E estimated by electroimmunoassay (Table V) consisted of 70–90% of ApoB and 10–30% of ApoE. Similar values were obtained for LP-B:E particles isolated by immunoprecipitation. No other apolipoproteins were detected by either double-diffusion analysis (Figure 5, pattern b) or electroimmunoassay. On gradient SDS–polyacrylamide gel electrophoresis (data not shown) LP-B:E particles displayed a slow-moving band characteristic of ApoB-100 and a fast-moving band of ApoE. The neutral lipid composition of LP-B:E particles (Table V) was also characterized by a high percentage of triglycerides (63%) and relatively low but similar percentages of cholesterol (19%) and cholesterol esters (18%). LP-B:E particles were shown by electron microscopy to represent a relatively wide distribution of spherical particles ranging in size from 220 to

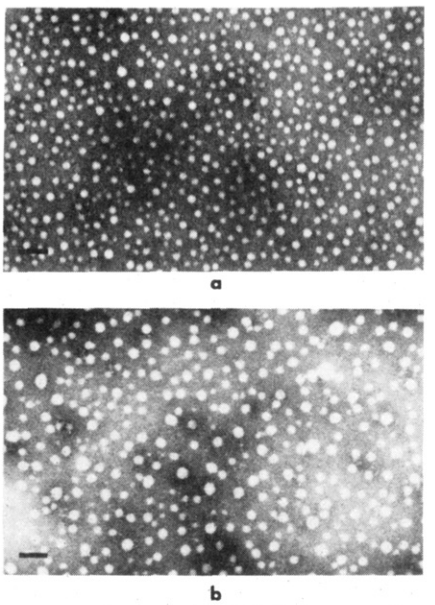

FIGURE 6: Electron micrographs of LP-B (pattern a) and LP-B:E (pattern b) particles.

500 Å (Figure 6, pattern b). Approximately 40% of all particles had diameters between 200 and 300 Å, 40% had diameters between 300 and 400 Å, 18% had diameters between 400 and 500 Å, and 2% of all particles had an average diameter of 500 Å. These results agree with the immunological detection of LP-B:E particles in VLDL and LDL regions of the density spectrum (Figure 4).

To establish whether or not there was a precursor–product relationship between LP-B:E and LP-B particles, the total ApoB and ApoB associated with ApoE were measured in the cell culture supernatant fractions at various time intervals. In case of a hypothetical precursor–product relationship, all of the ApoB secreted in the form of LP-B:E particles would be converted, presumably by lipolytic degradation, into LP-B particles. Thus, the accumulation of total ApoB in the culture medium should coincide, at least in the early stages of incubation, with the accumulation of LP-B:E. However, the measurement of total ApoB and ApoB associated with ApoE by micro-ELISA assays failed to provide evidence for such relationship. As shown in Figure 7, the accumulation of LP-B rose sharply over the period of the first 8 h and reached a plateau approximately 10 h after the beginning of incubation. On the other hand, ApoB associated with ApoE in the form of LP-B:E particles accumulated at a much slower but constant rate. These results excluded LP-B:E as a major source of LP-B and indicated that LP-B particles are secreted independently of LP-B:E particles.

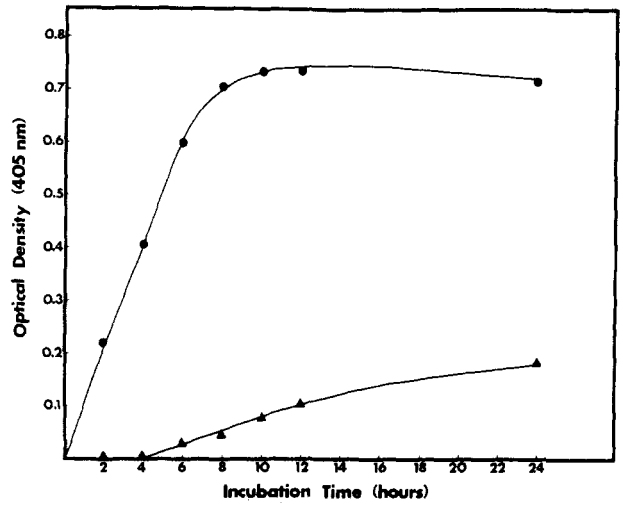

FIGURE 7: Secretion of LP-B (●) and LP-B:E (▲) particles as a function of incubation time.

The absence of lipolytic activities in the culture medium after 2, 4, 7, and 24 h of incubation provides further evidence that LP-B particles cannot be generated by the lipolytic degration of LP-B:E.

DISCUSSION

Evidence presented in this report shows that, in the culture medium of the human hepatoma cell line HepG2, ApoB and ApoA (A-I + A-II) form and constitute the two major populations of lipoprotein particles. In contrast to a sharp differentiation between these two lipoprotein groups, ApoE occurs in the form of three distinct populations of lipoprotein particles, one of which is a part of the ApoB-containing and the other a part of the ApoA-containing lipoproteins: the third subpopulation consists of particles with ApoE as the sole protein moiety. The available immunochemical and analytical evidence suggests that, in the culture medium, ApoC-III is not associated with ApoB-containing lipoproteins but occurs as a separate population of lipoprotein particles. It is quite possible that a part of ApoC-III may be associated with ApoA-containing particles. However, due to very low concentrations of ApoC peptides in the culture medium, their exact distribution in lipoprotein particles remains to be established by the use of a more sensitive analytical methodology.

Fractionation of culture medium lipoproteins into operationally defined "low-" and "high-density" lipoproteins by a single ultracentrifugation at $d = 1.063$ g/mL failed to result in the separation of ApoB- and ApoA-containing lipoproteins, because approximately 20% of ApoB-containing particles had hydrated densities greater than 1.063 g/mL and 10% of ApoA-containing lipoproteins occurred at densities lower than 1.063 g/mL. However, a complete separation of these two major groups of lipoproteins was accomplished by immunoprecipitation or immunoaffinity chromatography with antibodies to ApoB. A discriminate use of either of these two procedures revealed that ApoB-containing lipoproteins accumulating in the culture medium of HepG2 cells consist of two distinct types of lipoprotein particles, one of which contains ApoB and ApoE and the other containing only ApoB as their protein constituents. No immunological evidence was found for the occurrence of ApoB-containing lipoprotein particles with apolipoproteins A-I, A-II, C-I, C-II, C-III, or D as the additional protein constituents unless such lipoprotein particles were present at concentrations below the detection levels measurable by immunodiffusion and electroimmunoassay tests (5–10 μg/sample). A time study on the secretion of ApoB-containing particles showed that LP-B is not a degradation product of LP-B:E. A similarity in the lipid and protein composition of LP-B and LP-B:E particles isolated by immunoprecipitation and immunoaffinity chromatography indicates that neither of these two particle types is an isolation artifact generated by either method.

Electron microscopic and ultracentrifugal characterization of LP-B and LP-B:E particles showed that both lipoprotein species represent polydisperse systems of spherical particles heterogeneous with respect to the size and hydrated density but homogeneous with respect to the protein constituent(s). Thus, LP-B may be regarded as a distribution of particles ranging in size from 100 to 350 Å and in hydrated density from $d < 1.006$ g/mL to $d > 1.063$ g/mL with each particle containing ApoB as the sole protein. Similarly, LP-B:E particle distribution ranges in size from 200 to 550 Å and in hydrated density from $d < 1.006$ g/mL to $d = 1.063$ g/mL, with each particle having ApoB and ApoE as integral components of its protein moiety. Under the present experimental conditions, triglycerides were the major neutral lipid in both types of secreted ApoB-containing lipoproteins, accounting for the relatively large diameters and low hydrated densities of the majority of particles. The low values for cholesterol ester: cholesterol ratios may be ascribed to and explained by the apparent absence of LCAT activity in the culture medium (Koren et al., 1985). The physilogical conditions and factors determining and regulating the lipid composition of LP-B and LP-B:E particles remain to be established in the future.

So far, the formation and secretion of hepatic lipoproteins have been studied mainly in the rat by liver perfusion systems (Marsh, 1971, 1974; Hamilton et al., 1976; Dolphin et al., 1978; Marsh & Sparks, 1979; Noel et al., 1979; Dolphin, 1981), isolated hepatic Golgi apparatus (Mahley et al., 1969; Nestruck & Rubinstein, 1976; Swift et al., 1980), and cultured hepatocytes (Davis et al., 1979; Dashti et al., 1980; Bell-Quint & Forte, 1981; Haagaman & Van Golde, 1981). The general consensus appears to be that the newly secreted rat lipoproteins have density characteristics of VLDL and HDL particles with only a minor contribution, if any, by the LDL particles. However, the synthesis and secretion of LDL-like particles have been demonstrated in studies with isolated perfused liver from pig (Nakaya et al., 1977), guinea pig (Guo et al., 1982), nonhuman primates (Johnson et al., 1983; Jones et al., 1984), and cholesterol-fed rats (Noel et al., 1979; Dolphin, 1981; Swift et al., 1980). Although it was suggested (Guo et al., 1982; Jones et al., 1984) that some of these particles may represent the trapped plasma LDL washed out during the initial perfusion period, most particles, especially in cholesterol-fed animals, seem to be products of a de novo hepatic synthesis of LDL. In comparison with the corresponding plasma VLDL, the newly synthesized and secreted VLDL particles were shown to be rich in ApoB and ApoE and deficient in ApoC peptides (Hamilton et al., 1976; Dolphin et al., 1978; Noel et al., 1979; Nestruck & Rubinstein, 1976; Davis et al., 1979; Haggaman & Van Golde, 1981). By using immunochemical analyses, Dolphin et al. (1978) were the first to show that VLDL from normal rat perfusate only consisted of a complex lipoprotein with apolipoproteins B, C, and E as integral components of its protein moiety. In contrast, the VLDL isolated from liver perfusate of cholesterol-fed rats only contained the LP-B:E particles (Dolphin et al., 1978; Dolphin, 1981). The percentage composition of ApoB (78–85%) and ApoE (15–22%) in VLDL of secretory vesicles (Dolphin, 1981) was within the range of values characteristic for these two apolipoproteins in LP-B:E particles secreted by HepG2 cells. These results were con-

firmed by Davis et al. (1979), who provided immunochemical evidence that VLDL secreted by hepatocytes from normolipidemic rats consisted of LP-B:E rather than LP-B:C:E particles; the failure to detect ApoC peptides was ascribed to the apparently very low levels of these apolipoproteins in nascent VLDL. Like LP-B:E particles isolated from the HepG2 culture medium, LP-B:E particles secreted by rat hepatocytes had a low concentration of cholesterol esters and a high concentration of triglycerides (Davis et al., 1979). In contrast to the well-documented presence of LP-B:E particles in nascent VLDL secreted by rat hepatocytes or perfused liver, the occurrence of newly secreted LP-B particles has only been reported in the HDL isolated from rat liver perfusate (Fainaru et al., 1977); in this study the LP-B particles could not be sparated from particles containing ApoA-I and ApoE by ultracentrifugation or gel permeation chromatography but could only be separated by affinity chromatography or concanavalin A–Sepharose. The lipid composition of LP-B was not reported (Fainaru et al., 1977). One of the possible explanations for difficulties in isolating LP-B particles may be that in all reported studies rat lipoproteins were fractionated by ultracentrifugation and/or gel filtration, procedures that are inadequate for separating and identifying lipoprotein particles characterized by similar hydrated densities and sizes but different apolipoprotein composition. This also applies to studies with the perfused livers from pig (Nakaya et al., 1977) and nonhuman primates (Johnson et al., 1983; Jones et al., 1984).

There are some important differences in the composition and source of ApoB-containing lipoprotein particles between the HepG2 cell culture medium and normal human plasma. The major ApoB-containing lipoprotein in human plasma is LP-B (Lee & Alaupovic, 1974, 1970); Although present mainly within the density range 1.019–1.063 g/mL (Lee & Alaupovic, 1974), LP-B particles have also been detected in the HDL (Kostner & Alaupovic, 1972; Kostner, 1972) and VLDL regions.[2] Whereas LP-B particles detected in the culture medium are distinct secretory products of HepG2 cells, plasma LP-B particles seem to be mainly products of the lipolytic degradation of ApoB-containing triglyceride-rich VLDL (Deckelbaum et al., 1979; Alaupovic et al., 1986). Most of the plasma ApoB-containing lipoproteins with densities <1.019 g/mL are associated with ApoE and/or ApoC peptides (Osborne & Brewer, 1977; Alaupovic, 1972; Alaupovic et al., 1972, 1986; Lee & Alaupovic, 1974; Curry et al., 1976b; Fellin et al., 1974; Patsch et al., 1975). However, similarly to various rat liver model systems, there is a virtual absence in culture medium of LP-B and LP-B:E particles associated with ApoC peptides. The occurrence in human plasma of LP-B:E particles has been suggested by several investigators (Koren et al., 1983; Castro & Fielding, 1984; Gibson et al., 1984), but a detailed characterization of these particles has not yet been reported. It appears that one of the main reasons for these compositonal differences is the lack of interaction in the culture medium between the triglyceride-rich LP-B and LP-B:E particles and ApoC peptides. In plasma, the corresponding newly secreted LP-B and LP-B:E particles interact with ApoC peptides, most of which are derived from ApoA-I- and ApoA-II-containing lipoproteins of high densities, to form LP-B:C and LP-B:C:E particles. By acquiring ApoC peptides, the transformed triglyceride-rich, hepatic lipoproteins are compositionally suitable to undergo hydrolysis catalyzed by lipoprotein lipase. Lipolytic degradation of these particles results in the dissociation of cholesterol- and phospholipid-rich ApoC peptides and ApoE and the formation of cholesterol ester rich LP-B particles (Eisenberg et al., 1978; Tam & Breckenridge, 1983; Alaupovic et al., 1986).

In summary, results of this study have shown that, in the culture medium of HepG2 cells, apolipoproteins A-I, A-II, B, and E are the major apolipoproteins. ApoC peptides and ApoD are present in very small amounts. ApoB-containing lipoproteins consist of two polydisperse populations of spherical LP-B and LP-B:E particles, both of which are characterized by triglycerides as the main lipid component. These two fundamental, hepatic ApoB-containing lipoproteins differ from the corresponding plasma lipoproteins mainly with respect to the content of ApoC peptides and the ratio of triglycerides and cholesterol esters. Their specific functional roles in the secretion and transport of lipids remain to be established.

ACKNOWLEDGMENTS

We thank Jim Fesmire and Randy Whitmer for technical assistance and Julie Wen for assistance with electron microscopy. We also thank Anne Harris-Boxeur for artwork and for preparation of the manuscript.

Registry No. Cholesterol, 57-88-5.

[2] Sequential immunoprecipitation of normolipidemic and some hyperlipoproteinemic VLDL with antisera to apolipoproteins E, C-III, and C-II showed the presence of complex lipoproteins LP-B:C-I:C-II:C-III and LP-B:C-I:C-II:C-III:E and simple lipoproteins LP-C-III and LP-B as major types of lipoprotein particles (Alaupovic, 1984b).

REFERENCES

Alaupovic, P. (1972) *Protides Biol. Fluids 19*, 9–19.

Alaupovic, P. (1984a) in *Clinical and Metabolic Aspects of High-Density Lipoproteins* (Miller, N. E., & miller, G. J., Eds.) pp 1–44, Elsevier, Amsterdam.

Alaupovic, P. (1984b) presented at the IVth Pan-American Congress of Biochemistry, Buenos Aires, Argentina, November 4–8, 1984; Abstract 145.

Alaupovic, P., Lee, D. M., & McConathy, W. J. (1972) *Biochim. Biophys. Acta 260*, 689–707.

Alaupovic, P., Wang, C.-S., McConathy, W. J., Weiser, D., & Downs, D. (1986) *Arch. Biochem. Biophys. 244*, 226–237.

Avrameas, S. (1969) *Immunochemistry 6*, 43–52.

Bell-Quint, J., & Forte, T. (1981) *Biochim. Biophys. Acta 663*, 83–98.

Bisgaier, C. L., & Glickman, R. M. (1983) *Annu. Rev. Physiol. 45*, 625–636.

Carlson, L. A., & Holmquist, L. (1982) *Clin. Chim. Acta 124*, 163–178.

Castro, G. R., & Fielding, C. J. (1984) *J. Lipid Res. 25*, 58–67.

Curry, M. D., Alaupovic, P., & Suenram, C. A. (1976a) *Clin. Chem. (Winston-Salem, N.C.) 22*, 315–322.

Curry, M. D., McConathy, W. J., Alaupovic, P., Ledford, J. H., & Popovic, M. (1976b) *Biochim. Biophys. Acta 439*, 413–425.

Curry, M. D., McConathy, W. J., & Alaupovic, P. (1977) *Biochim. Biophys. Acta 491*, 232–241.

Curry, M. D., Gustafson, A., Alaupovic, P., & McConathy, W. J. (1978) *Clin. Chem. (Winston-Salem, N.C.) 24*, 280–286.

Curry, M. D., McConathy, W. J., Fesmire, J., & Alaupovic, P. (1980) *Biochim. Biophys. Acta 617*, 503–513.

Curry, M. D., McConathy, W. J., Fesmire, J. D., & Alaupovic, P. (1981) *Clin. Chem. (Winston-Salem, N.C.) 27*, 543–548.

Dashti, N., McConathy, W. J., & Ontko, J. A. (1980) *Biochim. Biophys. Acta 618*, 347–358.

Dashti, N., Wolfbauer, G., Koren, E., Knowles, B., & Alaupovic, P. (1984) *Biochim. Biophys. Acta 794*, 373–384.

Dashti, N., Wolfbauer, G., & Alaupovic, P. (1985) *Biochim. Biophys. Acta 833*, 100–110.
Dashti, N., Lee, D. M., & Mok, T. (1986) *Biochem. Biophys. Res. Commun. 137*, 493–499.
Davis, R. A., Engelhorn, S. C., Pangburn, S. H., Weinstein, D. B., & Steinberg, D. (1979) *J. Biol. Chem. 254*, 2010–2016.
Deckelbaum, R. J., Eisenberg, S., Fainaru, M., Barenholz, Y., & Olivecrona, T. (1979) *J. Biol. Chem. 254*, 6079–6087.
Dolphin, P. J. (1981) *J. Lipid Res. 22*, 971–989.
Dolphin, P. J., Wong, L., & Rubinstein, D. (1978) *Can. J. Biochem. 56*, 673–683.
Eisenberg, S., Chajek, T., & Deckelbaum, R. (1978) *Pharmacol. Res. Commun. 10*, 729–738.
Fainaru, M., Felker, T. E., Hamilton, R. L., & Havel, R. J. (1977) *Metab., Clin. Exp. 26*, 999–1004.
Fellin, R., Agostini, B., Rost, W., & Seidel, D. (1974) *Clin. Chim. Acta 54*, 325–333.
Galfrē, G., & Milstein, C. (1981) *Methods Enzymol. 73*, 3–46.
Gibson, J. C., Rubinstein, A., & Brown, W. V. (1984) *Clin. Chem. (Winston-Salem, N.C.) 30*, 1784–1788.
Gordon, J. I., Bisgaier, C. L., Sims, H. F., Sachdev, O. P., Glickman, R. M., & Strauss, A. W. (1984) *J. Biol. Chem. 259*, 468–474.
Guo, L. S. S., Hamilton, R. L., Ostwald, R., & Havel, R. J. (1982) *J. Lipid Res. 23*, 543–555.
Haagaman, H. P., & Van Golde, L. M. G. (1981) *Arch. Biochem. Biophys. 208*, 395–402.
Hamilton, R. L., Williams, M. C., Fielding, C. J., & Havel, R. J. (1976) *J. Clin. Invest. 58*, 667–680.
Havekes, L., Van Hinsbergh, V., Kempen, H. J., & Emeis, J. (1983) *Biochem. J. 214*, 951–958.
Hoeg, J. M., Demosky, S. J., Jr., Edge, S. B., Gregg, R. E., Osborne, J. C., Jr., & Brewer, H. B., Jr. (1985) *Arteriosclerosis (Dallas) 5*, 228–237.
Illingworth, D. R., Lindsey, S., & Hagernenas, F. C. (1984) *Exp. Cell Res. 155*, 518–526.
Johnson, F. L., St. Clair, R. W., & Rudel, L. L. (1983) *J. Clin. Invest. 72*, 221–236.
Jones, L. A., Teramoto, T., Juhn, D. J., Goldberg, R. B., Rubenstein, A. H., & Getz, G. S. (1984) *J. Lipid Res. 25*, 319–335.
Knowles, B. B., Howe, C. C., & Aden, D. P. (1980) *Science (Washington, D.C.) 209*, 497–499.
Koren, E., Solter, D., Knight, C., & Alaupovic, P. (1983) *Arteriosclerosis (Dallas) 3*, 510a.
Koren, E., McConathy, W. J., Lacko, A. G., & Knowles, B. (1985) *Cancer Biochem. Biophys. 7*, 301–307.
Koren, E., Knight-Gibson, C., Wen, G., DeBault, L. E., & Alaupovic, P. (1986a) *Biochim. Biophys. Acta 876*, 101–107.
Koren, E., Solter, D., Lee, D. M., Reiner, Z., McConathy, W. J., Dashti, N., & Alaupovic, P. (1986b) *Biochim. Biophys. Acta 876*, 91–100.
Kostner, G. (1972) *Biochem. J. 130*, 913–917.
Kostner, G., & Alaupovic, P. (1972) *Biochemistry 11*, 3419–3428.
Kuksis, A., Myher, J. J., Marai, L., & Geher, K. (1975) *J. Chromatogr. Sci. 13*, 423–430.
Lee, D. M., & Alaupovic, P. (1970) *Biochemistry 9*, 2244–2252.
Lee, D. M., & Alaupovic, P. (1974) *Biochem. J. 137*, 155–167.
Lee, D. M., Valente, A. J., Kuo, W. H., & Maeda, H. (1981) *Biochim. Biophys. Acta 666*, 133–146.
Lee, D. M., Koren, E., Singh, S., & Mok, T. (1984) *Biochem. Biophys. Res. Commun. 123*, 1149–1156.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Mahley, R. W., Hamilton, R. L., & LeQuire, V. S. (1969) *J. Lipid Res. 10*, 433–439.
Marsh, J. B. (1971) in *Plasma Lipoproteins* (Smellie, R. M. S., Ed.) Biochem. Soc. Symp. No. 33, pp 89–98, Academic, London and New York.
Marsh, J. B. (1974) *J. Lipid Res. 15*, 544–550.
Marsh, J. B., & Sparks, C. E. (1979) *J. Clin. Invest. 64*, 1229–1237.
McConathy, W. J., & Alaupovic, P. (1976) *Biochemistry 15*, 515–520.
McConathy, W. J., Koren, E., Wieland, H., Campos, E. M., Lee, D. M., Kloer, H. U., & Alaupovic, P. (1985) *J. Chromatogr. 342*, 47–66.
Morris, K. M., Goldberger, G., Colten, H. R., Aden, D. P., & Knowles, B. B. (1982) *Science (Washington, D.C.) 215*, 399–400.
Nakaya, N., Chung, B. H., Patsch, J. R., & Taunton, O. D. (1977) *J. Biol. Chem. 252*, 7530–7533.
Nestruck, A. C., & Rubinstein, D. (1976) *Can. J. Biochem. 54*, 617–628.
Noel, S.-P., Wong, L., Dolphin, P. J., Dory, L., & Rubinstein, D. (1979) *J. Clin. Invest. 64*, 674–683.
Osborne, J. C., Jr., & Brewer, H. B., Jr. (1977) *Adv. Protein Chem. 31*, 253–337.
Patsch, J. R., Sailer, S., & Braunsteiner, H. (1975) *Eur. J. Clin. Invest. 5*, 45–55.
Rash, J. M., Rothblat, G. H., & Sparks, C. E. (1981) *Biochim. Biophys. Acta 666*, 294–298.
Schwartz, A. L., Fridovich, S. E., Knowles, B. B., & Lodish, H. F. (1981) *J. Biol. Chem. 256*, 8878–8881.
Swift, L. L., Manowitz, N. R., Dunn, G. D., & LeQuire, V. S. (1980) *J. Clin. Invest. 66*, 415–425.
Tam, S. P., & Breckenridge, W. C. (1983) *J. Lipid Res. 24*, 1343–1357.
Tam, S. P., Archer, T. K., & Deeley, R. G. (1985) *J. Biol. Chem. 260*, 1670–1676.
Wahl, P. W., Warnick, G. R., Albers, J. J., Hoover, J. J., Walden, C. E., Bergelin, R. O., Ogilvie, J. T., Hazzard, W. R., & Knopp, R. J. (1981) *Atherosclerosis (Shannon, Irel.) 39*, 111–124.
Wang, C.-S., Bass, H. B., Downs, D., & Whitmer, R. K. (1981) *Clin. Chem. (Winston-Salem, N.C.) 27*, 663–668.
Wolfbauer, G., & Dashti, N. (1983) *Arteriosclerosis (Dallas) 3*, 496a.
Zannis, V. I., Breslow, J. L., SanGiacomo, T. R., Aden, D. P., & Knowles, B. B. (1981) *Biochemistry 20*, 7089–7096.