Polarized secretion of newly synthesized lipoproteins by the Caco-2 human intestinal cell line

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Abstract Lipoprotein secretion by Caco-2 cells, a human intestinal cell line, was studied in cells grown on inserts containing a Millipore filter (0.45 μm), separating secretory products from the apical and basolateral membranes into separate chambers. Under these conditions, as observed by electron microscopy, the cells formed a monolayer of columnar epithelial cells with microvilli on the apical surface and tight junctions between cells. The electrical resistances of the cell monolayers were 250–500 ohms/cm^2. Both ^{14}C-labeled lipids and ^{35}S-labeled proteins were used to assess lipoprotein secretion. After a 24-hr incubation with [^{14}C]oleic acid, 60–80% of the secreted triglyceride (TG) was in the basolateral chamber; 40% of the TG was present in the $d < 1.006$ g/ml (chylomicron + VLDL) fraction and 50% in the $1.006 < d < 1.063$ g/ml (LDL) fraction. After a 4-hr incubation with [^{35}S]methionine, apolipoproteins were found to be major secretory products with 75–100% secreted to the basolateral chamber. Apolipoproteins B-100, B-48, E, A-I, A-IV, and C-III were identified by immunoprecipitation. The $d < 1.006$ g/ml fraction was found to contain all of the major apolipoproteins, while the LDL fraction contained primarily apoB-100 and apoE; the HDL ($1.063 < d < 1.21$ g/ml) fraction principally contained apoA-I and apoA-IV. Mn-heparin precipitated all of the [^{35}S]methionine-labeled apoB-100 and B-48 and a majority of the other apolipoproteins, and 80% of the [^{14}C]oleic acid-labeled triglyceride, but only 15% of the phospholipid, demonstrating that Caco-2 cells secrete triglyceride-rich lipoproteins containing apoB. Secretion of lipoproteins was dependent on the lipid content of the medium; prior incubation with lipoprotein-depleted serum specifically reduced the secretion of lipoproteins, while addition of both LDL and oleic acid to the medium maintained the level of apoB-100, B-48, and A-IV secretion to that observed in the control cultures. — **Traber, M. G., H. J. Kayden, and M. J. Rindler.** Polarized secretion of newly synthesized lipoproteins by the Caco-2 human intestinal cell line. *J. Lipid Res.* 1987. **28:** 1350–1363.

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Studies of the absorption of dietary lipids and lipid-soluble vitamins, their intracellular transport and secretion in lipoproteins in humans have been hampered by the lack of an adequate model system. A tissue culture model of intestinal cells has been difficult to develop as the cells must not only grow, but also differentiate in culture. Caco-2 cells, isolated from a human colon carcinoma (1), have been demonstrated to spontaneously differentiate in cell culture and to display the morphology and many of the characteristics of intact intestine. Caco-2 cells, as initially characterized by Pinto et al. (2), have alkaline phosphatase and sucrase-isomaltase activities at 50% of the levels observed in normal human intestine, and aminopeptidase at 10%. These authors also described the structural polarity of Caco-2 cells — microvilli on the apical surface and the presence of tight junctions — and concluded, based on the structural and functional differentiation pattern, that although Caco-2 cells were isolated from a tumor of the colon, the cells had characteristics of mature enterocytes. The ability of Caco-2 cells to synthesize and transport microvillar hydrolases was studied by Hauri et al. (3), who concluded that these integral membrane proteins were expressed in a polarized fashion, confined to the apical surface, as is typical of enterocytes in vivo. The polarized nature of Caco-2 cells has been confirmed physiologically, as Caco-2 cells possess a cAMP-regulated mucosal chloride conductance characteristic of secretory cells (4). Other investigators have used Caco-2 cells in studies of the suppression of sucrase activity (5), the sodium-dependent transport of phosphate (6), the characteristics of folic acid uptake (7), and the post-translational processing of microvillar hydrolases (8).

Caco-2, like other cell lines derived from transporting epithelia, form fluid-filled domes and have a ouabain-sensitive Na^+, K^+ ATPase (9). Dome formation by Caco-2 monolayers suggests that the cells are secreting electrolytes and water from the basolateral membrane, and that the junctions formed between the cells can prevent macromolecular transport across the cell monolayers. Thus, lipoprotein secretion out of the basolateral membrane

Abbreviations: BSA, bovine serum albumin; TCA, trichloroacetic acid; LPDS, lipoprotein-depleted serum; PAGE, polyacrylamide gel electrophoresis; TLC, thin-layer chromatography; FCS, fetal calf serum; VLDL, very low density lipoproteins; LDL, low density lipoproteins; TG, triglycerides; PL, phospholipids; CE, cholesteryl esters.

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might be restricted in cells grown on standard tissue culture dishes, although Hughes et al. (10) have reported that Caco-2 cells could synthesize and secrete apolipoproteins, which floated at lipoprotein densities and were characterized as lipoproteins by electron microscopy.

In order to study lipoprotein secretion by Caco-2 cells, we have cultured cells under conditions where secretion from the basolateral membrane is not restricted, by growing the cells on porous (0.45 μm) filters. This system was initially developed for MDCK cells (a kidney cell line) (11) to allow uptake of nutrients by cells from the basolateral surface, similar to nutrient uptake in vivo. The structural and functional polarization of MDCK (11, 12) and Caco-2 cells, as shown in these studies, is well conserved under these conditions. Another advantage to the filter units (available commercially) is that they allow additions to the apical side of the cells, and collection of secretory products from the basal chamber with minimal contamination of the added substance.

The purpose of the studies described in this report was to develop a model system for studying newly synthesized lipoproteins of human intestinal origin. We demonstrate that Caco-2 cells secrete lipoproteins that contain both newly synthesized lipids and apolipoproteins. By employing the filter chamber technique, we have determined that lipoproteins synthesized by Caco-2 cells are secreted from the basolateral surface and have many of the characteristics of typical lipoproteins. Caco-2 cells, grown on filter units, represent a useful model system for studying the characteristics and regulation of intestinal cell lipoprotein synthesis.

METHODS

Cell culture

Caco-2 cells (#HTB37; American Type Culture Collection, Rockville, MD) were cultured at 37°C with 5% CO_2 in Dulbecco's minimal essential medium (DMEM) (4.5 g glucose/l), 20 mM HEPES (pH 7.3), penicillin (200 U/ml), streptomycin (200 μg/ml), fungizone (5 mg/ml) (all from Irvine Scientific, Irvine, CA), polymixin (200 U/ml; GIBCO, Grand Island, NY) and 20% fetal calf serum (FCS; GIBCO). Stock cultures of cells between passages 23 and 40 were grown in 75-cm^2 flasks (Corning Glassworks, Corning, NY). When the cell layer became nearly confluent, the cells were disassociated with 0.5% trypsin and 0.2 g/ml EDTA (GIBCO), and replated at approximately 10^4 cells/cm^2 into new flasks. For experiments, 3 ml of medium containing 1–1.2 $\times$ 10^6 cells was plated onto presoaked Millicell-HA inserts (30 mm; Millipore, Bedford, MA) and placed in six-well dishes (35 mm; Falcon). The following day the chambers were rinsed three times and the medium was replaced, 1.5 ml on each side; subsequently, the medium was changed every 2–3 days. Although the cells were plated on the inserts at nearly confluent densities, the cells usually took 6 to 10 days to form a tight monolayer as assayed by electrical resistance (see below). All of the experiments described were performed using cells grown on inserts; all of the studies describing secretion to the apical versus basolateral sides compare the contents of medium obtained from inside the insert (apical) to that outside the insert (basolateral).

Measurement of electrical resistance

Electrical resistance was measured by applying an electrical potential across the Millicell insert membrane, essentially as described (11, 13). Electrodes were mounted on a ringstand, so that one pair of recording and current electrodes was placed in the medium inside the chamber and one pair outside of the insert, in the culture dish. The current required to effect a 3.3 mv potential difference was measured on a microammeter, and the resistance was calculated according to Ohm's law. When a resistance of 250 ohm/cm^2 was obtained (background resistance of the filter was $<$ 30 ohm/cm^2), cells were used for study of secretion to the apical versus basolateral sides of the cells. At this level of electrical resistance, the monolayers prevented the diffusion of ^{125}I-labeled bovine serum albumin (a gift from Dr. E. Croze, N.Y.U. Medical Center) (in 4 hr $<$ 1% diffused from one side to the other).

Electron microscopy

Cell layers typical of those used for analysis of secretion of lipids or proteins were washed in phosphate-buffered saline (Dulbecco's). The filter was detached from the holder and fixed with 2% glutaraldehyde (Polysciences, Warrington, PA) in 0.1 M sodium cacodylate buffer (pH 7.0) overnight at 4°C. The filters with attached cells were further processed by standard techniques. Ultrathin sections were cut with a Sorvall MT2B microtome (Dupont Instruments, Wilmington, DE) and examined with a Philips 301 electron microscope (Philips Electronic Instruments, Mahwah, NJ) at 80 kV.

Incorporation of [^{14}C]oleic acid into lipids

[1-^{14}C]Oleic acid (Amersham, Arlington Heights, IL) incorporation was studied by incubating Caco-2 cells with oleic acid substrate ([^{14}C]oleic acid bound to bovine serum albumin (BSA), 4:1, mol:mol, ratio) prepared as described (14), harvesting the medium from the apical and the basolateral chambers, and quantitating the ^{14}C content of the lipid fractions. In some experiments the cellular lipids and the lipids in the apical medium was also analyzed. Specifically, monolayers, washed once with medium, were incubated with the addition of oleic acid substrate (sp act 3.7 Ci/mol, 20 μCi/ml; 0.1 ml was used per ml of medium, except where other amounts are indicated) to the standard culture medium for 4, 20, or 24 hr, as indicated. Subsequently, the medium from the inside

and the outside of the inserts was collected, centrifuged at 400 *g* for 5 min to remove any cell debris, and aliquots were taken for analysis. The medium and the lipoprotein fractions (isolated as described below) were extracted with chloroform–methanol 2:1 and the lipids were quantitated.

Intracellular lipids were analyzed by washing the cells three times with buffer C (50 mM Tris, pH 7.4, 0.15 M NaCl, 2 mg BSA/ml), followed by three washes with buffer D (buffer C without BSA). The cells were then scraped off the inserts, centrifuged at 500 *g* for 5 min in buffer D, and resuspended; aliquots were taken for lipid analysis and for protein quantitation by the method of Lowry et al. (15).

To determine the distribution of incorporated [^{14}C]oleic acid into radioactive lipids, medium or cells were extracted with chloroform–methanol 2:1, the water layer was acidified, and an aliquot of the chloroform layer with added carrier lipids (authentic triolein, oleic acid, and cholesteryl oleate; Alltech Associates, Inc., Applied Science Labs, Deerfield, IL) was plated onto a thin-layer chromatography (TLC) plate (silica gel G; Fisher Scientific Co., Pittsburgh, PA). The TLC plate was developed in petroleum ether–ethyl ether–acetic acid 140:60:0.8, and the lipids were located by exposure to iodine vapor. The area corresponding to each lipid was placed in a scintillation vial with Betafluor (National Diagnostics, Sommerville, NJ) and counted. (When the substrate was analyzed by this protocol, less than 1% of the ^{14}C-counts were detected in the other lipid fractions.) The counts were corrected for efficiency of counting. The nmol or pmol of [^{14}C]oleic acid incorporated, based on the specific activity of the substrate, are reported.

Incorporation of [^{35}S]methionine

To study the secretion of newly synthesized proteins, Caco-2 cells were incubated with [^{35}S]methionine (500 Ci/mmol, New England Nuclear, Boston, MA). Serum and unlabeled methionine were removed from the cell layer, as well as from the outer chamber, by rinsing the inserts three times with serum-free, methionine-free MEM (Irvine Scientific), and incubating the cells with the last rinse for 30 min at 37°C. The medium was replaced with a total of 3 ml/chamber of methionine-free medium supplemented with 0.25% FCS and 125 μCi of [^{35}S]methionine/ml. Following incubation for 4 hr at 37°C with 5% CO_2, the medium from the apical and basolateral sides was collected separately and centrifuged (400 *g* for 5 min) to remove cellular debris. To inhibit proteolysis, 5 mM EDTA and 10 U/ml trasylol (aprotinin; FBA Pharmaceuticals, New York, NY) were then added. The distribution of apolipoproteins in lipoprotein fractions was analyzed immediately upon collection of the medium. The remaining medium was stored at −20°C in the presence of 2% FCS for later analysis by immunoprecipitation.

Isolation of lipoproteins by ultracentrifugation

Lipoproteins were isolated using the newly available TL100 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) as described by Dr. Herbert K. Naito of The Cleveland Clinic Foundation, Cleveland, OH (personal communication). Immediately upon collection, medium (as described above) was aliquoted (0.75 ml) into polycarbonate tubes (11 × 34 mm; Beckman Instruments, Inc.), and overlayered with 0.25 ml of dialysis buffer (0.15 M NaCl, 0.3 mM EDTA, pH 7.4). The density < 1.006 g/ml fraction was isolated by centrifuging the samples in a TLA-100.2 fixed-angle rotor (Beckman Instruments, Inc.) at 435,680 *g* for 5 min (chylomicron fraction for [^{14}C]oleic acid incorporation studies) or for 2.5 hr (chylomicrons and very low density lipoproteins), and the upper 0.25 ml collected by slicing the tubes with a Centritube Slicer (Beckman Instruments, Inc.). The bottom fraction was collected and the density was adjusted to 1.063 g/ml with solid KBr. The samples were then centrifuged at 435,680 *g* for 2.5 hr and the top ($1.006 < d < 1.063$ g/ml, LDL) and bottom fractions were recovered as described above. The density of the bottom fraction was adjusted to 1.21 g/ml with solid KBr and the samples were refrigerated overnight. The following day the samples were centrifuged at 435,680 *g* for 4 hr, then the $1.063 < d < 1.21$ g/ml (HDL) and $d > 1.21$ g/ml fractions were isolated by tube slicing. The entire procedure from the commencement of radiolabeling with [^{35}S]methionine to final isolation of the lipoproteins took a maximum of 30 hr.

Immediately upon isolation, the lipoproteins were precipitated with 20% trichloroacetic acid (TCA) after addition of 5 μg of BSA, as a nonspecific carrier, and refrigerated until all fractions had been isolated and precipitated. In addition, 50- to 100-μl aliquots of the initial apical and basolateral media were also precipitated with TCA for the analysis of total protein secretion. The TCA precipitates were washed three times by repeated centrifugation (1000 *g*) and resuspension in 5% TCA with unlabeled methionine. The 5% TCA was carefully removed, and the samples were resuspended in 40 μl of sample buffer. The pH was adjusted with Tris base (if necessary), and the samples were analyzed by PAGE (see below).

For the study of the effect of lipids and lipoproteins on the synthesis of apolipoproteins, lipoprotein-depleted serum (LPDS) was prepared from outdated pooled human plasma (acid citrate dextrose as anticoagulant), obtained from the N.Y.U. Medical Center Blood Bank. The plasma was set to a density of 1.21 g/ml, centrifuged at 100,000 *g* for 48 hr using an L5-65 ultracentrifuge (Beckman), and the lipoproteins were removed by tube slicing and discarded. The $d > 1.21$ g/ml fraction was dialyzed against dialysis buffer, clotted by the addition of thrombin, and sterilized by filtration with a 0.45 μm filter unit (Nalge, Rochester,

NY). LDL was isolated from Watanabe rabbit serum using the protocol described above for isolation of the 1.006 < d < 1.063 g/ml fraction. The LDL was dialyzed, sterilized by filtration, and used within 1 month of isolation. The protein contents were measured according to the method of Lowry et al. (15). The oleic acid bound to BSA (5 mM, 4.5:1, mol/mol, 0.1 ml added/ml of medium) for addition to medium was prepared identically to that of the radioactive substrate and sterilized by filtration.

Immunoprecipitation of apolipoproteins

This was performed essentially as described previously (16), except that the medium was not concentrated. Briefly, sodium dodecyl sulfate (SDS) was added to a final concentration of 0.8% to aliquots (generally 50–300 μl) of medium. The samples were boiled for 2 min and, after cooling, an equal volume of solution A (190 mM NaCl, 50 mM Tris, pH 7.4, 6 mM EDTA, 2.5% Triton X-100 (Sigma, St. Louis, MO), 10 U/ml trasylol) was added. After an overnight incubation at 4°C with the appropriate antibodies to human apolipoproteins (5–10 μl), and a 2-hr incubation at room temperature with protein A linked to Sepharose-CL4B beads (Sigma), the beads were washed four times with solution A, once in buffer D, and resuspended in 40 μl of sample buffer for analysis by PAGE (see below). The antibodies used were: rabbit anti-human apoB (Calbiochem Behring Corp., La Jolla, CA, as well as that generously provided by Dr. Michael Schotz of the VA Wadsworth Medical Center, Los Angeles, CA); rabbit anti-human apoA-I (Calbiochem Behring); goat anti-human apoE and rabbit anti-human A-II (generously provided by Drs. Richard Gregg and Bryan Brewer of the NIH, Bethesda, MD); rabbit anti-human apoA-IV (generously provided by Drs. Charles Bisgaier and Robert Glickman of Columbia University College of Physicians and Surgeons, New York, NY); and rabbit anti-human apoC-I and rabbit anti-human C-III (generously provided by Dr. Petar Alaupovic, Oklahoma Medical Research Foundation, Oklahoma City, OK).

Heparin-Mn precipitation

Precipitation of apoB-containing lipoproteins was performed as described by Warnick and Albers (17). Precipitation reagent (50 μl) (14 mg/ml of heparin and 0.92 M $MnCl_2$) was added to 0.5 ml of medium after the addition of 5 μl of plasma from a Watanabe rabbit. The sample was mixed and incubated at 4°C for 30 min, centrifuged at 10,000 *g* for 1 min, and the supernatant was transferred to a second tube for precipitation with TCA (see above) or for extraction with chloroform–methanol (see above). The Mn-heparin precipitate was dissolved in 20 μl of 1 M citrate, then reprecipitated by the addition of 1 ml of 0.92 M $MnCl_2$. The precipitate was washed three times by repeated centrifugation and resuspension of $MnCl_2$ solution before resuspension in sample buffer for PAGE analysis or extraction with chloroform–methanol 2:1.

Polyacrylamide gel electrophoresis

PAGE was performed essentially as described previously (16), according to the method of Laemmli (18). Samples were resuspended in sample buffer (10% SDS, 20% glycerol, 100 mM Tris, pH 7.3, 5% beta-mercaptoethanol, 0.05% bromophenol blue), boiled for 2 min, and run on polyacrylamide gels (gradient or a single concentration containing 0.1% SDS, as described in the figure legends) with a stacker gel of 4% polyacrylamide. Radioactive molecular weight standards used were purchased from BRL (Bethesda, MD). The gels were fixed (10% acetic acid in 30% methanol) and prepared for fluorography either by the method of Bonner and Laskey (19), or using Enhance (New England Nuclear) according to the manufacturer's instructions. The dried gels were exposed at −70°C using Kodak AR film (Eastman Kodak, Rochester, NY). Scanning of the autoradiograms was performed on a Hoefer Scientific densitometer, using a Perkin Elmer chart recorder. Relevant bands were cut from the chart paper and weighed on a Mettler balance.

RESULTS

Electron microscopy of Caco-2 cells

The morphology of a typical monolayer of Caco-2 cells cultured on cellulose filters and processed for electron microscopy is shown in **Fig. 1.** The tall epithelial cells formed a highly interdigitated, columnar monolayer with tight junctions between cells. As expected for cells having the properties of differentiated small intestine (2), microvilli were present on the apical surface of many cells, while the basal surface was attached to the filter support. Large translucent inclusions, which appear to be lipid droplets, were present in the cytoplasm of most cells.

Incorporation of [^{14}C]oleic acid and secretion of ^{14}C-labeled lipids

To determine whether Caco-2 cells could secrete newly synthesized lipids, cells were incubated with [^{14}C]oleic acid substrate added to the apical chamber; then the intracellular ^{14}C-labeled lipids and those in the basolateral medium were quantitated. **Table 1** shows the intracellular concentration of ^{14}C-labeled lipids. Cells incubated for 4 hr with [^{14}C]oleic acid incorporated the fatty acid into both phospholipids (PL) and triglycerides (TG), with lesser amounts incorporated into cholesteryl esters (CE); TG was the predominant form of the ^{14}C-labeled lipids secreted into the medium. Increasing the amounts of the [^{14}C]oleic acid substrate did not change this pattern of [^{14}C]oleic acid incorporation or secretion;

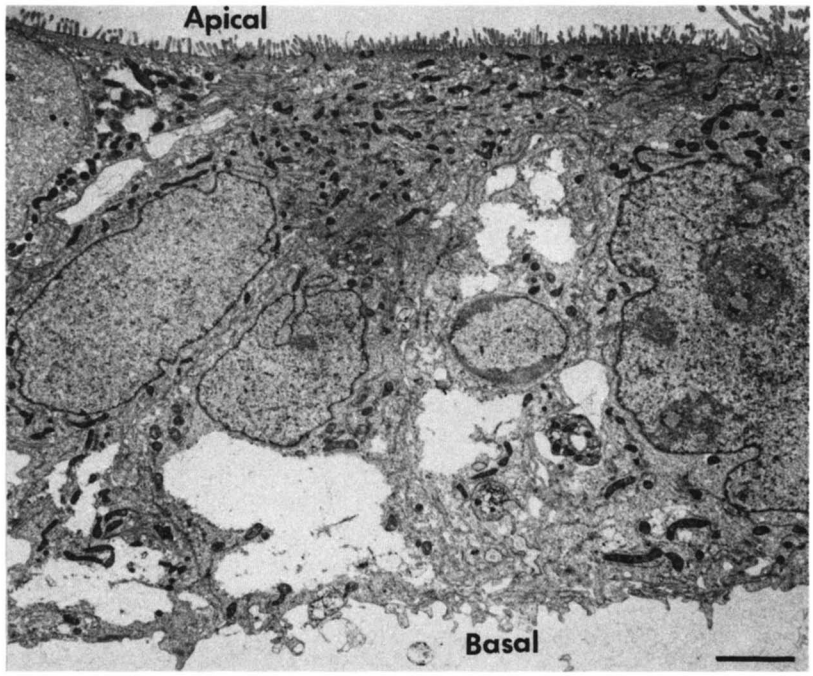

Fig. 1. Electron micrograph of Caco-2 cells grown on filters. Cells grown for 8 days on 0.45-μm filters with an electrical resistance of > 250 ohm/cm^2 were fixed and processed by standard techniques for electron microscopy as described in Methods. Note the microvilli at the apical membrane and the filter at the basal side below the cells. Bar = 2.7 μm.

therefore 3 μCi was chosen for the remaining experiments. As is shown in **Table 2,** a longer incubation with [^{14}C]oleic acid resulted in a 30% increase in the amount of TG recovered in the medium (4.8 vs. 3.5 nmol/mg). In this experiment, the [^{14}C]oleic acid substrate was removed after the first 24 hr, then the incubation was continued for a subsequent 24 hr. There was no appreciable difference in the rates of secretion of TG between days 1 and 2 (4.8 ± 1.4 nmol/mg protein and 3.8 ± 0.3 nmol/mg protein, respectively). Nor was the rate of lipid secretion high enough to result in a decrease in the cellular lipid content, since the cellular TG remained constant for the 2 days (Table 2). In all experiments, the fraction of the TG detected in the medium ranged from 3 to 8% of that present intracellularly.

TABLE 1. Effect of increasing amounts of substrate on the secretion of [^{14}C]oleic acid-labeled lipids by Caco-2 cells

	[^{14}C]Oleic Acid Incorporated					
	Cellular			Basolateral Medium		
Substrate	PL	TG	CE	PL	TG	CE
μCi	*nmol/mg protein*					
1.0	25.3	23.3	0.7	0.4	0.8	0.1
2.0	34.3	42.6	1.0	0.4	1.8	0.2
3.0	33.3	55.5	1.2	0.4	3.5	0.4

Following a 4-hr incubation with [^{14}C]oleic acid substrate, the lipids from Caco-2 cells grown on filters and from the medium collected from the basolateral chambers were extracted with chloroform–methanol 2:1, isolated by TLC, and quantitated as described in Methods. The averages of duplicate incubations are shown.

Intestinal cells take up dietary lipids at their luminal surface and secrete lipoproteins from the basolateral membrane. Given the polarized morphology and the biochemical properties of Caco-2 monolayers, it seemed likely that the secretion of lipids, presumably in the form of lipoproteins, might be polarized. Therefore, the content of [^{14}C]oleic acid-labeled lipids in the media from the apical and basolateral chambers of cell monolayers that had tight junctions, as demonstrated by their electrical resistance, was analyzed. As discussed above, both PL and TG are major intracellular products of [^{14}C]oleic acid incorporation. However, in four experiments, newly synthesized TG was preferentially secreted to the basolateral medium (60–80%), while equal amounts of PL were found in both the apical and basolateral medium (data not shown). These observations suggest that the TG were secreted in newly synthesized lipoproteins. Therefore, the ability of Caco-2 monolayers to secrete triglyceride-rich lipoproteins in a polarized fashion was investigated. The media from the apical and basolateral chambers of cells with electrical resistance, which had been incubated for

TABLE 2. Effect of duration of incubation on secretion of ^{14}C-labeled lipids by Caco-2 cells

	[^{14}C]Oleic Acid Incorporated					
	Cellular			Basolateral Medium		
Time	PL	TG	CE	PL	TG	CE
hr	*nmol/mg protein*					
24	39	125	1.1	0.6 ± 0.1	4.8 ± 1.4	0.2 ± 0.1
48	52 ± 2	145 ± 8	1.5 ± 0.1	1.0 ± 0.2	3.8 ± 0.3	0.1 ± 0.1

Confluent monolayers (6) of cells grown on filters were incubated with 3.0 μCi of oleic acid substrate for 24 hr, then the medium from six basolateral chambers and the cells from two inserts were collected, extracted, and analyzed. The remaining inserts were washed three times with medium containing 20% FCS, and incubated for an additional 24 hr without substrate before analysis. Shown are the cellular contents of ^{14}C-labeled lipids (24 hr, averages of two incubations, and 48 hr, means ± SD, n = 4) and the lipids secreted into the basolateral chamber (mean ± SD, n = 6 for 24 hr, and n = 4 for 48 hr).

24 hr with [^{14}C]oleic acid substrate, was collected. The medium from both chambers was immediately centrifuged for 5 min and the lipoproteins present in the d < 1.006 g/ml fraction (chylomicrons) were isolated and quantitated. **Fig. 2,** from a representative experiment, demonstrates that 76–82% of the lipids present in this lipoprotein fraction were secreted to the basolateral side.

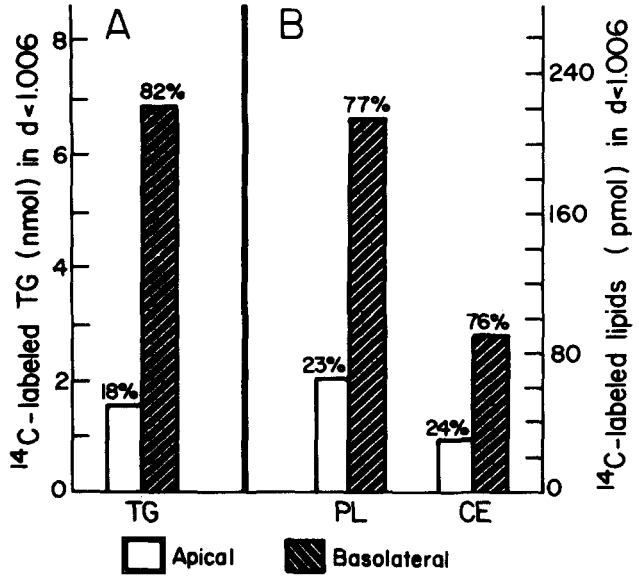

Fig. 2. Polarized secretion of triglyceride-rich lipoproteins. Caco-2 cells, grown on filters (with an electrical resistance of > 250 ohm/cm^2) were incubated with [^{14}C]oleic acid substrate for 20 hr. The medium was then collected from the apical and basolateral chambers. The lipoproteins in the d < 1.006 g/ml fraction were isolated by centrifugation at 436,680 *g* for 5 min and the lipids were extracted and analyzed as described in Methods. Panel A shows the TG (nmol) recovered in the d < 1.006 g/ml lipoprotein fraction and indicates the percentage found in the apical (empty bar) and basolateral (filled bar) chambers. In panel B, similar data are shown for PL and CE, with the units in pmol (right hand scale).

Distribution of ^{14}C oleic acid-labeled lipids in lipoprotein fraction

Inasmuch as the small intestine secretes triglyceride-rich lipoproteins and high density lipoproteins (20), the production of these lipoproteins by Caco-2 cells was investigated. The medium was collected from the basolateral chamber of six inserts containing cells incubated with [^{14}C]oleic acid substrate for 20 hr. Lipoproteins were immediately isolated either without altering the density of the medium (d 1.006 g/ml for 5 min) so that triglyceride-rich lipoproteins would float to the top of the tube, or by increasing the density of the medium to 1.21 g/ml and centrifuging for 4 hr such that all lipoproteins would float to the top. The d < 1.006 g/ml fraction of the medium, which encompasses the chylomicron particles, contained 64 ± 7% of the total TG and 68 ± 17% of the total CE, but only 11 ± 6% of the total PL. The d < 1.21 g/ml fraction, which includes all of the lipoproteins, contained 91 ± 8% of the total TG, 68 ± 19% of the total CE, and 67 ± 19% of the total PL.

The distribution of lipoproteins secreted by Caco-2 cells analyzed by sequential ultracentrifugation is depicted in **Table 3.** In this experiment, 42% of the total TG was present in the d < 1.006 g/ml fraction centrifuged for 2 hr, and 52% in the 1.006 < d < 1.063 g/ml fraction. The HDL fraction (1.063 < d < 1.21 g/ml) contained 6% of the total TG, but 18% of the total PL and 14% of the total CE. The distribution of newly synthesized lipids in these fractions is shown in **Fig. 3.** Both the d < 1.006 g/ml and the 1.006 < d < 1.063 g/ml (LDL) fractions contained 90% TG. In contrast, the 1.063 < d < 1.21 g/ml (HDL) fraction contained 63% TG and 34% PL. PL was the major form of labeled lipid in the d > 1.21 g/ml fraction. It should be noted that the free cholesterol content of the lipoproteins was not measured; therefore these are overestimates of the percent of each lipid in the lipoprotein fractions.

TABLE 3. Sequential isolation of ^{14}C-labeled lipids in lipoproteins secreted by Caco-2 cells

Density	TG	PL	CE
g/ml		%	
d < 1.006	42[a]	24	25
1.006 < d < 1.063	52	27	28
1.063 < d < 1.21	6	18	14
d > 1.21	<1	30	32

Caco-2 cells were incubated with [^{14}C]oleic acid substrate (3 μCi) for 20 hr. The medium from three inserts was pooled and the lipoproteins were isolated by sequential ultracentrifugation at 435,680 *g*, using the TL100A rotor in the TL100 ultracentrifuge with density adjustments made by the addition of KBr. Each lipoprotein fraction was obtained by tube slicing following ultracentrifugation for the indicated length of time: d < 1.006 (2 hr), 1.006 < d < 1.063 (2.5 hr), 1.063 < d < 1.21 (4 hr). The lipids were extracted from each fraction with chloroform–methanol 2:1, separated by TLC, and quantitated as described in Methods.

[a] Percentage distribution of each lipid in each fraction.

Polarized secretion of apolipoproteins

To characterize the proteins secreted by Caco-2 cells, medium was collected from the apical and basolateral chambers of cells incubated for 4 hr with [^{35}S]methionine, and the TCA-precipitable proteins were subjected to PAGE on a 7–13% gradient gel. As demonstrated by TCA precipitation of aliquots of medium from the apical and basolateral chambers, all of the proteins detectable in the medium were selectively secreted to the basolateral chamber (**Fig. 4,** compare lanes a and b). Immunoprecipitation of the media from both chambers using antibodies to human apolipoproteins documented that apolipoproteins were also secreted in a polarized fashion by Caco-2 cells (Fig. 4). Specifically, apoA-I (lanes c,d), apoE (lanes e,f), and apoA-IV (lanes g,h) were detected nearly exclusively in the basolateral medium. In contrast to adult human intestine which produces only apoB-48 (21), both apoB-100 and apoB-48 (lanes i,j) were synthesized by Caco-2 cells; these were primarily secreted into the basolateral chamber as well. Other major radiolabeled products included those at 400, 90, 53, and 23 kDa (lane b). The 400-kDa band is a major secretory product of these cells and was a frequent contaminant of immunoprecipitates, including control, nonimmune serum (data not shown).

The secretion of other apolipoproteins by the Caco-2 cells was also investigated. Apolipoproteins A-II and C-I were not detectable by our immunoprecipitation techniques, even when detergent was left out of the procedure (data not shown). However, apoC-III was detectable and, as shown in **Fig. 5,** appeared as a broad diffuse band by PAGE on a 12.5% gel. Since apoC-III required a separate gel for analysis, it was not specifically identified in the lipoprotein fractions described below.

Distribution of apolipoproteins in lipoprotein fractions

To characterize the lipoproteins secreted by Caco-2 cells, medium was collected from the basolateral chamber following a 4-hr incubation with [^{35}S]methionine. The medium was subjected to sequential ultracentrifugation to isolate the apolipoproteins that float at the densities of the major human lipoprotein classes. A typical experiment is shown in **Fig. 6,** in which half of the isolated lipoproteins, dissolved in sample buffer, was applied to a 7–13% gradient polyacrylamide gel (Fig. 6A) and the other half to a 6% gel (Fig. 6B) for better separation of the apoBs. Quantitation of the distribution of each of the apolipoproteins by densitometric scanning of the autoradiograms in Fig. 6 is shown in **Table 4.** The distribution of the two apoBs was different, a total of 98% of apoB-100 was found in the d < 1.006 g/ml and 1.006 < d < 1.063 g/ml fractions, while apoB-48 was nearly equally distributed in all of the lipoproteins. Most (68%) of apoA-IV was found in the d > 1.21 g/ml fraction, while the largest percentage (63%) of apoE was present in the 1.006 < d < 1.063 g/ml fraction, and the largest percentage (49%) of apoA-I was in the 1.063 < d < 1.21 g/ml fraction. The distribution of the 90-kDa protein, which was found primarily in the d > 1.21 g/ml fraction, is shown for comparison. This fraction (lipoprotein-depleted, Fig. 6 lane d) also contained apoA-I, A-IV, and E. Although the general background of the d > 1.21 g/ml fraction was high, very little apoB-100 was visible in this experiment or in five other similar trials. This result was more readily apparent when the medium was set to a density of 1.21 g/ml and fractions were analyzed directly (data not shown).

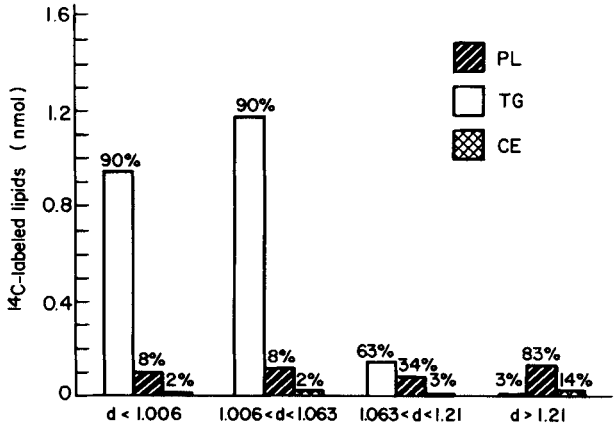

Fig. 3. Composition of ^{14}C-labeled lipids in lipoproteins. Lipoprotein fractions were isolated as described in Table 3 from Caco-2 cells grown on filters and incubated with [^{14}C]oleic acid substrate. The [^{14}C]oleic acid incorporated (nmol) into each lipid in the lipoprotein fraction is shown by the height of the bars. The percentage (%) of [^{14}C]oleic acid in TG, PL, and CE for each isolated lipoprotein fraction is noted above the bars.

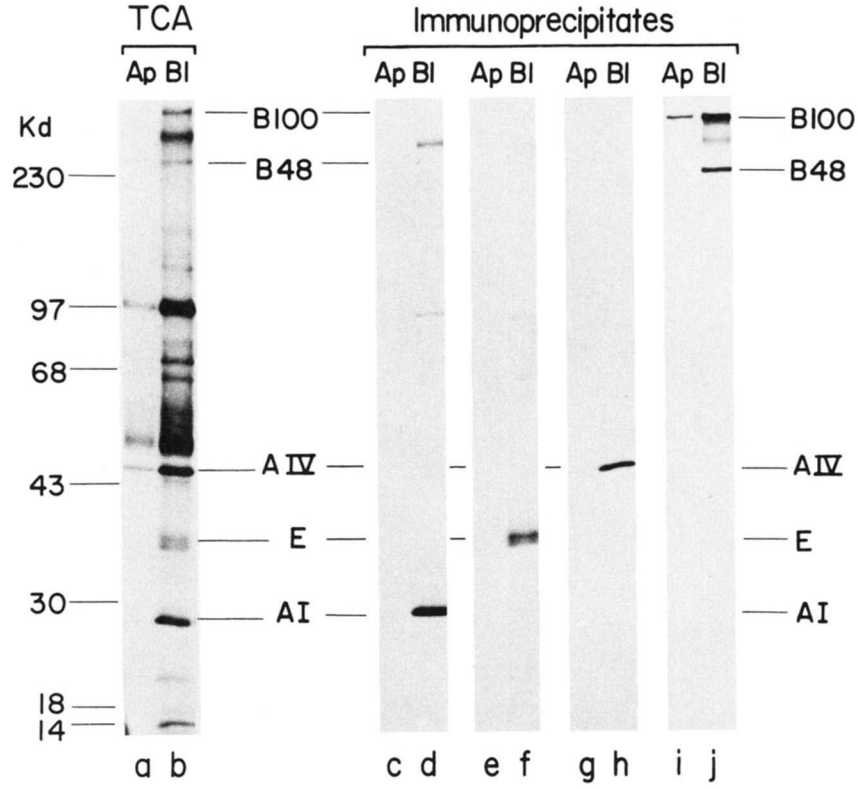

Fig. 4. Polarized secretion of apolipoproteins. Media was collected from the apical (Ap) or basolateral (Bl) chambers from Caco-2 cells (with electrical resistance > 250 ohm/cm^2) incubated with [^{35}S]methionine for 4 hr. Aliquots of the medium were precipitated with either TCA (lanes a and b), or with antibodies to human apolipoproteins: apoA-I (lanes c, d), apoE (lanes e, f), apoA-IV (lanes g, h), or apoB (lanes i, j), then subjected to PAGE on a 7–13% gradient gel as described in Methods. Lanes a, c, e, g, and i are samples from the apical medium, while lanes b, d, f, h, and j are the corresponding basolateral samples. The positions of molecular weight standards are noted on the left side of the figure.

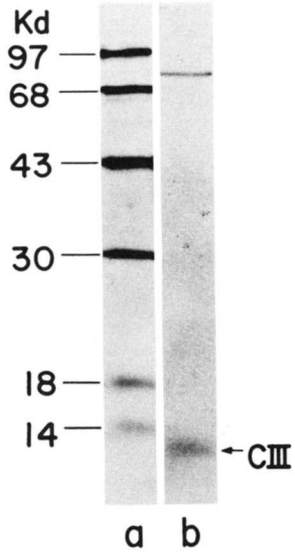

Fig. 5. Secretion of apoC-III. Medium was collected from the basolateral chamber from Caco-2 cells incubated with [^{35}S]methionine for 4 hr. After immunoprecipitation with antiserum to human apoC-III (lane b), the sample was subjected to PAGE on a 12.5% gel as described in Methods. Molecular weight standards were run in lane a.

The typical apolipoprotein composition of Caco-2 lipoproteins is shown in **Table 5.** The d < 1.006 g/ml (chylomicrons and VLDL) was found to contain 34% apoB-100, 17% apoA-IV, 20% apoE, and 27% apoA-I, along with the detectable amounts of apoB-48. In preliminary experiments the chylomicron fraction (isolated in a 5-min spin) also contained apoB-100. The 1.006 < d < 1.063 g/ml (LDL) fraction contained 57% apoB-100 and 30% apoE. The 1.063 < d < 1.21 g/ml fraction (HDL) contained 46% apoA-IV and 44% apoA-I with traces of apoE and B-48.

Mn-heparin precipitation of lipoproteins

To demonstrate that Caco-2 cells secrete intact lipoproteins, the apolipoprotein B-containing lipoproteins were precipitated with Mn-heparin reagent and the distribution of newly synthesized apolipoproteins and ^{14}C-labeled lipids was analyzed. Results typical of three separate experiments are shown in **Fig. 7.** When the medium from [^{35}S]methionine-labeled cells was analyzed, both apoB-100 and apoB-48 were absent from the supernatant, but apoA-IV, E, and A-I were present in both the precipitate (lane b) and the supernatant (lane a), consistent with

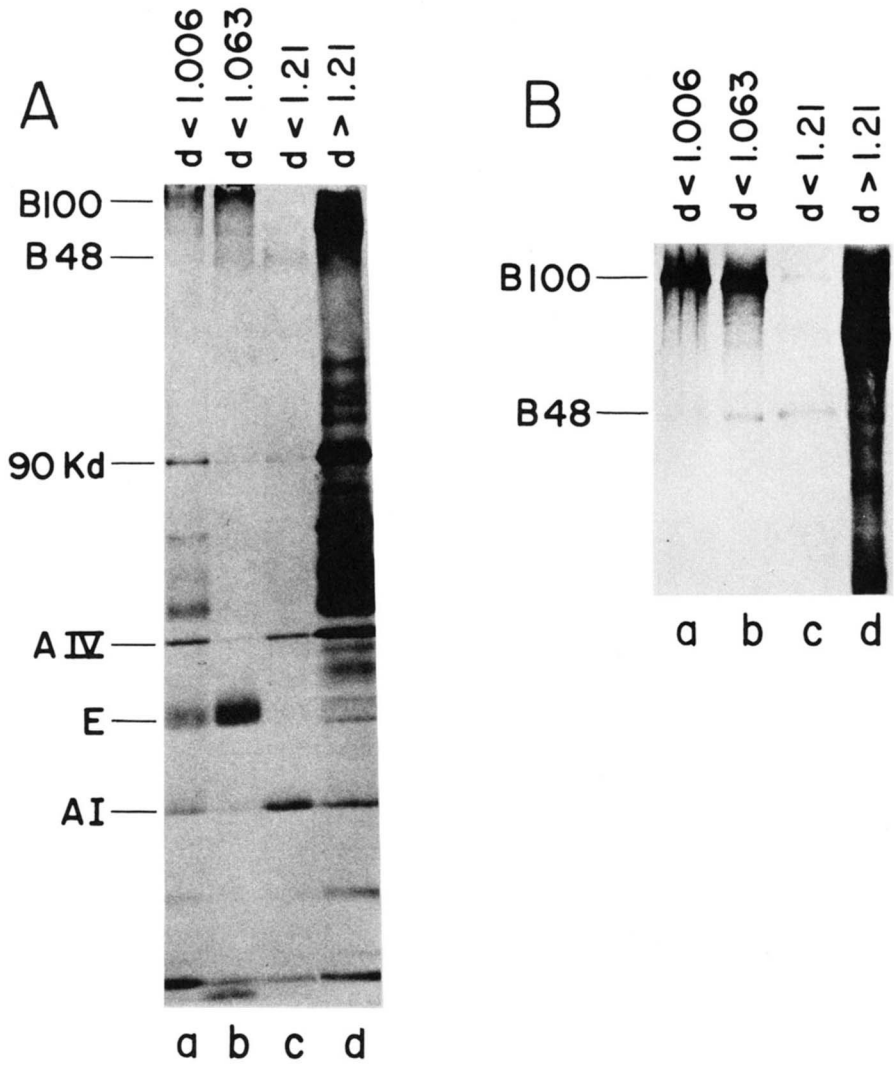

Fig. 6. Sequential isolation of lipoproteins secreted by Caco-2 cells. As described in detail in Methods, medium from cells incubated for 4 hr with [^{35}S]methionine was collected from the basolateral side of three inserts. Following addition of protease inhibitors, the lipoprotein fractions were isolated by sequential ultracentrifugation using a TL100A rotor in a TL100 ultracentrifuge with density adjustments made by the addition of KBr. Immediately upon completion of ultracentrifugation, the lipoprotein fractions were recovered by tube slicing and precipitated with 20% TCA. Following extensive washing, the precipitates were resuspended in sample buffer for analysis by PAGE; half of the sample was applied to a 7–13% gradient gel (panel A) or a 6% gel (panel B). Identification of the apolipoproteins was made by comparison to immunoprecipitated samples and to molecular weight standards. In each panel the lanes correspond to: a) d < 1.006 g/ml (2 hr at 435,680 *g*); b) 1.006 < d < 1.063 g/ml (2.5 hr at 435,680 *g*); c) 1.063 < d <1.21 g/ml (4 hr at 435,680 *g*); and d) d > 1.21 g/ml.

the presence of "HDL"-like particles in the supernatant. Addition of the Mn-heparin reagent to medium from cells that had been incubated with [^{14}C]oleic acid substrate resulted in the precipitation of 80% of the TG, but 85% of the PL remained in the supernatant. These results demonstrate that precipitation by Mn-heparin of the apoB-containing lipoproteins results in the simultaneous precipitation of the apoB- and TG-containing lipoproteins secreted by Caco-2 cells.

Regulation of lipoprotein secretion by lipids

Since the intestine secretes chylomicrons in response to uptake of dietary lipids, the response of Caco-2 cells to variations in the lipid content of the medium was investigated. Cells were preincubated for 40 hr in medium containing either 20% FCS or 10% LPDS (lipoprotein-depleted serum) with or without the addition of oleic acid (0.5 mM), or LDL (100 μg/ml), or both. As can be seen

TABLE 4. Sequential isolation of ^{35}S-labeled apolipoproteins in lipoproteins secreted by Caco-2 cells

Density	Apolipoproteins					
	B-100	B-48	A-IV	E	A-I	90 kDa
g/ml			%			
d < 1.006	48	18	18	26	22	3
1.006 < d < 1.063	50	28	2	63	5	<1
1.063 < d < 1.21	<1	38	13	2	49	1
d > 1.21	<1	17	68	9	24	95

The autoradiograms shown in Fig. 6 were analyzed by densitometric scanning and the distribution for each ^{35}S-labeled apolipoprotein into the lipoprotein fractions was calculated. The 90 Kd protein does not appear to be a lipoprotein and is included for comparison as a control.

from **Fig. 8,** preincubation with LPDS (lanes c and d) resulted in a marked specific decrease in apolipoprotein synthesis compared to cells incubated with FCS (lanes a and b). The specificity of this decrease in apolipoprotein synthesis was established from the fact that the secretion of other proteins (i.e., 90 kDa) was unaffected by the lipid content of the medium.

Quantitation by densitometric scanning of two separate experiments of the apolipoproteins secreted in response to the lipid content of the medium is shown in **Table 6.** For this analysis the amount of the 90-kDa protein was arbitrarily set to 100 to standardize for differences in the incorporation and recovery of [^{35}S]methionine (the minor variations in the quantity of this protein had no relation to the lipid content of the medium) and the amounts of the apolipoproteins in each incubation condition were adjusted accordingly. The percent of control level was calculated by dividing the amount of apolipoprotein secreted in response to the preincubation medium by the amount of the same apolipoprotein secreted in medium containing FCS. These data demonstrate that secretion of all of the apolipoproteins was decreased to 19–65% of that observed in the control (20% FCS) in response to preincubation with 10% LPDS. The addition of LDL to the medium resulted in the maintenance of apoA-IV at control levels, and apoB-48 and B-100 to 83% and 76% of control levels, respectively. In response to the addition of oleic acid to the medium, both B-48 and B-100 were maintained at control levels, but apoA-IV only reached 72% of control levels. Addition of both LDL and oleic acid maintained the levels of synthesis of apoA-IV, B-48, and B-100 at control levels, but apoA-I and E remained at ~50% of control. These results suggest that the secretion, and perhaps the synthesis, of apolipoproteins is regulated by the presence of exogenous lipid in the incubation medium. The amount of cholesterol in the medium appears to affect apoA-IV secretion, while apoB synthesis appears to be regulated by the presence of fatty acid.

DISCUSSION

Caco-2 cells, when grown on Millipore filters, exhibit physiological, morphological, and biochemical characteristics of small intestinal epithelia. In metabolic labeling experiments using [^{14}C]oleic acid or [^{35}S]methionine, cell monolayers synthesized and secreted lipoproteins, as well as a wide variety of ^{35}S-labeled protein products, including those at 400, 90, 53, and 23 kDa, which were exported from the basolateral surface. No radiolabeled products were found to be secreted preferentially from the apical domain. This polarized pattern of secretion is what would be expected from the intestine in vivo, since, aside from secretory component which is derived from a membrane-bound precursor by proteolytic cleavage (22), no proteins that are specifically secreted out of enterocytes via the brush border membrane have been identified.

Analysis of [^{14}C]oleic acid incorporation into cellular lipids indicated that the major intracellular products were PL and TG, with a low level of CE synthesis. In the medium however, PL was present at only 10% of the level of TG, which was the major secretory product, while CE was barely detectable. Thus Caco-2 cells, like the liver-derived cell line HepG2 (23, 24), have a very limited ability to

TABLE 5. Composition of ^{35}S-labeled apolipoproteins in lipoproteins secreted by Caco-2 cells

Apolipoprotein	Density		
	d < 1.006 g/ml	1.006 < d < 1.063 g/ml	1.063 < d < 1.21 g/ml
		%	
B-100	34	57	2
B-48	2	7	4
A-IV	17	2	46
E	20	30	4
A-I	27	3	44

The [^{35}S]methionine composition of apolipoproteins in each lipoprotein fraction, prepared by sequential ultracentrifugation as described in Fig. 6, is shown. The data were obtained by densitometric scanning of an autoradiogram of a typical experiment.

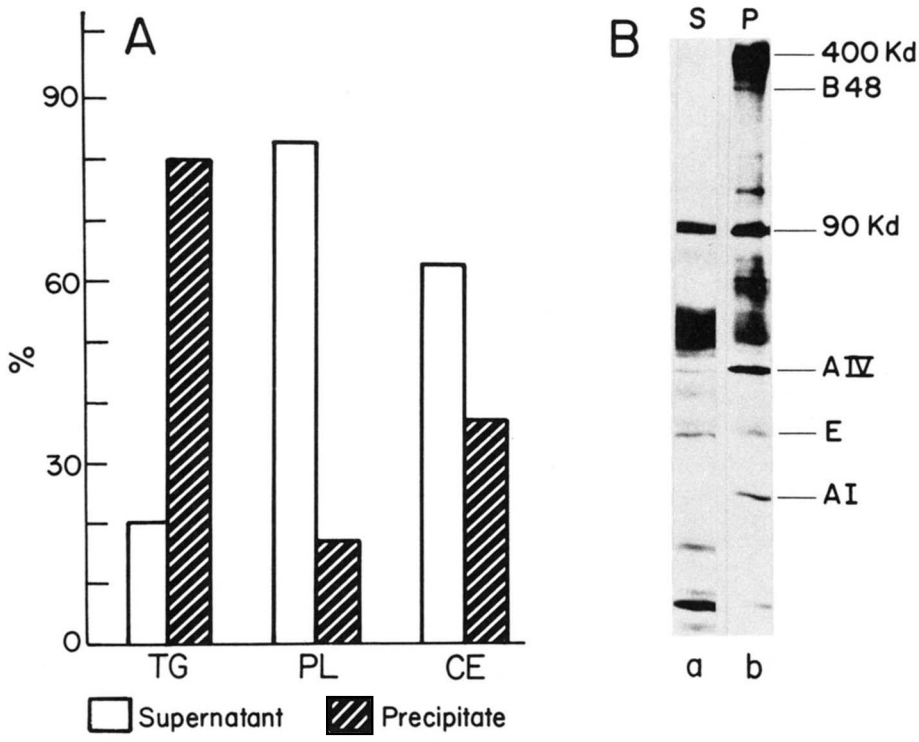

Fig. 7. Mn-heparin precipitation of ^{14}C-labeled lipids and ^{35}S-labeled proteins. Caco-2 cells were incubated either with [^{14}C]oleic acid for 20 hr or with [^{35}S]methionine for 4 hr and then the medium was collected. As described in Methods, to 0.5 ml of medium was added 5 μl of Watanabe rabbit plasma and 50 μl of precipitation reagent. The samples were incubated at 4°C for 30 min, centrifuged at 10,000 *g* for 1 min, and the supernatant was removed and precipitated with TCA ([^{35}S]methionine) or extracted with chloroform–methanol 2:1 ([^{14}C]oleic acid). The Mn-heparin precipitates were dissolved, reprecipitated, washed by repeated centrifugation, and solubilized in sample buffer for analysis by PAGE ([^{35}S]methionine) or extracted with chloroform–methanol 2:1 ([^{14}C]oleic acid) for analysis by TLC. Panel A shows the percentage of each lipid that precipitated (filled bar) or remained in the supernatant (open bar) following addition of Mn-heparin reagent to the medium. Panel B depicts the corresponding [^{35}S]methionine-labeled proteins in the supernatant (lane a) or the precipitate (lane b).

synthesize or secrete CE. The majority (60–80%) of the TG found in the medium was present in the basolateral chamber, and indeed, as indicated in Fig. 2, all of the lipids present in the triglyceride-rich lipoprotein d < 1.006 g/ml fraction were secreted in a polarized fashion (~80% appearing on the basolateral side).

Radiolabeling of cell monolayers with [^{35}S]methionine followed by immunoprecipitation of the newly synthesized proteins revealed that apolipoproteins are major secretory products. These included apoA-IV, A-I, C-III, E, B-100, and B-48. Densitometric scannings of the autoradiograms (not shown) indicated that apoA-I had a shoulder of slightly higher molecular weight that may represent a small amount of pro-A-I with the six amino acid "pro" segment, which is cleaved by a factor present in serum or plasma (25). A similar observation has been reported for Caco-2 cells (10) and for HepG2 cells (26). In addition, on a 7–13% gradient gel, apoE ran as a doublet, previously attributed to genetic polymorphism in the human population and variable sialylation of the apoE polypeptide (27). A surprising finding was the production of both apoB-48 and apoB-100 by the Caco-2 cells since adult human intestine secretes chylomicrons, which contain apoB-48 but not apoB-100 (21, 28). In our studies, B-100 was generally the more predominant form of the two apolipoproteins, although its synthesis varied considerably, from approximately the same amount as B-48 to as much as 10 times greater over the course of the nearly 1 year during which these experiments were performed (stock cultures were replenished every 15 passages). Glickman, Rogers, and Glickman (21) have reported that the form of apoB synthesized by the intestine depends upon the developmental stage of the tissue. In short term incubations with [^{3}H]leucine, intestinal segments from human fetuses at 11 weeks of gestation produced more apoB-100 than B-48, while those from fetuses at 16 weeks made approximately equal amounts of the two apolipoproteins; only B-48 was synthesized by adult intestinal segments (obtained by biopsy). Caco-2 cells would appear, therefore, to resemble the intestine in its fetal condition. That these tumorigenic cells resemble their natural counterparts at an early stage of development is supported by the work of Pinto et al. (2)

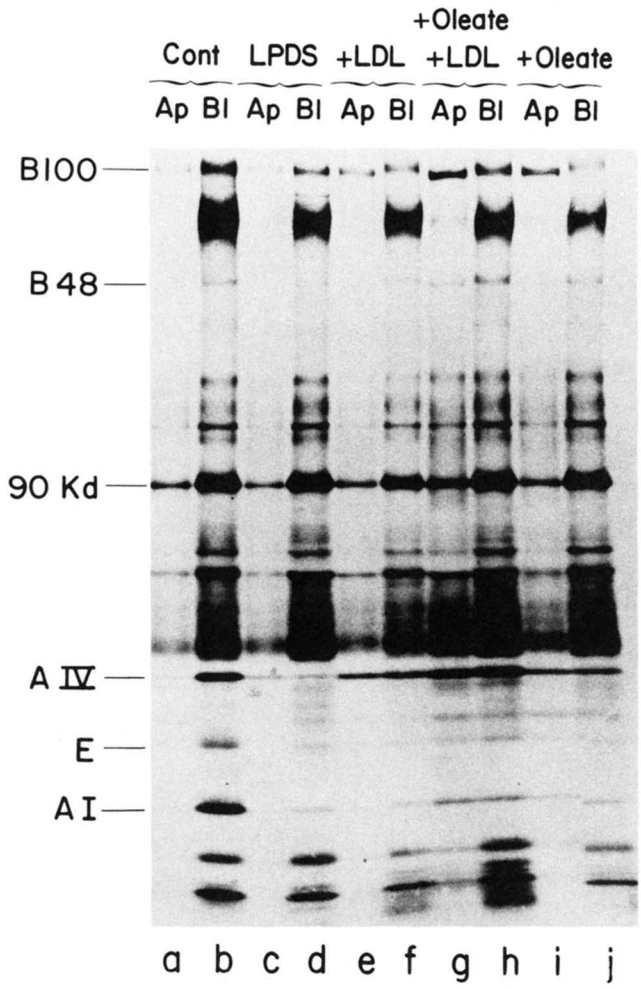

Fig. 8. Incubation with LPDS results in a decrease in apolipoprotein synthesis. Caco-2 cells, grown on filters with a resistance of > 150 ohm/cm^2, were preincubated for 40 hr in the modified media as indicated below, then were labeled for 4 hr with [^{35}S]methionine. TCA precipitates prepared from the apical (Ap; lanes a, c, e, g, i) or basolateral (Bl; lanes b, d, f, h, j) medium were analyzed by PAGE on a 7–13% gradient gel. The preincubation medium was supplemented with: 20% FCS (a, b), 10% LPDS (c, d), 10% LPDS + LDL (e, f), 10% LPDS + LDL + oleate (g, h) or 10% LPDS + oleate (i, j). The LDL was added at 100 μg/ml and the oleate (bound to BSA) at 0.5 mM.

who, based on a study of glycogen content of Caco-2 cells, concluded that they resembled enterocytes from 15-week-old human fetuses. In addition, the cells secrete alpha-fetoprotein (Traber, M. G., H. J. Kayden, and M. J. Rindler, unpublished observations), which is also a product of the fetal liver and intestine, but not the adult (29). At the molecular level, it has not yet been established unambiguously whether apoB-100 and B-48 are derived from a single gene or even a single mRNA species (30, 31). Thus, studies in Caco-2 cells could provide insight into the factors responsible for the developmental transition between the two forms of apoB.

Analysis of the distribution of lipids and of apolipoproteins demonstrated that Caco-2 cells secrete particles that can be isolated at the densities of the major lipoproteins present in human plasma. This contrasts with the human adult small intestine which synthesizes only chylomicrons and HDL (20), as characterized by the apolipoprotein content of the particles. In general, the apolipoprotein content of the lipoprotein fractions secreted by Caco-2 cells was not unlike that of lipoproteins in human plasma (except for the absence of apoA-II); however, the lipid composition was unusual due primarily to the limited CE content. The TG-rich lipoproteins in the d < 1.006 g/ml fraction (chylomicron and VLDL) contained apoB (primarily B-100), E, A-IV, and A-I. Particles that floated in the LDL density range (1.006 < d < 1.063 g/ml) were markedly enriched in apoB-100 and E, but were also high in TG, which comprises 90% of the ^{14}C-labeled lipids in this fraction. With respect to their lipid composition, these particles did not resemble normal plasma LDL, which contains 50% CE (20), nor were they comparable to βVLDL from type III patients, an apoE-rich LDL containing 36% CE and 20% TG (32). However, the particles in the LDL density range do resemble VLDL secreted by the liver, which contains apoB-100 and apoE, with 60% TG, 20% PL, and 20% CE (20). The HDL density fraction of the medium (1.063 < d < 1.21 g/ml) was unusual with respect to both the apolipoprotein and lipid composition. Caco-2 "HDL" consisted primarily of apoA-I and A-IV, with no B-100, but with detectable amounts (4%, Table 5) of B-48. These particles were also found to have a distinctive lipid composition: 63% TG and 34% PL with only 3% CE, unlike plasma HDL which contains 8% TG, 50% PL, and 30% CE (20). Very little B-100, but some apoB-48, A-I, and E, along with a major proportion of A-IV, was found in the d > 1.21 g/ml "lipoprotein-free" fraction of the medium. Bisgaier et al. (33) have previously reported that apoA-IV exists both as a free

TABLE 6. Regulation of apolipoprotein synthesis by Caco-2 cells in response to the lipid content of the medium

Medium	Apolipoprotein					
	B-100	B-48	A-IV	E	A-I	90 kDa
FCS	100	100	100	100	100	100
LPDS	65	37	30	27	19	100
LPDS + LDL	76	83	112	28	30	100
LPDS + oleate	101	100	72	43	30	100
LPDS + LDL + oleate	106	159	126	48	51	100

The autoradiograms from two experiments carried out as described in Fig. 8 were subjected to densitometric analysis. The total of secreted apolipoproteins in the medium was calculated from the sum of the separate values obtained for the two chambers. The amount of the 90-kDa protein was taken as a constant for each sample and used to normalize each value for differences in [^{35}S]methionine incorporation and protein recovery. The percent of control level was calculated by dividing the amount of apolipoprotein secreted in response to the preincubation medium by the amount of the same apolipoprotein secreted in medium containing FCS. Shown are the averages of the percentages for the two experiments.

apolipoprotein and in association with chylomicron and HDL particles.

The secretion of both "alpha" and "beta" lipoproteins by Caco-2 cells, i.e., non-apoB-containing and apoB-containing lipoproteins, was demonstrated by the results of the Mn-heparin precipitation experiments. When the precipitation procedure was applied to the basolateral medium after the Caco-2 cells were labeled with [^{14}C]oleic acid, 80% of the TG was precipitated while 85% of the PL was found in the supernatant. Only the lipoproteins containing apoB are precipitated using this method (17), while HDL particles remain in the supernatant. No ^{35}S-labeled apoBs were detected in the supernatant after this treatment. These studies confirm that Caco-2 cells secrete lipoproteins that contain both apoB and TG.

Hughes et al. (10) have reported that Caco-2 cells synthesize and secrete apolipoproteins that float at lipoprotein densities, which could be observed by electron microscopy. These authors found that the cells produced apolipoproteins B-100, B-48, A-I, E, and C-III, but not apoA-IV, nor was mRNA for apoA-IV found by hybridization with a cDNA probe. We detected apoA-IV by immunoprecipitation, and a protein of identical molecular weight was identifiable in autoradiograms of the $d < 1.006$ and $1.063 < d < 1.21$ g/ml density fractions of the medium. Furthermore, Hughes et al. (10) did not observe any apoB in the $d < 1.006$ g/ml fraction (except when the cells were incubated with unlabeled fatty acids), and reported that the HDL fraction contained both B-100 and B-48. We observed that Caco-2 cells grown in 20% FCS secrete lipoproteins that float at $d < 1.006$ g/ml which contain both forms of apoB. ApoB-100 is a major component of this fraction, and is absent from the HDL fraction.

While the results of the two studies are difficult to reconcile, some of these discrepancies may be ascribed to differences in the cell lines, differences in the incubation conditions, and methodological differences. Among these is the growth of the cells on filters, which may promote their differentiation and allows the unimpeded recovery of the lipoproteins. There were also differences in the method of lipoprotein isolation, although it is not clear whether the shorter period of time required for the fractionation of the medium (30 hr in our studies vs. perhaps 96 hr) was an important factor. Hughes et al. (10) concentrated the medium by dialysis against dry polyethylene glycol before sequential ultracentrifugation to isolate the lipoproteins. Because of the small amount of lipoproteins actually produced, these steps may have contributed to greater loss or degradation of apoB and apoA-IV. Finally, our radiolabeling experiments were conducted either in the presence of serum in the medium (for [^{14}C]oleic acid) or within 4.5 hr after the replacement of 20% with 0.25% FCS (for [^{35}S]methionine) while Hughes et al. (10) used an 18-hr incubation in serum-free medium. As shown in Fig. 8, the presence or absence of lipid in the culture medium may have profound and very specific effects on secretion of apolipoproteins, especially apoA-IV.

Indeed, the replacement of fetal calf serum in the medium with lipoprotein-depleted human serum resulted in reduced secretion of all of the apolipoproteins measured (Fig. 8, Table 6), ranging from fivefold (apoA-I) to nearly twofold (apoB-100). The addition of LDL to the preincubation medium resulted in the maintenance of apoA-IV secretion at control levels, suggesting that its secretion may be regulated by the cholesterol available to the cell. Fat feeding in humans has also been demonstrated to increase the synthesis and secretion of apoA-IV (33). Although inclusion of oleic acid in the preincubation medium did maintain the levels of secretion of apoB-48 and B-100, even the presence of LDL and oleic acid together was unable to maintain the secretion of apoA-I and E to control levels. This finding suggests that there must be other factors in the serum that influence apolipoprotein production as well. Further experiments using specific cDNA probes to assess the levels of mRNA under the various culture conditions are necessary before we can conclude that the regulation of secretion is at the level of gene expression. Nonetheless, the Caco-2 cell, especially when cultured on permeable supports, appears to be a suitable model system not only for analysis of the intestinal handling of dietary lipids and vitamins, but also for the study of the regulation of apolipoprotein synthesis by extracellular lipids and drugs.

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