Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7545–7549, December 1981
Cell Biology

Mouse macrophages synthesize and secrete a protein resembling apolipoprotein E

(cholesterol excretion/lipoprotein receptors/acetylated low density lipoprotein/β-migrating very low density lipoprotein)

SANDIP K. BASU*, MICHAEL S. BROWN*, Y. K. HO*, RICHARD J. HAVEL†, AND JOSEPH L. GOLDSTEIN*

*Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235; and †Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, California 94140

Contributed by Michael S. Brown, August 14, 1981

ABSTRACT **Monolayers of mouse peritoneal macrophages were shown to synthesize and secrete a protein that resembles apoprotein E (apoE), a normal constituent of plasma lipoproteins. Synthesis and secretion were studied by incubation of macrophages with L-[^{35}S]methionine and analysis of the ^{35}S-labeled proteins secreted into the culture medium. The ^{35}S-labeled protein resembling apoE showed the following properties: (*i*) it floated in the ultracentrifuge at a density <1.215 g/ml, indicating that it was associated with lipid; (*ii*) by NaDodSO$_4$/polyacrylamide gel electrophoresis, its M_r of 35,000 was identical to that of authentic apoE obtained from mouse plasma very low density lipoprotein; (*iii*) its isoelectric point of 5.4 was the same as that of authentic mouse apoE; (*iv*) it comigrated with authentic mouse apoE after two-dimensional isoelectric focusing/NaDodSO$_4$/polyacrylamide gel electrophoresis; and (*v*) it was quantitatively precipitated by a monospecific antibody directed against rat apoE. Synthesis and secretion of the apoE-like protein was stimulated 3- to 8-fold when the macrophages were loaded with cholesterol by incubation with either acetylated low density lipoprotein (acetyl-LDL) or β-migrating very low density lipoprotein from cholesterol-fed rabbits. When the cells were incubated with acetyl-LDL, the apoE-like protein composed ≈2% of the total ^{35}S-labeled protein synthesized by the cells and ≈10% of the total ^{35}S-labeled protein secreted into the medium. The current findings suggest a role for apoE in the plasma transport of cholesterol excreted from cholesterol-loaded macrophages.**

Macrophages play a quantitatively significant role in cholesterol metabolism. They take up and degrade cholesterol-containing materials, such as effete red blood cells, senescent granulocytes, and cell debris. Although macrophages do not take up large amounts of naturally occurring plasma lipoproteins, they possess a remarkable capacity to internalize plasma low density lipoprotein (LDL) that has been made negatively charged by chemical modifications such as acetylation (1), maleylation (1), and treatment with malondialdehyde (2, 3). Macrophages also take up large amounts of β-migrating very low density lipoprotein (β-VLDL), a cholesterol-rich lipoprotein that accumulates in plasma of cholesterol-fed animals (4, 5).

Acetyl-LDL and β-VLDL are taken up by macrophages through receptor-mediated endocytosis (1, 4). After binding to the cell surface, the lipoproteins are internalized and delivered to lysosomes, where their cholesteryl esters are hydrolyzed. Much of the liberated cholesterol is retained in the cytoplasm, where it is reesterified and stored as cholesteryl esters (6). When the culture medium contains high density lipoprotein (HDL) or other cholesterol-binding substances, the stored cholesteryl esters are hydrolyzed and the cholesterol is excreted from the macrophages (7, 8).

The question arises as to whether sterol excretion by macrophages is accompanied by the synthesis and secretion of any of the protein components of plasma lipoproteins. Thus, in the current studies we incubated monolayers of mouse peritoneal macrophages with acetyl-LDL to load them with cholesterol. We then incubated the cells with [^{35}S]methionine under conditions in which cholesterol excretion was known to be maximal. We found that the macrophages synthesized and secreted a protein that floated in the lipoprotein fraction at ρ <1.215 g/ml. This protein is similar to, if not identical with, apoprotein E (apoE), a normal constituent of certain plasma lipoproteins, including chylomicron remnants, VLDL, and HDL (9). One function of apoE is to serve as a signal that allows cholesterol-carrying lipoproteins to bind to hepatic receptors, thereby promoting their uptake by the liver (10–12). The current results raise the possibility that apoE may be synthesized by cholesterol-loaded macrophages in order to facilitate the transport of excreted cholesterol from scavenger cells to the liver.

METHODS

Antibodies. A rabbit antiserum to bovine albumin and a rabbit antibody to human β-lipoprotein (IgG fraction) were obtained from Cappel Laboratories (Cochranville, PA). A rabbit antiserum to rat apoE was prepared as described (13).

Lipoproteins. Human LDL (ρ 1.019–1.063 g/ml) and HDL$_3$ (ρ 1.125–1.215 g/ml) were prepared by centrifugation (1). LDL was acetylated with repeated additions of acetic anhydride (1). β-VLDL (ρ <1.006 g/ml) was prepared from plasma of cholesterol-fed rabbits (5). Mouse VLDL (ρ <1.006 g/ml), prepared by centrifugation of normal plasma (220,000 × g, 16 hr, 4°C), was delipidated by extraction with acetone/ethanol (1:1 vol/vol) at −20°C. The apoVLDL precipitate was dissolved in buffer as indicated in legends and centrifuged at 10,000 × g for 10 min at 4°C; the supernate was used for electrophoresis or isoelectric focusing (IEF). Lipoprotein concentrations are given in terms of protein content (14).

Mouse Macrophage Monolayers. Peritoneal cells were harvested from unstimulated male Swiss–Webster mice (20–30 g) (1, 6). The cells were suspended in Dulbecco's modified Eagle's medium (DME medium) containing 20% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at 4–6 × 10^6 cells per ml. Two-milliliter aliquots of cells were dispensed into 60 × 15 mm plastic Petri dishes and incubated in a humidified CO$_2$ (5%) incubator at 37°C. After 1–2 hr at 37°C, the dishes were washed four times with 3 ml of DME medium to remove nonadherent cells. The adherent cells were incu-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: apo, apoprotein; DME medium, Dulbecco's modified Eagle's medium; IEF, isoelectric focusing; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; β-VLDL, β-migrating VLDL.

bated for 18 hr at 37°C in 2 ml of DME medium containing 20% fetal calf serum, after which further additions were made.

Radiolabeling of Proteins with [^{35}S]Methionine and Preparation of ^{35}S-Labeled Lipoprotein Fraction. After 18 hr in culture (day 1), each macrophage monolayer was washed once with 2 ml of DME medium. The dishes then received 2 ml of DME medium containing acetyl-LDL or other additions as indicated in legends. After incubation for 24 hr at 37°C (day 2), the medium was replaced with 2 ml of methionine-free DME medium supplemented with L-[^{35}S]methionine at 40 μCi/ml (1 Ci = 3.7×10^{10} becquerels), 40 μM unlabeled L-methionine, and HDL or other additions as indicated. After incubation for 24 hr at 37°C (day 3), the medium from 3–10 dishes was combined, adjusted to density 1.215 g/ml with solid KBr (15), and centrifuged at 63,000 rpm in a type 65 Beckman rotor for 48 hr at 4°C. Each 13-ml centrifuge tube was sliced under the cap to obtain 2 fractions: a top *lipoprotein fraction* at $\rho < 1.215$ g/ml (1–2 ml) and a bottom *nonlipoprotein fraction* at $\rho > 1.215$ g/ml (11–12 ml). Both fractions were dialyzed at 4°C against 25 mM NH_4HCO_3, then lyophilized, and stored at −20°C.

Electrophoresis. Electrophoresis was conducted in one dimension on 10% polyacrylamide slab gels containing 0.1% $NaDodSO_4$ (16). Samples were applied in 100 μl of buffer O [10% (vol/vol) glycerol/5% (vol/vol) 2-mercaptoethanol/2.3% $NaDodSO_4$/62.5 mM Tris·HCl (pH 6.8)] (16) after heating at 90°C for 3 min. Electrophoresis was carried out at 25 mA per slab gel at 10°C for 3–4 hr. Gels were stained with Coomassie blue G250 and destained. For fluorography, each slab gel was soaked in 160 ml of EN^{3}HANCE (New England Nuclear) for 1 hr at room temperature, washed for 1 hr with cold water, dried, and kept in contact with an x-ray film (Kodak XAR-5 film) at −70°C for 18–48 hr. Methods for two-dimensional IEF/$NaDodSO_4$/polyacrylamide gel electrophoresis (16) and for IEF in tube gels (17) are described in the referenced articles. Gels were calibrated with the following M_r standards: phosphorylase b, 94,000; bovine serum albumin, 68,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 21,000; and lysozyme, 14,000.

RESULTS

In the standard protocol, monolayers of mouse peritoneal macrophages were incubated for 24 hr in the presence of acetyl-LDL so as to load the cells with cholesteryl esters (1, 6). The cells were then incubated for 24 hr with HDL under conditions in which excretion of cholesterol is known to occur (7, 8). During the excretory period, the medium contained [^{35}S]methionine. After excretion, the medium was subjected to centrifugation at a density of 1.215 g/ml, and the secreted ^{35}S-labeled proteins that floated in the lipoprotein fraction were analyzed.

Fig. 1 presents an autoradiogram of a $NaDodSO_4$/polyacrylamide gel, lane 1 showing the total radiolabeled proteins secreted into the medium by macrophages incubated in the presence of [^{35}S]methionine. Many bands were observed, including a major band with a M_r of 35,000. When the medium was subjected to centrifugation at ρ = 1.215 g/ml, the M_r 35,000 protein was the predominant radiolabeled band in the lipoprotein fraction (Fig. 1, lane 2). The M_r 35,000 band contained ≈80% of the total ^{35}S radioactivity present in the lipoprotein fraction, as determined by scintillation counting of consecutive 5-mm sections of the gel in Fig. 1, lane 2. Fig. 1, lane 3, shows a Coomassie blue-stained gel of mouse apoVLDL. The M_r of the apoE band is 35,000, which is the same as that of the major ^{35}S-labeled protein secreted by the macrophages (Fig. 1, lane 2). The other prominent Coomassie blue-stained bands in apoVLDL correspond to apoB (two bands at top of gel) and the

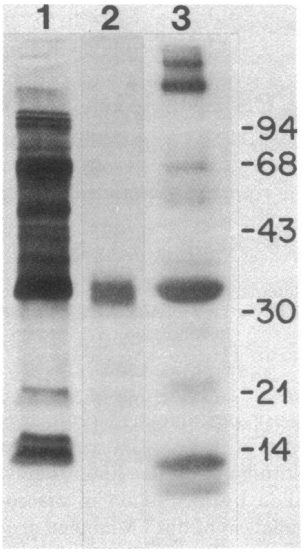

FIG. 1. $NaDodSO_4$/polyacrylamide gel electrophoresis of ^{35}S-labeled proteins produced by macrophages. On day 1, cells received DME medium and acetyl-LDL at 50 μg/ml. On day 2, medium was replaced with DME medium with [^{35}S]methionine and HDL at 100 μg/ml. On day 3, medium was centrifuged to obtain the lipoprotein fraction. Aliquots were subjected to electrophoresis and fluorography. Lane 1, autoradiogram of unfractionated medium (88,000 cpm applied). Lane 2, autoradiogram of lipoprotein fraction (9800 cpm). Lane 3, Coomassie blue stain of mouse plasma apoVLDL (50 μg). M_r ($\times 10^{-3}$) standards are indicated.

C apoproteins (prominent bands at bottom of gel, M_r <14,000) (Fig. 1, lane 3).

By IEF, apoE from mouse apoVLDL showed three bands at an isoelectric point of about 5.4 (Fig. 2*A*). This charge heterogeneity is characteristic of apoE from human and animal species (9). In a parallel gel, a sample of the ^{35}S-labeled lipoprotein fraction secreted by macrophages was subjected to IEF, after which the gel was sliced and radioactivities were measured. A single major peak of ^{35}S radioactivity was seen. The isoelectric point of this protein corresponded to that of apoE (Fig. 2*B*).

The macrophage-produced protein also migrated similarly to authentic mouse apoE when subjected to two-dimensional

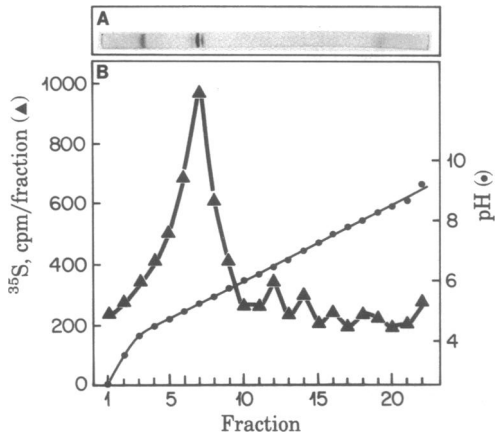

FIG. 2. IEF gels of mouse apoVLDL (*A*) and the ^{35}S-labeled lipoprotein fraction (*B*). The ^{35}S-labeled lipoprotein fraction was prepared from the medium of mouse macrophages as in Fig. 1. IEF was performed in a 7.5% polyacrylamide gel (5 × 125 mm tube) containing 6 M urea and 2% Ampholine, pH 3.5–10 (17). Each lyophilized sample (10,000 cpm of ^{35}S-labeled lipoprotein fraction or 50 μg of mouse apoVLDL) was dissolved in 50 μl of buffer containing 20 mM *N*-ethylmorpholine·HCl (pH 8.6) and 8 M urea and mixed with 2 ml of gel prior to polymerization. Each gel was run with 1.5% H_3PO_4 (anode) and 2.5% ethylenediamine (cathode) at 150 V at 5°C for 20 hr. After electrophoresis, the gel containing apoVLDL was fixed, stained with Coomassie blue, destained, and photographed (*A*). The gel containing the ^{35}S-labeled lipoprotein fraction (*B*) was sliced into 5-mm sections. Each gel slice was shaken with 10 ml of Econofluor containing 7% Protosol (both from New England Nuclear) and 0.5% water for 24 hr at 37°C, after which the content of ^{35}S radioactivity was determined in a liquid scintillation counter (▲). The pH of each gel slice was measured in a parallel gel after incubating each 5-mm section in 1 ml of water for 2 hr at 24°C (●).

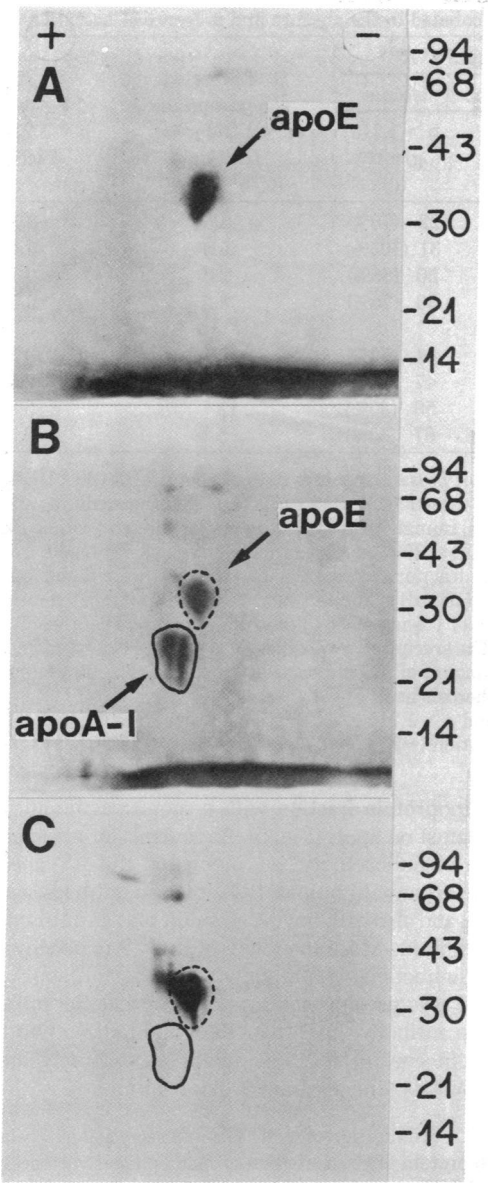

FIG. 3. Two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel of mouse apoVLDL and the ^{35}S-labeled lipoprotein fraction. The ^{35}S-labeled lipoprotein fraction was prepared from the medium of mouse macrophages as in Fig. 1. Each lyophilized sample was dissolved in 100 μl of solution containing 9.5 M urea, 2% Nonidet P40, 2% Ampholine (pH 3.5–10), and 5% 2-mercaptoethanol. Samples were subjected to IEF (16), after which the tube gels were loaded onto 10% polyacrylamide slab gels containing 0.1% NaDodSO$_4$ as described for nonequilibrated gels (16). After electrophoresis, the gels were stained with Coomassie blue, destained, and prepared for fluorography as indicated. (*A*) Coomassie blue-stained gel of mouse apoVLDL (50 μg applied). (*B*) Coomassie blue-stained gel of a mixture of mouse apoVLDL (50 μg applied) and ^{35}S-labeled lipoprotein fraction secreted in the presence of unlabeled human HDL (100,000 cpm applied). (*C*) Autoradiogram of the gel in *B*. The dashed and solid circles show the position of the two major Coomassie blue-stained spots in *B*, apoE and apoA-I, respectively. Autoradiogram in *C* was exposed for 24 hr. M_r ($\times 10^{-3}$) standards are indicated. The anode (acid side of the gel) is on the left.

IEF/NaDodSO$_4$ gel electrophoresis. Fig. 3*A* shows a Coomassie blue stain of a two-dimensional gel of apoE of apoVLDL. To compare the mobility of apoE with the ^{35}S-labeled protein secreted by macrophages, the macrophages were allowed to secrete the labeled protein in the presence of added HDL and the lipoprotein fraction was isolated by centrifugation at ρ 1.215 g/ml. The radioactive lipoprotein fraction was then mixed with apoVLDL so as to give a mass of apoE that could be visualized by Coomassie blue staining. Fig. 3*B* shows a Coomassie blue stain of the two-dimensional gel containing the mixture of proteins. Two prominent spots are seen. One corresponds to apoA-I, which was present in human HDL added during the incubation (solid circle). The other corresponds to mouse apoE, which was added in the form of apoVLDL prior to electrophoresis (dashed circle). Fig. 3*C* shows an autoradiogram of the same gel as that shown in Fig. 3*B*. The major radiolabeled spot coincides with the authentic mouse apoE, as indicated by the dashed circle. This spot appears to have several components whose relative mobilities resemble those of the isoforms of human apoE on two-dimensional gels (18). The apoA-I spot (indicated by solid circle) was devoid of ^{35}S radioactivity (Fig. 3*C*).

To analyze these data quantitatively, we incubated mouse macrophages in the absence or presence of acetyl-LDL and then incubated them with [^{35}S]methionine in the absence or presence of HDL or LDL. The medium was subjected to centrifugation at ρ 1.215 g/ml (Table 1). Prior incubation with acetyl-LDL caused a 2.5-fold stimulation of total protein synthesis and secretion by the macrophages (column a + b, compare groups 1–4 with groups 5–8). There was a more pronounced stimulation of the synthesis and secretion of the protein in the lipoprotein fraction, as indicated by the 6- to 8-fold increase in ^{35}S radioactivity (column c, compare groups 1–4 with groups 5–8). When cells had been incubated in the absence of acetyl-LDL, about 4% of the secreted protein floated at $\rho < 1.215$ g/ml. When cells had been incubated with acetyl-LDL, about 10% of the secreted proteins floated at $\rho < 1.215$ g/ml.

Fig. 4 shows an autoradiogram of the NaDodSO$_4$ gel of the secreted ^{35}S-labeled lipoprotein fractions from the experiment of Table 1. Prior incubation with acetyl-LDL markedly stimulated the secretion of the M_r 35,000 protein that resembles apoE

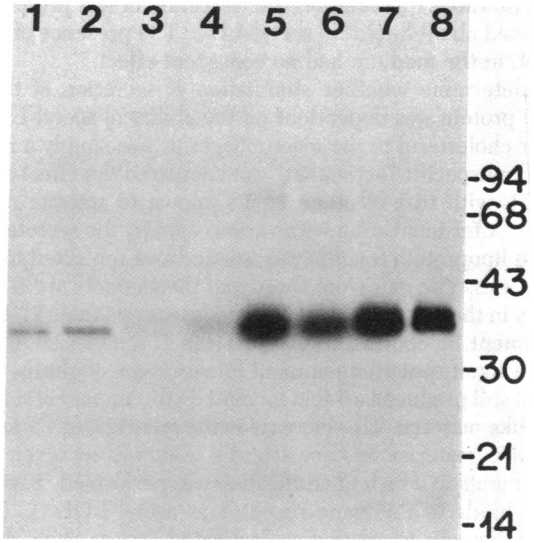

FIG. 4. Autoradiograms of NaDodSO$_4$/polyacrylamide gels of ^{35}S-labeled lipoprotein fraction secreted by mouse macrophages under different metabolic conditions. Macrophages were processed as in Table 1. An aliquot (100 μl) of the dialyzed ^{35}S-labeled lipoprotein fraction (total volume, 1 ml) was lyophilized and dissolved in 100 μl of buffer O, and 90 μl was loaded onto a polyacrylamide slab gel. After electrophoresis and fluorography, the M_r 35,000 band was cut from each lane and soaked in 100 μl of water for 1 hr, after which the sample was prepared for scintillation counting as described for Fig. 2 (see Table 1, column e). Lanes 1–8 refer to the corresponding groups in Table 1. Autoradiograms were exposed for 18 hr. M_r ($\times 10^{-3}$) standards are indicated.

Table 1. Synthesis and secretion of ^{35}S-labeled proteins by mouse macrophages incubated in the absence and presence of acetyl-LDL

Group	Additions to medium: Day 1	Day 2	Day 3	^{35}S precipitable by trichloroacetic acid, cpm × 10^{-5}: Cells (a)	Medium (b)	Cells + medium (a + b)	Fractionated medium: $\rho < 1.215$ g/ml (c)	$\rho > 1.215$ g/ml* (d)	% of secreted ^{35}S-protein in $\rho < 1.215$ g/ml fraction [(c/b) × 100]	M_r 35,000 ^{35}S-protein in $\rho < 1.215$ g/ml fraction,† cpm × 10^{-5} (e)
1	—	—	—	94	33	127	1.2	22 (70%)	3.6	0.33
2	—	—	HDL	86	32	118	1.7	31 (103%)	5.3	0.42
3	—	HDL	—	63	36	99	1.0	30 (86%)	2.8	0.18
4	—	LDL	—	100	46	146	1.6	33 (75%)	3.5	0.57
5	Ac-LDL	—	—	231	73	304	8.6	44 (72%)	12	3.3
6	Ac-LDL	—	HDL	178	58	236	5.3	47 (90%)	9.1	2.3
7	Ac-LDL	HDL	—	199	85	284	9.9	58 (80%)	12	3.8
8	Ac-LDL	LDL	—	334	113	447	6.0	67 (65%)	5.3	2.7

On day 1, macrophages received 2 ml of DME medium in the absence (groups 1–4) or presence (groups 5–8) of acetyl-LDL (Ac-LDL) at 50 μg/ml. After incubation for 24 hr at 37°C, the monolayers were divided into eight groups (three monolayers per group), and the medium was replaced with 2 ml of DME medium, [^{35}S]methionine, and one of the following: no lipoproteins, human HDL at 100 μg/ml, or human LDL at 100 μg/ml. On day 3, the media from the three monolayers for each group were combined, and HDL at 100 μg/ml was added to the pooled media from groups 2 and 6. All media were then centrifuged at $\rho = 1.215$ g/ml and the upper and lower fractions were collected. The cells were dissolved in buffer containing 50 mM Tris/maleate (pH 6), 2 mM $CaCl_2$, and 0.2% Nonidet P40. Aliquots (100 μl) of the cell suspension, unfractionated medium, and fractionated medium were precipitated with 10% trichloroacetic acid in the presence of 0.4 mg of bovine serum albumin. The precipitates were washed twice with 10% trichloroacetic acid containing 1 mM unlabeled L-methionine. The precipitates were dissolved in 0.1 M NaOH and neutralized with 1 M acetic acid, and radioactivities were measured in 10 ml of Aquasol. The amount of ^{35}S radioactivity in the M_r 35,000 protein of the $\rho < 1.215$ g/ml fraction was determined after $NaDodSO_4$/polyacrylamide gel electrophoresis as in Fig. 4.

* Numbers in parentheses represent % recovery of total radioactivity in $\rho < 1.215$ and $\rho > 1.215$ g/ml fractions. Recovery = [(c + d)/b] × 100.

† Values were not corrected for procedural losses that occurred during both centrifugation (≈20%) and electrophoresis (≈25%).

[compare groups 1–4 (no acetyl-LDL) with groups 5–8 (with acetyl-LDL)]. The presence of HDL was not required for secretion [compare group 5 (no HDL) with group 7 (with HDL)]. For purposes of relative quantitation, the M_r 35,000 bands shown in Fig. 4 were cut out from the gel and subjected to scintillation counting. No correction was made for incomplete recovery during the centrifugation and the electrophoresis. As shown in Table 1 (column e), the secretion of this protein was stimulated about 8-fold by acetyl-LDL. The presence of HDL or LDL in the medium had no consistent effect.

To determine whether stimulation of secretion of the M_r 35,000 protein was dependent on the ability of acetyl-LDL to deliver cholesterol to the macrophages or was simply a reflection of nonspecific "activation," we compared the effect of acetyl-LDL with that of other agents known to activate macrophages. After incubation with various agents, the secreted ^{35}S-labeled lipoprotein fraction was isolated and subjected to electrophoresis. The gels were sliced and the amounts of ^{35}S radioactivity in the M_r 35,000 bands were measured (Table 2). In this experiment, in contrast to that of Table 1, acetyl-LDL did not cause a significant stimulation of total protein synthesis. However, it still produced a 6-fold increase in the amount of secreted apoE-like material. This increase in the secretion of ^{35}S-labeled apoE-like material was consistently observed in seven other experiments in which quantification was performed. Fucoidin, which binds to the same receptor as acetyl-LDL (1, 3) and causes the cells to assume an "activated" appearance, did not significantly increase the production of apoE-like material. Bacterial lipopolysaccharide did not stimulate secretion of the M_r 35,000 protein. In contrast, rabbit β-VLDL, a lipoprotein that binds to a macrophage receptor distinct from the acetyl-LDL receptor (4, 5) and delivers cholesterol, stimulated synthesis of the M_r 35,000 protein by 3-fold. The relative stimulation of synthesis of the apoE-like protein by β-VLDL and acetyl-LDL was correlated with the ability of these two lipoproteins to increase the cholesteryl ester content of the cells (Table 2).

To confirm the similarity between the apoE-like protein secreted by macrophages and plasma apoE, we incubated the ^{35}S-labeled lipoprotein fraction with a monospecific antibody directed against rat apoE (Fig. 5). As controls for nonspecific precipitation of radioactivity, we incubated the ^{35}S-labeled lipoprotein fraction with human LDL plus an antibody against human LDL and also with bovine albumin plus an antibody against bovine albumin. More than 90% of the ^{35}S radioactivity in the lipoprotein fraction was precipitated by the anti-apoE, whereas only 2.3% was precipitated in a control reaction mixture containing no antibody. Less than 4% of the radioactivity was precipitated in control reaction mixtures containing anti-LDL/LDL or anti-bovine albumin/bovine albumin.

Table 2. Stimulation of synthesis and secretion of ^{35}S-labeled M_r 35,000 protein after incubation of macrophages with acetyl-LDL and β-VLDL

Additions to medium on day 1	Total ^{35}S-protein synthesized, cpm × 10^{-5}	M_r 35,000 ^{35}S-protein in $\rho < 1.215$ g/ml fraction, cpm × 10^{-5}	Content of cellular cholesterol, μg sterol/mg protein: Free	Esterified
None	339	0.54	29	1.2
Acetyl-LDL	432	3.2	63	145
β-VLDL	447	1.5	51	27
Fucoidin	430	0.82	32	2.0
Lipopolysaccharide	377	0.73	21	1.6

On day 1, macrophages received 2 ml of DME medium plus one of the following: no addition, acetyl-LDL at 50 μg/ml, rabbit β-VLDL at 200 μg/ml, fucoidin at 75 μg/ml, or lipopolysaccharide at 50 μg/ml. After incubation for 24 hr at 37°C, medium was replaced with 2 ml of DME medium containing [^{35}S]methionine and human HDL at 100 μg/ml. On day 3, medium from each group was centrifuged to obtain the $\rho < 1.215$ fraction. The total amount of ^{35}S-labeled protein synthesized (trichloroacetic acid-insoluble radioactivity in cells plus medium) was determined as in Table 1. The amount of ^{35}S radioactivity in the M_r 35,000 protein of the $\rho < 1.215$ g/ml fraction was determined as in Fig. 4 and Table 1. No correction was made for procedural losses, which averaged ≈50%. Cholesterol content of the cells was measured by gas/liquid chromatography (6).

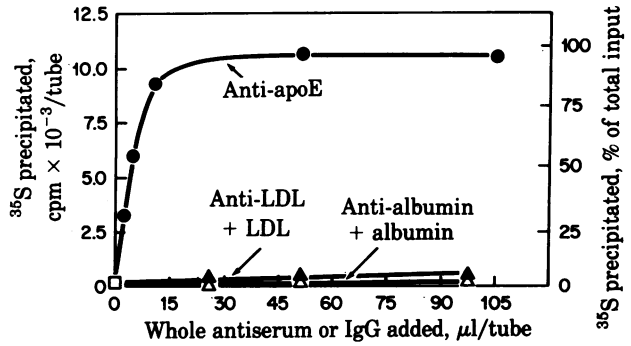

FIG. 5. Immunoprecipitation of secreted ^{35}S-labeled lipoprotein fraction by antiserum to rat apoE. The ^{35}S-labeled lipoprotein fraction secreted by mouse macrophages was prepared as in Fig. 1. Each immunoprecipitation mixture (150 μl) contained 20 mM Tris·HCl (pH 7.5), 0.2% Nonidet P40, 0.25% sodium deoxycholate, 7 mM EDTA, ^{35}S-labeled lipoprotein fraction (11,030 cpm), and the indicated volume of one of the following: □, no antibody; ●, antibody against rat apoE (whole serum 60 μg/μl); △, antibody against bovine albumin (whole serum 66 μg/μl) plus bovine albumin (10 μg); or ▲, antibody against human β-lipoprotein (IgG fraction, 15 μg/μl) plus human LDL (10 μg). Each tube was incubated at 37°C for 2 hr and then at 4°C for 20 hr. The tubes were centrifuged at 9400 × *g* for 10 min at 4°C and the precipitate was washed with 150 μl of the above buffer. Each precipitate was dissolved in 150 μl of buffer O, after which the radioactivity of a 100-μl aliquot was measured in 10 ml of Aquasol.

DISCUSSION

The current results indicate that mouse peritoneal macrophages secrete a protein that is remarkably similar to plasma apoE. The protein floats at $\rho < 1.215$ g/ml, indicating that it binds lipid. It has the same M_r as authentic mouse apoE (35,000) and the same isoelectric point (5.4). It also comigrates with authentic mouse apoE on two-dimensional IEF/NaDodSO$_4$/polyacrylamide gel electrophoresis. Finally, it is precipitated by a monospecific antibody directed against rat apoE.

The mouse macrophages synthesized a small amount of the apoE-like protein in the basal state. Synthesis and secretion were stimulated 6- to 8-fold when the cells were loaded with cholesterol by incubation with acetyl-LDL and 3-fold when the cells were incubated with β-VLDL, which delivers lesser amounts of cholesterol. There was no significant stimulation when cells were incubated with fucoidin or bacterial lipopolysaccharide, which are "activators" of macrophages. Thus, the synthesis of the apoE-like protein appears to be modulated by the cholesterol content of the cells.

The one puzzling finding of this study was that the secretion of the apoE-like protein was not absolutely dependent on the presence of HDL. Previous studies have shown that cholesterol-loaded macrophages do not secrete cholesterol unless they are incubated with HDL or some other acceptor for the excreted cholesterol (7, 8). Yet the current data show that cholesterol-loaded macrophages secrete similar amounts of apoE-like protein whether or not HDL is present. In the absence of HDL, the apoE-like protein is secreted, but there is no net secretion of cholesterol (7, 8). Thus, if apoE plays a role in cholesterol excretion by macrophages, it cannot act alone. It is conceivable that an apoE–lipid complex carries cholesterol out of the macrophage, but if HDL is not present in the medium the cholesterol partitions back into the macrophage membrane. In the presence of HDL or other acceptor, this excreted cholesterol may be trapped in the medium, leading to net sterol secretion.

Although the present data are highly suggestive that the apoE-like material is authentic apoE, additional studies are necessary to document identity. It should be emphasized that the apoE-like protein is not a trace product of macrophages. After loading with acetyl-LDL, about 10% of the total secreted protein floated at $\rho < 1.215$ g/ml. About 80–90% of the ^{35}S-labeled protein in this fraction was the apoE-like material as judged by gel electrophoresis and immunoprecipitation. Thus, about 10% of the total protein secreted by cholesterol-loaded macrophages may be apoE. This amounts to about 2% of the total protein synthesized by the macrophages (Table 1).

Whether apoE is synthesized by macrophages in the body of humans and animals is not yet known. In preliminary experiments, human macrophages isolated from blood and maintained in culture for several days were shown to secrete ^{35}S-labeled apoE-like material in response to acetyl-LDL. If such synthesis takes place *in vivo*, it implies a role for apoE in "reverse cholesterol transport" (19). ApoE-containing lipoproteins are known to be taken up avidly by lipoprotein receptors on the surface of hepatocytes (10–12, 20). It is possible that apoE may be synthesized by cholesterol-loaded macrophages in order to facilitate the transport of cholesterol from scavenger cells back to the liver.

We thank Floyd Cable, Julie Prlina, and Clyde Wetherill for excellent technical assistance. This research was supported by grants from the National Institutes of Health (HL 20948 and HL 14237).

1. Goldstein, J. L., Ho, Y. K., Basu, S. K. & Brown, M. S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 333–337.
2. Fogelman, A. M., Schechter, I., Seager, J., Hokom, M., Child, J. S. & Edwards, P. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2214–2218.
3. Brown, M. S., Basu, S. K., Falck, J. R., Ho, Y. K. & Goldstein, J. L. (1980) *J. Supramol. Struct.* **13**, 67–81.
4. Goldstein, J. L., Ho, Y. K., Brown, M. S., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1839–1848.
5. Mahley, R. W., Innerarity, T. L., Brown, M. S., Ho, Y. K. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 970–980.
6. Brown, M. S., Goldstein, J. L., Krieger, M., Ho, Y. K. & Anderson, R. G. W. (1979) *J. Cell Biol.* **82**, 597–613.
7. Ho, Y. K., Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 391–398.
8. Brown, M. S., Ho, Y. K. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 9344–9352.
9. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenberg, L. L. (Saunders, Philadelphia), 8th Ed., pp. 393–494.
10. Havel, R. J., Chao, Y.-S., Windler, E. E., Kotite, L. & Guo L. S. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4349–4353.
11. Sherrill, B. C., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1804–1807.
12. Windler, E. E. T., Kovanen, P. T., Chao, Y.-S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 10464–10471.
13. Fainaru, M., Havel, R. J. & Imaizumi, K. (1977) *Biochim. Biophys. Acta* **490**, 144–155.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
15. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353.
16. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
17. Utermann, G. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 1113–1121.
18. Zannis, V. I. & Breslow, J. L. (1981) *Biochemistry* **20**, 1033–1041.
19. Glomset, J. A. & Norum, K. R. (1973) *Adv. Lipid Res.* **11**, 1–65.
20. Brown, M. S., Kovanen, P. T. & Goldstein, J. L. (1981) *Science* **212**, 628–635.