Apolipoprotein E is secreted by cultured lipocytes of the rat liver

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Abstract Hepatic lipocytes, the retinoid-storing cells of the liver, share several characteristics with vascular smooth muscle cells. To determine whether they also share the characteristic of apolipoprotein E secretion, we have compared the relative mRNA expression and protein secretion of apolipoprotein E, apolipoprotein A-I, and apolipoprotein A-IV in early primary cultures of lipocytes, hepatocytes, and Kupffer cells. Expression of apolipoprotein mRNAs was detected using the polymerase chain reaction and oligonucleotide primers specific for apolipoprotein E, apolipoprotein A-I, and apolipoprotein A-IV. Cellular mRNA concentrations were compared by dot blot analysis, and apolipoprotein secretion was assessed by immunoblot analysis of culture media. Apolipoprotein E mRNA was found in all three cell types, whereas apolipoprotein A-I and A-IV mRNAs were detected only in hepatocytes. Hepatocyte, lipocyte, and Kupffer cell media all contained a M_r ~36,000 protein identified by an antibody specific for rat apolipoprotein E. The relative concentration of apolipoprotein E mRNA per microgram of total cellular RNA in lipocytes, hepatocytes, and Kupffer cells was 1.0, 3.0, and 1.6, respectively. The relative secretion of apolipoprotein E per cell was also lowest in lipocytes, being twofold greater in hepatocytes and 1.4-fold greater in Kupffer cells. The secretion of apolipoprotein E by lipocytes is not only an additional smooth muscle cell-like characteristic of the hepatic lipocyte, but also raises the possibility of retinol mobilization upon apolipoprotein secretion.—**Friedman, G., L. Liu, S. L. Friedman, and J. K. Boyles.** Apolipoprotein E is secreted by cultured lipocytes of the rat liver. *J. Lipid Res.* 1991. **32:** 107–114.

Supplementary key words Ito cell • perisinusoidal cell • fat-storing cell • stellate cell

Hepatic lipocytes are cells of diverse metabolic function that have many characteristics in common with vascular smooth muscle cells. Lipocytes (also known as Ito cells or stellate cells) are the primary cellular depot for retinoids, which are stored as droplets of retinyl esters within the cytoplasm (1, 2). Like vascular smooth muscle cells, they are an important source of extracellular matrix proteins, both in the normal and the fibrotic liver. Recent studies have shown that lipocytes synthesize collagens (3, 4), proteoglycans (5), fibronectin (6), and laminin (7). The relation of lipocytes to the endothelium of the liver sinusoids is analogous to the relation of pericytes and vascular smooth muscle cells to the endothelium of capillaries and larger vessels elsewhere in the body. Lipocytes are situated in the subendothelial space of Disse and extend numerous processes along the adventitial surface of sinusoidal endothelial cells (2). Like pericytes and smooth muscle cells, lipocytes contain desmin, an intermediate filament protein (8). This list of characteristics common to both lipocytes and vascular smooth muscle cells or pericytes suggests that lipocytes are the vascular smooth muscle cells of the hepatic sinusoid.

If lipocytes are pericytes or vascular smooth muscle cells, they should have additional characteristics in common with these cells. In particular, we sought to determine whether hepatic lipocytes synthesize apolipoprotein (apo) E. Cultured vascular smooth muscle cells synthesize and secrete substantial quantities of apoE (9–11), a plasma apolipoprotein that plays a central role in cholesterol homeostasis in mammals (for review, see refs. 12–14). Apolipoprotein E is a single-chain polypeptide of M_r ~34,000 in most species. In several species the sequences of the mRNA and the gene are known. Apolipoprotein E, like the apoB of several plasma lipoproteins, is a ligand for the low density lipoprotein receptor. Expression of this receptor is regulated by cellular cholesterol needs; expression is increased in response to a cholesterol deficiency and decreased in response to an excess of cholesterol (15). As a ligand for this receptor, apoE plays a major role in the delivery of cholesterol and other lipids to cells. Apolipoprotein E can also facilitate the movement of cholesterol out of cells (16–19). Apart from cholesterol homeostasis, apoE may participate in the regulation of

Abbreviations: apo, apolipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HDL, high density lipoprotein; PCR, polymerase chain reaction; bp. base pair.

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other cellular functions (9, 12). The liver is the major site of apoE synthesis: both hepatic parenchymal cells and Kupffer cells secrete apoE (20).

In this study, we have demonstrated that hepatic lipocytes in primary culture express apoE but not apoA-I or apoA-IV. Moreover, the concentration of apoE mRNA in early primary cultures of lipocytes, Kupffer cells, and hepatocytes was found to be similar.

MATERIALS AND METHODS

Cell culture

Lipocytes and Kupffer cells were obtained from male retired breeder Sprague-Dawley rats (450–600 g) by in situ perfusion of the liver with collagenase and Pronase, followed by density gradient separation of the cells as previously described (21). Kupffer cells were further purified by centrifugal elutriation (22, 23). Lipocytes and Kupffer cells were plated onto uncoated 35-mm plastic dishes at a density of 1–2 $\times$ 10^6 cells/dish and maintained in Medium 199 with 20% serum (10% horse: 10% calf) (Flow Laboratories, McLean, VA).

Hepatocytes were isolated from male Sprague-Dawley rats (250–300 g by perfusion with collagenase followed by centrifugal elutriation. These cells were plated at a density of 1 $\times$ 10^6 cells onto rat tail collagen (type 1) -coated 35-mm plastic dishes (24) and maintained in the same Medium 199 with 20% serum. Lipocytes, Kupffer cells, and hepatocytes were greater than 99%, 90%, and 95% pure, respectively, as assessed by specific fluorescent markers for lipocytes and Kupffer cells (21, 25).

For all cell types, the medium was replaced 24 h after plating and harvested 48 h later for analysis of apolipoprotein secretion. The cells were then harvested for RNA isolation. Cell counts were determined in triplicate using an automated cell counter (Sysmex, Kobe, Japan).

Immunoblots

Immunoblotting was performed as previously described (26), except that Carnation nonfat dry milk was used instead of gelatin for blocking. For protein separation, 10% reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used. Previously characterized rabbit antibodies (27, 28), kindly provided by Dr. Karl Weisgraber (Gladstone Foundation Laboratories for Cardiovascular Disease, San Francisco, CA), were used to detect rat apoE and rat apoA-I. A rabbit antibody raised against rat high density lipoproteins (HDL), kindly provided by Dr. Karl Weisgraber, was used to detect rat apoA-IV in the culture media.

RNA preparation and analysis

Total cellular RNA was extracted from cultured rat hepatic cells (lipocytes, hepatocytes, and Kupffer cells) with guanidine thiocyanate and sedimented through a cesium chloride cushion according to the method of Chirgwin et al. (29).

Dot blot analysis was performed with a template manifold apparatus (Schleicher & Schuell, Keene, NH). To ensure uniform dot size, total cellular RNA was used at different concentrations (1.0, 2.0, and 3.0 μg) and supplemented with yeast tRNA to provide a total of 3 μg of RNA per sample. The RNA samples were denatured by adding 1 M formaldehyde and heating at 60°C for 15 min, then diluted into 20 vol of 3 M NaCl containing 0.3 M trisodium citrate and applied to the nitrocellulose filters under vacuum. The filters were washed with additional diluent, baked at 80°C for 2 h, and then hybridized as described (30). Autoradiograms of filters were analyzed by quantitative scanning densitometry. Each of the three probes used for RNA quantification by dot blotting was first verified by Northern blot analysis of whole-liver RNA. Each of the three probes identified a single mRNA species of the appropriate molecular weight.

Apolipoprotein E mRNA was identified by a 5' end [^{32}P]ATP-labeled 300-base pair (bp) *Pst*I restriction fragment of the pGR124 cDNA clone (30). (This clone was kindly provided by Dr. John Taylor of the Gladstone Foundation Laboratories.)

Apolipoprotein A-IV mRNA was identified using an antisense cRNA probe corresponding to bases 1229–1626 of the apoA-IV genomic sequence (31). This probe was made using the polymerase chain reaction (PCR) followed by RNA production. The T7 RNA polymerase promoter was attached to the 5' end of the 3' primer used for the PCR (32). The 3' primer corresponded to bases 1626–1606 of the apoA-IV nucleotide sequence plus 29 bases of the T7 RNA polymerase promoter, and the 5' primer corresponded to bases 1229–1257 (**Table 1**). Rat liver cDNA was used as the template for the PCR. The PCR resulted in a 427-bp fragment. (The 427-bp amplified product was of the expected length and was verified by *Sac*I nuclease (New England Biolabs, Beverly, MA) treatment. *Sac*I nuclease cleaves the apoA-IV sequence at base pair 1359, yielding a 296- and a 131-bp fragment). A [^{32}P]UTP-labeled 398-base apoA-IV cRNA probe was then made by in vitro transcription using T7 RNA polymerase according to the manufacturer's directions (Promega, Madison, WI) and the 427-bp PCR-amplified oligonucleotide as a template.

Apolipoprotein A-I mRNA was identified by a 5' end [^{32}P]ATP-labeled antisense oligonucleotide corresponding to bases 1544–1573 of the apoA-I genomic sequence (31) (Table 1). The result was further verified by using a 301-base antisense cRNA probe corresponding to bases 1273–1573 of the apoA-I genomic sequence made in the same way as the apoA-IV cRNA probe. (The template cDNA of this probe was also verified by restriction cleavage using *Bsm*I (New England Biolabs, Beverly,

TABLE 1. Rat apolipoprotein E-, A-I-, and A-IV-specific oligonucleotides

Primer	Sequence	Nucleotide Number[a]
PCRE3	3' GTTGCTCCATTTGTGGTACGA 5'	2135–2115
PCRE5	5' CTCCCAAGTCACACAGGAACT 3'	1517–1537
PCRAI3	3' ACCTCTTACACTTTGTCTTCTAC 5'	1303–1281
PCRAI5	5' ATTTCGCCACTGTGTATGTGGA 3'	498–519
PCRAIV3	3' ATTTCCTTTTCAAATTGGTCTTATACCTT 5'	1626–1598
PCRAIV5	5' AGGACAAACTTGGGAACATTAACACCTAT 3'	1229–1257
Oligo-AI	3' TTCTTGGTGGGATGGGACTAGCTCATAGTA 5'	1573–1544
T7-AIV	3' ATTTCCTTTTCAAATTGGTCT*AGAGGGATATCACTCAGCATAATCCATGG* 5'	1626–1606

[a]Rat apoE (33), A-I (31), and A-IV (31) gene sequences.

MA), which yielded the expected 197- and 133-bp fragments. The latter contains the 29-bp T7 promoter.)

DNA amplification

First-strand cDNA was prepared from 2 μg of total cellular RNA using a cDNA synthesis kit (Amersham/Searle, Arlington Heights, IL). Oligo-d(T) was used to prime the first strand, and the reaction products were purified on NENsorb columns (New England Nuclear, Wilmington, DE) according to the manufacturer's instructions. The PCR was conducted using a kit containing *Thermus aquaticus* DNA polymerase and a DNA thermal cycler (both from Perkin-Elmer Cetus Instruments, Norwalk, CT). Ten to fifty nanograms of first-strand cDNA was used as a DNA template. In each reaction, 2.5 U of *Taq* polymerase and 100 pmol of each primer were used. Amplification of apoE, apoA-I, and apoA-IV sequences was carried out for 40 cycles; denaturation was for 2 min at 96°C, annealing for 1 min at 53°C, and extension for 3 min at 72°C. The amplified product was electrophoresed on a mixture of 3.1% Nusieve GTG agarose and 1% LE agarose (FMC Bioproducts, Rockland, ME), in 0.089 M Tris, 0.089 M boric acid, 2 mM EDTA, pH 8.0, and visualized after staining with 1% ethidium bromide.

Oligonucleotides

The oligonucleotides specific for apoE, apoA-I, and apoA-IV used as primers for PCR or for mRNA identification were synthesized on an Applied Biosystems 380B DNA synthesizer and purified on OPC cartridges (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The sequences of these oligonucleotides are given in Table 1.

RESULTS

Immunologic identification of apolipoprotein E in cultured lipocytes

The medium from lipocytes in early primary culture was tested for the presence of secreted apoE. The proteins within the culture medium were separated by SDS-PAGE, blotted, and probed with an antibody specific for rat apoE. As shown in **Fig. 1**, rat apoE (apparent M_r ~36,000) was identified within the media (middle lane). This antibody did not detect the bovine and equine apoE present in the serum used for the preparation of the medium (left lane). Rat apoE from the lipocyte culture medium had an apparent molecular weight slightly higher than that of the major form of apoE in rat serum (right lane); this may be due to increased sialylation of

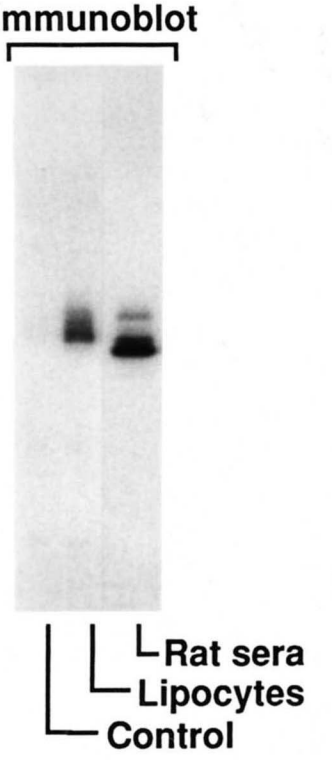

Fig. 1. Immunoblot identifying rat apoE in the culture medium of rat lipocytes. Rat lipocytes in early primary culture were incubated for 48 h in serum-containing medium. Fifty microliters of medium from the lipocytes (middle lane) and 10 μl of rat sera diluted 1:250 (right lane) were analyzed by SDS-PAGE and immunoblotting using a rat apoE-specific antibody. Medium that had not been in contact with cells was used as a control (left lane). Several molecular weight isoforms of apoE are identified in the lipocyte culture medium and in rat sera.

the apoE secreted by lipocytes. Higher-molecular-weight, sialylated forms of apoE have been reported in a variety of tissues, including the liver (34) and vascular smooth muscle (10).

Apolipoprotein E mRNA expression in cultured lipocytes

Lipocytes in early primary culture were tested for apoE mRNA. Total cellular RNA was collected, and cDNA was produced by reverse transcription of mRNA using an oligo-d(T) primer. This cDNA was then amplified by the PCR using primer pairs specific for rat apoE. Analysis of the amplified product by gel electrophoresis (**Fig. 2**) revealed the expected 218-bp fragment, indicating apoE mRNA amplification. Amplification of genomic DNA (which includes intron 3) with the same primer pair resulted in the production of the expected 618-bp DNA fragment, thus demonstrating both that the primers were specific for apoE and that apoE mRNA, not genomic DNA, was being detected in our RNA isolates. Both the 218-bp mRNA fragment and the 618-bp genomic fragment were also identified by a rat apo-E cDNA probe (30) using Southern blots (data not shown).

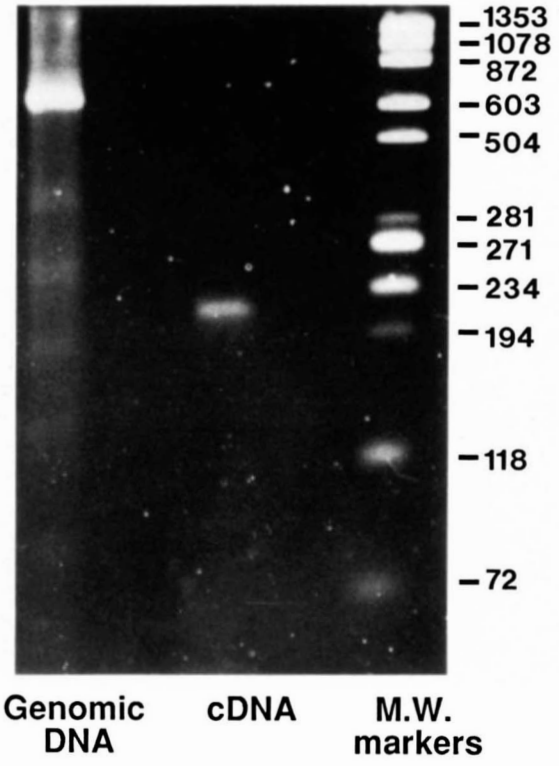

Fig. 2. Identification of apoE mRNA in cultured rat lipocytes. Rat genomic DNA and cDNA made from total lipocyte mRNA were amplified by the PCR using a primer pair specific for rat apoE. The PCR products were electrophoresed in an agarose gel and visualized with ethidium bromide. The expected 618-bp and 218-bp amplification products of the apoE gene and mRNA are visible in the left and center lanes, respectively. The molecular weight (M.W.) markers are visible in the right lane.

Apolipoprotein E secretion by cultured liver cells

We measured the secretion of apoE by cultured lipocytes, hepatocytes, and Kupffer cells to assess their potential contributions to total hepatic apoE production. Both Kupffer cells and hepatocytes are known to secrete apoE in culture (20). Media from early primary cultures of equal numbers of rat lipocytes, Kupffer cells, and hepatocytes were examined by SDS-PAGE and immunoblotting using a rat apoE-specific antibody. Lipocytes, Kupffer cells, and hepatocytes all secreted apoE (**Fig. 3**, left panel). However, the apoE secreted by cultured rat lipocytes and Kupffer cells was of a slightly higher apparent molecular weight than that secreted by hepatocytes. Per cell, the relative secretion of apoE, as determined by densitometry scans of immunoblots, was greatest from hepatocytes, followed by Kupffer cells and then lipocytes. The relative concentration of apoE in each was 2, 1.4, and 1, respectively.

Apolipoprotein A-I and A-IV secretion by cultured liver cells

Ramadori and co-workers (36) recently reported that hepatic lipocytes secrete apolipoproteins A-I and A-IV. To verify this, we immunoblotted culture medium from equal numbers of lipocytes, Kupffer cells, and hepatocytes with antibodies to rat apoA-I and rat HDL. The rat HDL antibody identifies apoA-I at 27 kDa, apoE at 34 kDa, apoA-IV at 45 kDa, β_2 glycoprotein at 58 kDa, and albumin at 66 kDa. As seen in Fig. 3, the rat apoA-I antibody (middle panel) and the rat HDL antibody (right panel) cross-reacted with the apoA-I in the serum of the culture medium. Thus, rat apoA-I secretion could not be determined. Rat apoA-IV (45 kDa), however, was specifically identified by the rat HDL antibody. Apolipoprotein A-IV was found only in medium obtained from cultured hepatocytes. Neither Kupffer cell nor lipocyte culture medium contained apoA-IV.

Apolipoprotein mRNA expression in cultured liver cells

To identify and quantitate the mRNAs specific for apoE, apoA-I, and apoA-IV in cultured hepatic cells, dot blots of total cellular RNA from lipocytes, Kupffer cells, and hepatocytes were used. These were probed with oligonucleotides specific for apoA-IV, and apoA-I, and apoE. The results are shown in **Fig. 4**. Apolipoprotein A-IV and apoA-I mRNAs were detected only in cultured hepatocytes, whereas apoE mRNA was detected in all three cell types. The level of apoE in each cell type was quantitated by densitometry (**Table 2**). Total cellular RNA from cultured lipocytes contained one-third the level of apoE mRNA found in cultured hepatocytes. Because of the larger size of the hepatocyte and the greater yield of total cellular RNA from these cells, however, the actual level of apoE mRNA per cell in lipo-

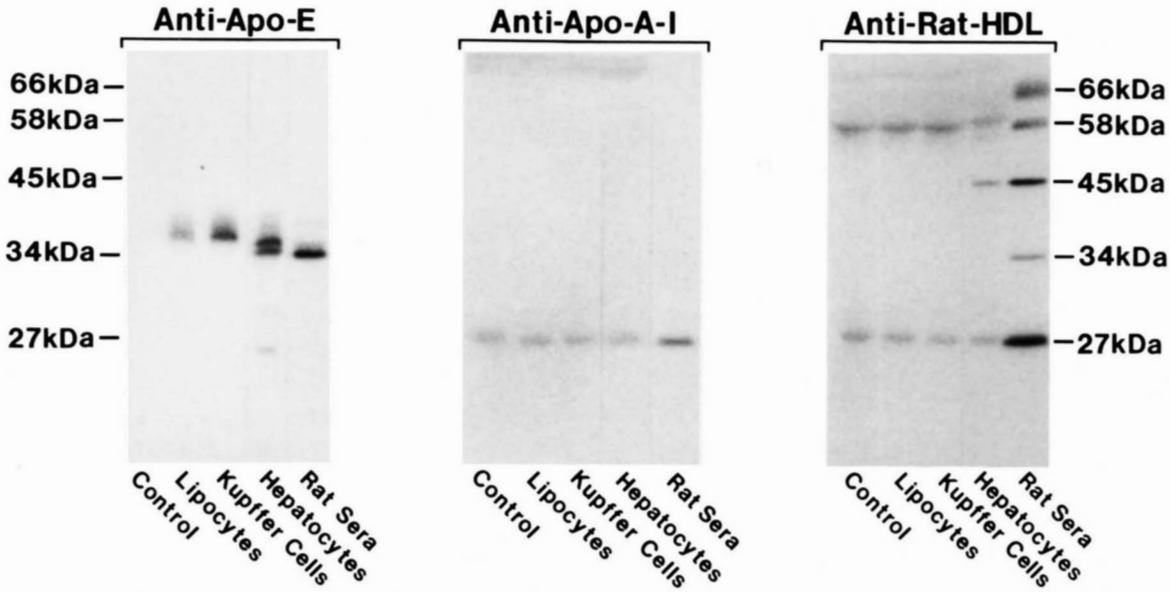

Fig. 3. Immunoblots identifying several rat apolipoproteins in the culture media of rat liver cells. Rat lipocytes, Kupffer cells, and hepatocytes were incubated for 48 h in serum-containing medium. Samples of medium incubated with an equal number of cells were analyzed by SDS-PAGE and immunoblotting using a rat apoE antibody (left panel), a rat apoA-I antibody (middle panel), and a rat HDL antibody (right panel). The rat HDL antibody identifies apoA-IV (45 kDa), apoA-I (27 kDa), albumin (66 kDa), and β_2 glycoprotein (58 kDa). Medium that had not been in contact with cells was used as a control for all antibodies. The rat apoE antibody identified apoE (34 kDa) in the media from all three cell types. The rat apoA-I antibody cross-reacted with apoA-I (27 kDa) in the culture media. The rat HDL antibody cross-reacted with β_2 glycoprotein (58 kDa) and with apoA-I (27 kDa) of the culture media. It identified rat apoA-IV (45 kDa) in medium from hepatocytes only. The rat HDL antibody detects apoE (37 kDa) only weakly, and so at this concentration it could not detect apoE in any of the media, although it does detect apoE in rat sera. Similarly, the rat HDL antibody detected albumin (66 kDa) only very weakly, and so at this concentration it could not detect albumin in the culture media of hepatocytes, particularly because they secrete less albumin with time in culture (35).

cytes was only about one ninth ($\sim 11\%$) of that in hepatocytes.

To confirm that apoA-IV and apoA-I mRNAs were indeed absent from cultured lipocytes and Kupffer cells, these messages were amplified with the PCR. Total cellular RNA was collected from each of the three cell types. After cDNA synthesis using an oligo-d(T) primer, PCR amplification of this mRNA-derived cDNA was performed using primers specific for apoE, apoA-I, and apoA-IV. The PCR technique was used to search for what might be rare apoA-I and apoA-IV messages. The PCR is a sensitive technique, having been shown to be capable of detecting mRNAs from a single cell (37); we reasoned that if mRNAs for apoA-I or apoA-IV were present in the $>1 \times 10^6$ cells we were using, they should be detectable. Analysis of the PCR-amplified products by gel electrophoresis (**Fig. 5**) detected the expected 218-bp DNA fragment corresponding to rat apoE mRNA in all three cultured hepatic cell types. However, using the primer pairs specific for rat apoA-I and apoA-IV, we detected the

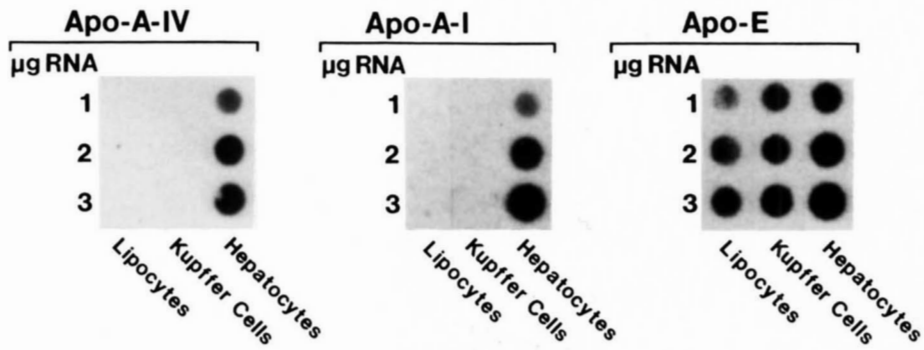

Fig. 4. Autoradiograms of dot blots on which the mRNAs for several apolipoproteins have been identified using labeled probes. Total cellular RNA was isolated from cultured rat lipocytes, Kupffer cells, and hepatocytes; blotted, and hybridized with labeled probes specific for apoA-IV, apoA-I, or apoE mRNA, respectively. Apolipoprotein E mRNA was detected in all three cell types, whereas apoA-I and apoA-IV mRNA were detected only in hepatocytes.

TABLE 2. Quantitation of apolipoprotein E mRNA in cultured liver cells

Cell Type	Relative Concentration of ApoE mRNA per μg of Cellular RNA	Total Yield of Cellular RNA per 10^6 Cells	Relative Concentration of ApoE mRNA per 10^6 cells
Lipocytes	1.0	4.6 μg	1
Kupffer cells	1.6	11.4 μg	4
Hepatocytes	3.0	13.8 μg	9

254-bp DNA fragment corresponding to apoA-I mRNA and 398-bp DNA fragment corresponding to apoA-IV mRNA only in cultured hepatocytes.

DISCUSSION

Apolipoprotein E is a major constituent of plasma lipoproteins in mammals (13, 14). The major site of apoE synthesis is the liver, but apoE is produced in a wide variety of tissues (30). In the rat liver, mRNA encoding apoE has been reported to represent ~1% of the total liver mRNA (20). Both hepatocytes and Kupffer cells have been shown to express apoE (20). In this study, we have shown that a third hepatic cell, the lipocyte, can secrete apoE in substantial quantities. Apolipoprotein E secretion by cultured lipocytes is approximately one-half of that of cultured hepatocytes, on a per cell basis. The concentration of apoE mRNA in the two cell types is also similar: per microgram of total cellular RNA, the concentration of apoE mRNA in cultured lipocytes was found to be approximately one-third of that in cultured hepatocytes.

The contribution of lipocytes to the total production of apoE by the liver is probably minimal. Assuming that the levels of secretion found in our culture system are indicative of those in vivo, one can calculate the probable contribution of lipocytes to hepatic apoE production. Hepatocytes are at least 6-fold more abundant than lipocytes in the liver (38). This relative abundance, combined with our finding that hepatocytes secrete approximately twice the level of apoE, suggests that hepatocytes contribute ~12-fold more apoE than do lipocytes to the total production of hepatic apoE. Thus, our results are in agreement with previous studies that have identified hepatocytes as the major hepatic source of apoE (20).

Hepatocytes, but not lipocytes or Kupffer cells, secrete two additional apolipoproteins, apoA-I, and apoA-IV. No mRNA encoding either of these apolipoproteins was identified in cultured lipocytes and Kupffer cells by dot blot

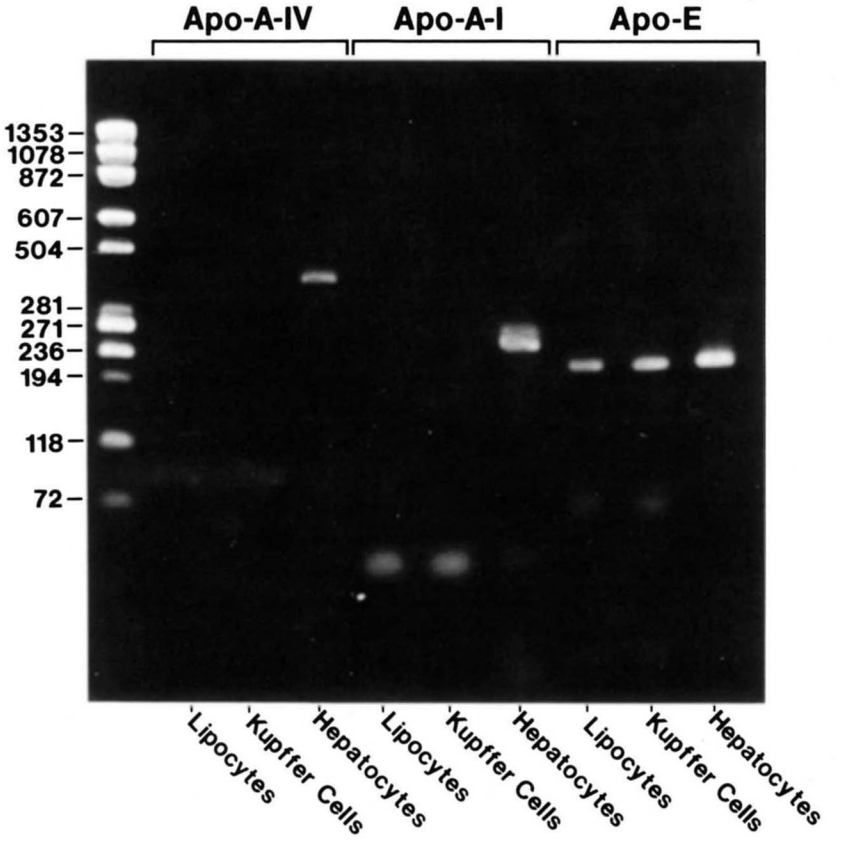

Fig. 5. Identification of apoA-IV mRNA, apoA-I mRNA, and apoE mRNA in cultured cells of the rat liver. Total cellular RNA was isolated from cultured lipocytes, Kupffer cells, and hepatocytes and used to make cDNAs. These were then amplified by the PCR using primer pairs specific for apoA-IV, apoA-I, or apoE RNA, respectively. The PCR reaction products were electrophoresed in an agarose gel, and the amplified products were visualized with ethidium bromide. The expected length of the amplified region of the apoA-IV mRNA is 398 bp; of the apoA-I mRNA, 254 bp; and of the apoE mRNA, 218 bp. Apolipoprotein A-IV and apoA-I mRNAs were identified in hepatocytes only, whereas apoE mRNA was found in all cell types.

analysis. Similarly, using the very sensitive technique of PCR amplification of specific sequences, we detected no mRNA for either apolipoprotein in cultured lipocytes and Kupffer cells. Our results are in contrast to those of Ramadori et al. (36), who presented evidence for the secretion of apoA-I and apoA-IV by lipocyte cultures. This apparent discrepancy remains to be resolved.

The secretion of apoE by lipocytes or the sequestering of other apolipoproteins by these cells could be important in retinoid metabolism. Retinoids absorbed by the intestine are transferred to the liver on large lipoprotein particles, chylomicrons (39). After uptake of these lipoproteins by hepatocytes, the dietary retinoids are transferred to lipocytes (39) for storage (1, 2). Recent experiments by Blomhoff, Berg, and Norum (40) suggest that retinol-binding protein may participate in the transfer of dietary retinol from the hepatocyte to the lipocyte. These investigators found that antibodies to retinol-binding protein block dietary retinol uptake by lipocytes (40). The mechanism of stored retinol mobilization and secretion by lipocytes, however, is unknown. Apolipoproteins, perhaps by virtue of their ability to form and stabilize lipoprotein particles, could help to mediate the transport of retinol into or out of the cell.

The secretion of apoE by lipocytes in early primary culture lends support to the identification of this cell as a vascular smooth muscle cell. Secretion of large amounts of apoE is typical of vascular smooth muscle cells in culture (9–11). It remains for future studies to determine whether lipocyte expression of apoE, like vascular smooth muscle cell expression of apoE, is modulated by the growth state of the cell (9). Similarly, the possible modulation of apoE expression and cell growth in lipocytes by those heparans and heparins capable of regulating apoE expression and cell growth in smooth muscle cells may also be of interest. Finally, the expression of apoE by lipocytes may be useful in distinguishing lipocytes from the portal fibroblasts of the liver, since fibroblasts have not been reported to produce apoE.

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REFERENCES

1. Hendriks, H. F. J., W. A. M. M. Verhoofstad, A. Brouwer, A. M. de Leeuw, and D. L. Knook. 1985. Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver. *Exp. Cell Res.* **160:** 138–149.
2. Wake, K. 1971. "Sternzellen" in the liver: perisinusoidal cells with special reference to storage of vitamin A. *Am. J. Anat.* **132:** 429–462.
3. Friedman, S. L., F. J. Roll, J. Boyles, and D. M. Bissell. 1985. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. *Proc. Natl. Acad. Sci. USA.* **82:** 8681–8685.
4. Saber, M. A., P. M. Novikoff, and D. A. Shafritz. 1990. Albumin and collagen mRNA expression in normal and analbuminemic rodent liver: analysis by in situ hybridization using biotinylated probes. *J. Histochem. Cytochem.* **38:** 199–207.
5. Arenson, D. M., S. L. Friedman, and D. M. Bissell. 1988. Formation of extracellular matrix in normal rat liver: lipocytes as a major source of proteoglycan. *Gastroenterology.* **95:** 441–447.
6. Ramadori, G., H. Rieder, T. Knittel, H. P. Dienes, and K-H. Meyer zum Büschenfelde. 1987. Fat storing cells (FSC) of rat liver synthesize and secrete fibronectin. *J. Hepatol.* **4:** 190–197.
7. Maher, J. J., S. L. Friedman, F. J. Roll, and D. M. Bissell. 1988. Immunolocalization of laminin in normal rat liver and biosynthesis of laminin by hepatic lipocytes in primary culture. *Gastroenterology.* **94:** 1053–1062.
8. Yokoi, Y., T. Namihisa, H. Kuroda, I. Komatsu, A. Miyazaki, S. Watanabe, and K. Usui. 1984. Immunocytochemical detection of desmin in fat-storing cells (Ito cells). *Hepatology.* **4:** 709–714.
9. Majack, R. A., C. K. Castle, L. V. Goodman, K. H. Weisgraber, R. W. Mahley, E. M. Shooter, and P. J. Gebicke-Haerter. 1988. Expression of apolipoprotein E by cultured vascular smooth muscle cells is controlled by growth state. *J. Cell Biol.* **107:** 1207–1213.
10. Hussain, M. M., N. L. R. Bucher, B. Faris, C. Franzblau, and V. I. Zannis, 1988. Tissue-specific posttranslational modification of rat apoE. Synthesis of sialated apoE forms by neonatal rat aortic smooth muscle cells. *J. Lipid Res.* **29:** 915–923.
11. Driscoll, D. M., and G. S. Getz. 1984. Extrahepatic synthesis of apolipoprotein E. *J. Lipid Res.* **25:** 1368–1379.
12. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
13. Mahley, R. W., T. L. Innerarity, S. C. Rall, Jr., and K. H. Weisgraber. 1984. Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res.* **25:** 1277–1294.
14. Mahley, R. W., and T. L. Innerarity. 1983. Lipoprotein receptors and cholesterol homeostasis. *Biochim. Biophys. Acta.* **737:** 197–222.
15. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
16. Stein, O., Y. Stein, M. Lefevre, and P. S. Roheim. 1986. The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from an ether analog of phosphatidylcholine. *Biochim. Biophys. Acta.* **878:** 7–13.
17. Gordon, V., T. L. Innerarity, and R. W. Mahley. 1983. Formation of cholesterol- and apoprotein E-enriched high density lipoproteins in vitro. *J. Biol. Chem.* **258:** 6202–6212.
18. Koo, C., T. L. Innerarity, and R. W. Mahley. 1985. Obligatory role of cholesterol and apolipoprotein E in the formation of large cholesterol-enriched and receptor-active high density lipoproteins. *J. Biol. Chem.* **260:** 11934–11943.

19. Fielding, C. J., J. Frohlich, K. Moser, and P. E. Fielding. 1982. Promotion of sterol efflux and net transport by apolipoprotein E in lecithin:cholesterol acyltransferase deficiency. *Metabolism.* **31:** 1023–1028.
20. Dawson, P. A., L. M. Lukaszewski, P. F. Ells, C. C. Malbon, and D. L. Williams. 1989. Quantification and regulation of apolipoprotein E expression in rat Kupffer cells. *J. Lipid Res.* **30:** 403–413.
21. Friedman, S. L., and F. J. Roll. 1987. Isolation and culture of hepatic lipocytes, Kupffer cells, and sinusoidal endothelial cells by density gradient centrifugation with Stractan. *Anal. Biochem.* **161:** 207–218.
22. Arthur, M. J. P., I. S. Bentley, A. R. Tanner, P. Kowalski Saunders, G. H. Millward-Sadler, and R. Wright. 1985. Oxygen-derived free radicals promote hepatic injury in the rat. *Gastroenterology.* **89:** 1114–1122.
23. Irving, M. G., F. J. Roll, S. Huang, and D. M. Bissell. 1984. Characterization and culture of sinusoidal endothelium from normal rat liver: lipoprotein uptake and collagen phenotype. *Gastroenterology.* **87:** 1233–1247.
24. Bissell, D. M., and P. S. Guzelian. 1980. Phenotypic stability of adult rat hepatocytes in primary monolayer culture. *Ann. N. Y. Acad. Sci.* **349:** 85–98.
25. Arthur, M. J. P., S. L. Friedman, F. J. Roll, and D. M. Bissell. 1989. Lipocytes from normal rat liver release a neutral metalloproteinase that degrades basement membrane (type IV) collagen. *J. Clin. Invest.* **84:** 1076–1085.
26. Boyles, J. K., R. E. Pitas, E. Wilson, R. W. Mahley, and J. M. Taylor. 1985. Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest.* **76:** 1501–1513.
27. Boyles, J. K., C. D. Zoellner, L. J. Anderson, L. M. Kosik, R. E. Pitas, K. H. Weisgraber, D. Y. Hui, R. W. Mahley, P. J. Gebicke-Haerter, M. J. Ignatius, and E. M. Shooter. 1989. A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J. Clin. Invest.* **83:** 1015–1031.
28. Boyles, J. K., L. M. Notterpek, and L. J. Anderson. 1990. Accumulation of apolipoproteins in the regenerating and remyelinating mammalian peripheral nerve: identification of apolipoprotein D, apolipoprotein A-IV, apolipoprotein E, and apolipoprotein A-I. *J. Biol. Chem.* **265:** 17805–17815.
29. Chirgwin, J. M., A. E. Przybyla,, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
30. Elshourbagy, N. A., W. S. Liao, R. W. Mahley, and J. M. Taylor. 1985. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc. Natl. Acad. Sci. USA.* **82:** 203–207.
31. Haddad, I. A., J. M. Ordovas, T. Fitzpatrick, and S. K. Karathanasis. 1986. Linkage, evolution, and expression of the rat apolipoprotein A-I, C-III, and A-IV genes. *J. Biol. Chem.* **261:** 13268–13277.
32. Stoflet, E. S., D. D. Koeberl, G. Sarkar, and S. S. Sommer. 1988. Genomic amplification with transcript sequencing. *Science.* **239:** 491–494.
33. Fukazawa, C., A. Matsumoto, and J. M. Taylor. 1986. Complete nucleotide sequence of the gene encoding rat apolipoprotein E. *Nucleic Acids Res.* **14:** 9527–9528.
34. Zannis, V. I., D. M. Kurnit, and J. L. Breslow. 1982. Hepatic apo-A-I and apo-E and intestinal apo-A-I are synthesized in precursor isoprotein forms by organ cultures of human fetal tissues. *J. Biol. Chem.* **257:** 536–544.
35. Bissell, D. M., D. M. Arenson, J. J. Maher, and F. J. Roll. 1987. Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. *J. Clin. Invest.* **79:** 801–812.
36. Ramadori, G., H. Rieder, F. Theiss, and K-H. Meyer zum Büschenfelde. 1989. Fat-storing (Ito) cells of rat liver synthesize and secrete apolipoproteins: comparison with hepatocytes. *Gastroenterology.* **97:** 163–172.
37. Brenner, C. A., A. W. Tam, P. A. Nelson, E. G. Engleman, N. Suzuki, K. E. Fry, and J. W. Larrick. 1989. Message amplification phenotyping (MAPPing): a technique to simultaneously measure multiple mRNAs from small numbers of cells. *BioTechniques.* **7:** 1096–1103.
38. De Leeuw, A. M., C. F. A. Van Bezooijen, and D. L. Knook. 1986. Changes in the ratio between sinusoidal and parenchymal cell numbers of the rat liver during development and aging. *In* Cells of the Hepatic Sinusoid. Vol. I. A. Kirn, D. L. Knook, and E. Wisse, editors. 463–470.
39. Blomhoff, R., P. Helgerud, M. Rasmussen, T. Berg, and K. R. Norum. 1982. In vivo uptake of chylomicron [^{3}H]retinyl ester by rat liver: evidence for retinol transfer from parenchymal to nonparenchymal cells. *Proc. Natl. Acad. Sci. USA.* **79:** 7326–7330.
40. Blomhoff, R., T. Berg, and K. R. Norum. 1988. Transfer of retinol from parenchymal to stellate cells in liver is mediated by retinol-binding protein. *Proc. Natl. Acad. Sci. USA.* **85:** 3455–3458.