Rabbit liver apolipoprotein A-I synthesis is under nonparenchymal cell paracrine control

Thomas J. Rea, Charles L. Bisgaier, Ronald B. DeMattos, and Michael E. Pape[1]

Atherosclerosis Research, Division of Therapeutics, Parke-Davis Pharmaceutical Research Division, Warner-Lambert, 2800 Plymouth Road, Ann Arbor, MI 48105

Abstract Apolipoprotein A-I (apoA-I), the primary protein of high density lipoprotein, originates from intestine and liver of almost all mammalian species. In contrast to most species, intact rabbit liver is only capable of producing minute amounts of apoA-I mRNA and protein. In this report we demonstrate that purified rabbit hepatic parenchymal cells have apoA-I mRNA levels approximately 50-fold higher than intact liver after 48 h in monolayer culture. Investigations of the differential between in vivo and in vitro expression showed that conditioned media from nonparenchymal cells, a cell population essentially absent in parenchymal cell cultures, inhibited the elevation of apoA-I mRNA in a specific, concentration-dependent, and reversible fashion. Furthermore, at a concentration of nonparenchymal cell-conditioned media that inhibited apoA-I mRNA levels by > 80% compared to control, there were only slight changes in apoB, apoE, LDL receptor, LCAT, 7α-hydroxylase, hepatic lipase, HMG-CoA reductase, and albumin mRNA levels. Metabolic labeling of parenchymal cell secreted proteins with [^{35}S]methionine followed by apoA-I immunoprecipitation revealed that apoA-I synthesis and secretion corresponded to the changes observed for apoA-I mRNA. Initial biochemical characterization of the nonparenchymal cell media revealed that the inhibitory factor was > 30 kDa, heat-stable to 70°C, and still active after urea denaturation and renaturation. These data suggest that, in rabbits, hepatic parenchymal–nonparenchymal communication in the form of a secreted factor may attenuate liver apoA-I expression in vivo.—**Rea, T. J., C. L. Bisgaier, R. B. DeMattos, and M. E. Pape.** Rabbit liver apolipoprotein A-I synthesis is under nonparenchymal cell paracrine control. *J. Lipid Res.*, 1994. **35:** 1274–1282.

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Atherosclerosis is a multifactorial disease that is a leading cause of death in developed countries. Epidemiological data reveal that elevated levels of plasma low density lipoproteins (LDL) are positively correlated while high density lipoproteins (HDL) are negatively correlated with the disease (1, 2). Apolipoprotein A-I is the primary protein moiety of HDL and, thus, may be a major contributor to HDL's protective role in coronary heart disease development (3). Indeed, transgenic mice overexpressing human apoA-I are protected from diet-induced atherosclerosis (4).

ApoA-I intestinal and liver gene expression and metabolism have been studied in several animal models of atherosclerosis. In all species studied, except the rabbit, liver is a major site of apoA-I synthesis as inferred from apoA-I mRNA levels and protein synthesis in that organ (5–13). Published studies have consistently demonstrated that rabbit liver contains at least 100-fold less apoA-I mRNA than small intestine (8–10, 14). Given the low basal levels of apoA-I mRNA in rabbit liver, we explored cell culture models to investigate possible factors limiting its expression.

MATERIALS AND METHODS

Isolation of rabbit liver cell types

Male New Zealand white rabbits (2–2.5 kg; Kuiper, Gary, Indiana) were maintained on a chow diet (ad libitum). All animal procedures were in accordance with the Parke-Davis Animal Treatment Guidelines. Rabbit parenchymal and nonparenchymal cells were isolated using modifications (14) of the two-step method of Seglen (15). Parenchymal cells were further purified by sedimentation through a Percoll gradient (16). The resulting parenchymal cells were washed with media and plated on collagen type I-coated 100-mm dishes in complete media (Collaborative Research). Nonparenchymal cells from supernatants of parenchymal cell washes were pelleted, resuspended in Gey's balanced salt solution (GIBCO BRL, Gaithersburg, MD), and treated with 0.05% pronase E for 1.5 h at 39°C to destroy contaminating parenchymal cells. After protease treatment, nonparenchymal cell populations had less than 0.5% contamination by parenchymal cells as assessed by light microscopy.

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; apoA-I, apolipoprotein A-I; LCAT, lecithin:cholesterol acyltransferase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; CETP, cholesteryl ester transfer protein.

[1]To whom correspondence should be addressed. e-mail: papem@aa.wl.com.

Cells were cultured in HEPES-buffered Dulbecco's modified Eagle media containing 1 × MEM nonessential amino acids, 0.4 mg/ml fructose, 10% heat-inactivated fetal bovine serum, 20 ng/ml epidermal growth factor, 12 nM insulin, 1.6 μM hydrocortisone, 10 μg/ml gentamycin, and 0.2% bovine serum albumin (complete media). In some experiments, specific media components were omitted from complete media. Nonparenchymal cell conditioned media were prepared after 24 h of culture by clarification (centrifugation at 600 *g*, 7 min) and sterile ultrafiltration (0.22 μm cellulose acetate). Washout experiments involved media removal, two rinses with fresh media followed by media replacement.

Quantitation of mRNA levels

Total RNA was isolated and mRNA levels were measured as previously described (17, 18). Autoradiographic images were analyzed using the Molecular Dynamics 400E PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Rabbit mRNA specific probes and internal standards were synthesized from pBluescript II SK (+) plasmids containing partial rabbit cDNAs for the genes of interest. Cloned cDNAs were prepared in polymerase chain reactions using conserved primers based on published homologous sequences (14). In each RNAse protection assay, an internal standard RNA was included to normalize mass values, thus controlling for sample process variation. Resulting data are represented either as single sample points or as the mean plus and minus the standard error of the mean (SEM). Typically, 10–30 pg of internal standard and 30–50 μg total RNA was used in each protection assay. In some internal standard/RNAse protection assays, protected probe fragments migrated as multiple bands upon electrophoretic separation on polyacrylamide (e.g., HMG-CoA reductase, hepatic lipase, albumin). We have observed this phenomenon previously; it is most likely due to secondary structure of the RNA fragments, which could be related to resistance of the probe to RNAse activity (14, 18).

Cellular labeling and immunoprecipitation of rabbit apoA-I

Parenchymal cells were labeled for 4 h with [^{35}S]methionine (50 μ Ci/ml; Amersham). Immunoprecipitation of labeled cell apoA-I was performed by modification of the method of Davidson and Glickman (13). Cell supernatant apoA-I was immunoprecipitated in NETTAM buffer (0.15 M NaCl, 5 mM EDTA, 50 mM Tris-HCl, 0.1% BSA, 1.25% Triton X-100, 0.625% SDS, 2 mM methionine, pH 7.4). Antigen-antibody complexes were collected using protein A Sepharose (Pharmacia). Immunoprecipitated apoA-I was resolved after denaturation with SDS and 2-mercaptoethanol by electrophoresis and autoradiography (4–20% gradient SDS-polyacrylamide gels; Novex).

Biochemical characterization of the inhibitory factor

Concentrated preparations of nonparenchymal cell conditioned media for preliminary biochemical characterization were generated by pressure ultrafiltration through Amicon Diaflo Ultrafilters (YM series). The concentrates were then treated as described in the figure legends. After the various treatments, concentrates were diluted to a final 1 × concentration using complete media and filter-sterilized.

RESULTS

We analyzed rabbit parenchymal cell cultures for the expression of apoA-I and eight other genes whose products are involved in lipid and lipoprotein metabolism. Shown in **Fig. 1** is the mRNA level for each of the corresponding genes in cultured parenchymal cells over 8 days. Unlike intact liver, isolated rabbit parenchymal cells in culture expressed copious amounts of apoA-I mRNA. By 24 h, the apoA-I mRNA levels were 30- to 50-fold higher than in intact liver, a level maintained for at least 8 days. HMG-CoA reductase and LDL-receptor mRNAs were also elevated relative to intact liver levels but to a much lesser extent. The slight elevation of these mRNAs, i.e., relative to apoA-I elevation, was probably related to maintenance of essential cell cholesterol homeostasis. Levels of other mRNAs analyzed either increased slightly (apoE, hepatic lipase), stayed approximately the same (apoB), or decreased slightly (LCAT, albumin) compared to intact liver. Cholesteryl ester transfer protein and 7α-hydroxylase mRNAs were undetectable at any time after culturing parenchymal cells (data not shown). These data suggested that the apoA-I mRNA elevation was specific because the other mRNA levels were either unaffected or changed minimally.

Others have reported that various external factors can influence apoA-I gene expression (19–29). However, changes in apoA-I mRNA levels are relatively refractory to most pharmacological, hormonal, or dietary perturbations with maximum changes of about 2-fold in most cases and 6-fold in rare instances. In contrast, maximum apoA-I mRNA changes in our culture system were one to two orders of magnitude greater than the liver amounts. The possibility existed that an exogenous factor in the culture media mediated the apoA-I mRNA elevation. To explore this issue, isolated parenchymal cells were cultured with exclusion of specific media components. As shown in **Fig. 2A,** cells in either serum or serum-free media, in which either epidermal growth factor, insulin, or hydrocortisone was deleted, still displayed a 25- to 45-fold increase in apoA-I mRNA after 24 h as compared to intact liver levels. Furthermore, 10% serum from chow-fed or cholesterol-fed rabbits did not affect the apoA-I mRNA elevation (Fig. 2B). We also plated hepatocytes onto

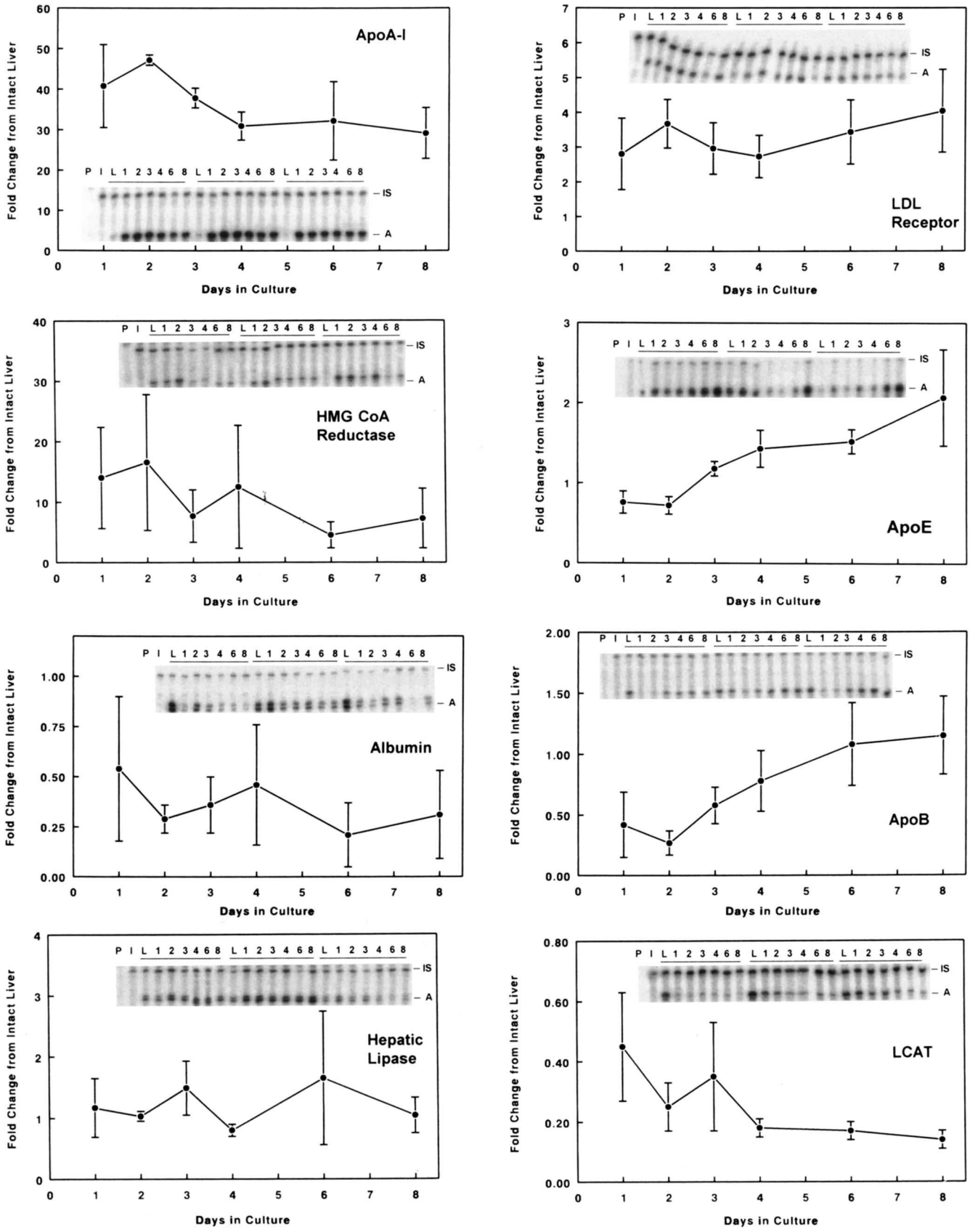


Fig. 1. mRNA levels in cultured parenchymal cells. Purified rabbit liver parenchymal cells were cultured on collagen type I matrix in complete media. Inset shows primary data from RNAse protection assays for mRNA isolated from cells cultured from three separate rabbit livers for up to 8 days. All mRNA mass values were normalized by plotting the fold change relative to liver mRNA mass for each gene. Plotted data show the mean and standard error of the mean for each time point (n = 3). "P" indicates control hybridization reactions that include only the specific probe for each gene. "I" indicates control hybridization reactions including probe and internal standard RNA. "L" indicates liver tissue samples. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

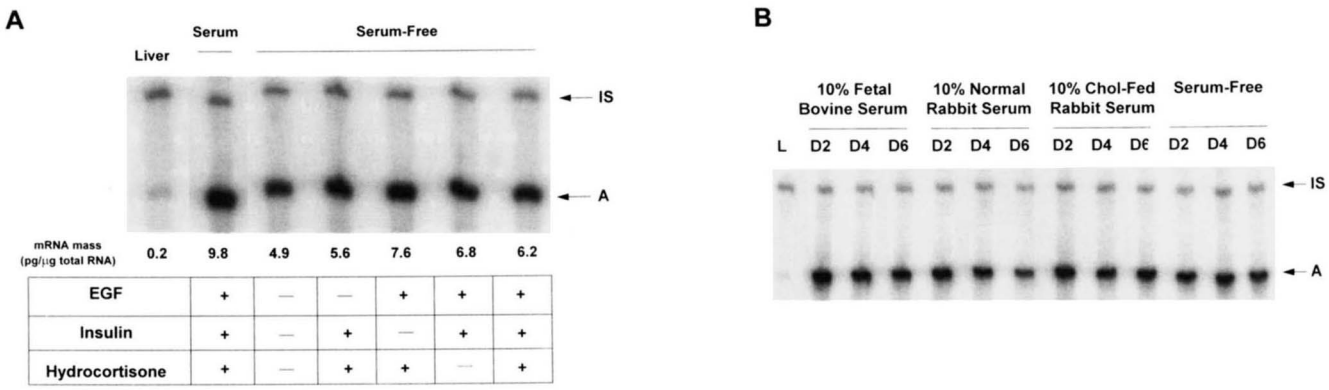


Fig. 2. Effect of media components on the expression of apoA-I mRNA in parenchymal cells. Purified primary rabbit hepatic parenchymal cells were isolated and cultured in various media on collagen I-coated plates. After the defined treatments, total RNA was isolated and apoA-I mRNA was assayed as in Fig. 1. Panel A: parenchymal cells were cultured for 24 h on collagen type I matrix in culture media modified by exclusion of a specific hormone or growth factor supplement. Grid legend indicates media modification. Media contained all other supplements described in Materials and Methods. Panel B: parenchymal cells were cultured for 2, 4, and 6 days (D2, D4, D6, respectively) in complete media with one of the following: 10% fetal bovine serum, normal rabbit serum, cholesterol-fed rabbit serum, or no serum. "L" shows liver tissue sample. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

different matrices (collagen I, collagen type IV, laminin, fibronectin) in an attempt to identify culture matrix variables contributing to the induction. Induction of apoA-I mRNA was observed in all cases (data not shown). These data suggested that apoA-I mRNA induction in the parenchymal cells was not solely due to media or other culture components, but primarily related to their removal from the in vivo liver environment.

Approximately 35% of liver cells are nonparenchymal (Kupffer cells, endothelial cells, lipocytes; (30)), which primarily line the sinusoids of the organ. These sinusoidal lining cells are essentially removed during our parenchymal cell isolation procedures. This fact led us to hypothesize that a nonparenchymal cell interaction (e.g., tight junctions), matrix component, or soluble factor inhibited the expression of apoA-I mRNA in liver. To test this hypothesis, apoA-I mRNA levels were measured in co-cultures of hepatic parenchymal and nonparenchymal cells. These cultures displayed steady-state apoA-I mRNA levels that were similar to amounts observed in intact liver (data not shown). To determine whether nonparenchymal cells secrete a soluble paracrine factor(s) that inhibited apoA-I mRNA expression, we incubated isolated parenchymal cells with nonparenchymal cell conditioned media. A concentration-dependent decrease in apoA-I mRNA accumulation was observed in parenchymal cells after a 24-h exposure (**Fig. 3**). This effect was reversible as evidenced by the accumulation of apoA-I mRNA when nonparenchymal cell conditioned media was removed and replaced with fresh media. In an alternative concentration effect experiment, we plated different numbers of nonparenchymal cells per 100-mm plate, collected the conditioned media, and exposed parenchymal cells to this media. A concentration-dependent response to the inhibitory activity was also observed in this experiment (**Fig. 4**). We have observed in numerous experiments a marked elevation of apoA-I mRNA in parenchymal cells after 24 h in culture relative to liver, as well as a marked inhibition of apoA-I mRNA accumulation in response to nonparenchymal cell conditioned media (**Table 1**). Thus, it appeared that nonparenchymal cells secreted a factor that inhibited apoA-I mRNA accumulation in parenchymal cells.

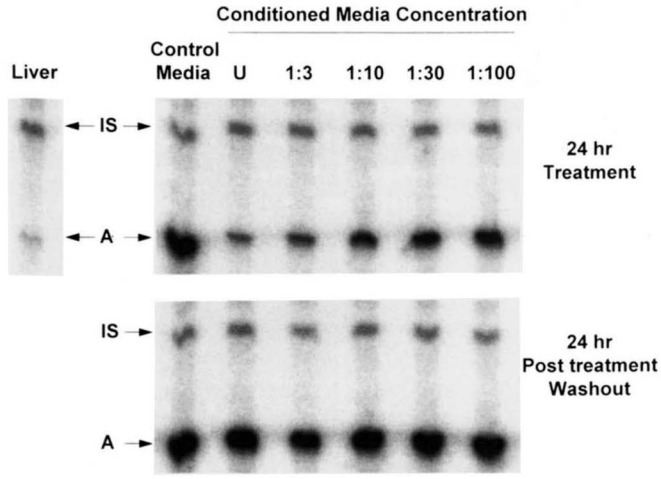


Fig. 3. Decrease in apoA-I mRNA induction is concentration-dependent and reversible. Upper panel: primary hepatocytes were cultured for 24 h in the presence of undiluted ("U") or various dilutions (1:*n*, where *n* is the fold dilution) of nonparenchymal cell conditioned media (24-h treatment). Lower panel: in a duplicate set of hepatocytes, after the 24-h treatment, culture media was removed and replaced with complete media (washout). For both sample series RNA was harvested from the cells and analyzed as described in Fig. 1 for apoA-I mRNA content. RNA from liver tissue (L) and control media cultures are shown for comparison. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

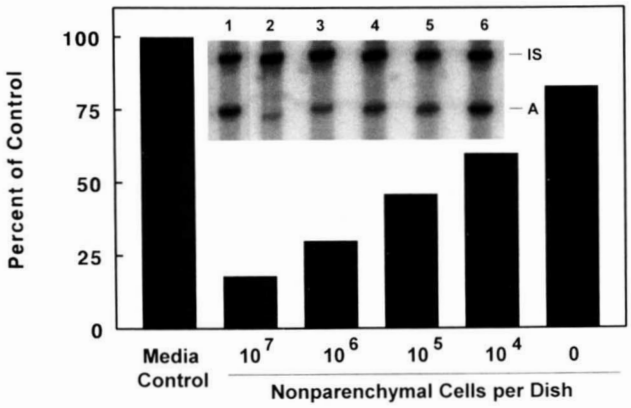


Fig. 4. Dose-dependent decrease in apoA-I mRNA based on nonparenchymal cell number. Rabbit liver nonparenchymal cells were plated at various densities on collagen type I-coated plates. Conditioned media from these cells was used for culture of freshly prepared hepatocytes. ApoA-I RNA content was analyzed after a 24-h incubation. Inset shows protection assay primary data from media control (lane 1) and decreasing numbers of nonparenchymal cells per 100-mm dish from 10^7 cells (lane 2) to no cells (lane 6). Bar graph shows apoA-I mass as percentage of control. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

Our initial characterization of cultured hepatic parenchymal cells demonstrated that a variety of plasma lipid regulating genes were functional in vitro. To determine whether the attenuation of cultured hepatocyte apoA-I mRNA levels by nonparenchymal cell conditioned media was specific to that particular gene product or, instead, a generalized phenomenon for many hepatocyte functions, we determined the steady state mRNA levels of nine genes expressed by the rabbit hepatocyte in the presence or absence of nonparenchymal cell conditioned media (**Fig. 5**). Only apoA-I mRNA mass changed significantly in the presence of nonparenchymal cell conditioned media. These data show the specificity for apoA-I modulation among the category of genes we have tested.

To determine whether apoA-I secretion rates changed concomitantly with apoA-I mRNA levels, radiolabeled apoA-I was immunoprecipitated from parenchymal cell supernatant after a 4-h incubation in either control or nonparenchymal cell-conditioned media and [^{35}S]methionine. Cells grown in control media showed appreciable levels of apoA-I synthesis; however, detectable amounts of apoA-I were not observed in cells treated with nonparenchymal cell-conditioned media (**Fig. 6,** left panel). The reduction in apoA-I secretion/synthesis appears to be greater than the reduction in apoA-I mRNA levels (Fig. 6, right panel) suggesting an additional level of translational or post-translational control for apoA-I expression in this system. These data demonstrated that the level of apoA-I synthesis/secretion was concordant with apoA-I mRNA levels in hepatic parenchymal cells.

To investigate the nature of the inhibitory activity, we performed several preliminary biochemical studies. In two separate experiments, we estimated the molecular weight of the factor by filtering nonparenchymal cell conditioned media through molecular weight cutoff membranes. As shown in **Fig. 7,** the factor is greater than 30,000 daltons. Further studies revealed that the factor was still active after heating at 70°C (**Fig. 8**) and after exposing to protein denaturing concentrations of urea followed by dialysis (data not shown). Treatment of concentrated nonparenchymal cell conditioned media with proteinase K coupled to oxirane-activated macroporous acrylic beads did not abolish its ability to inhibit apoA-I mRNA accumulation in cultured parenchymal cells (data not shown).

DISCUSSION

We initially chose the rabbit hepatocyte model to study apoA-I mRNA metabolism because of the low levels of that mRNA in vivo. The advantage of low levels of the mRNA, we reasoned, was that it provided an ideal model

TABLE 1. ApoA-I mRNA levels in rabbit liver, cultured parenchymal cells (PC), and cultured parenchymal cells treated with nonparenchymal cell (NPC)-conditioned media

Tissue/Cell-Treatment (n = 12)[a]	ApoA-I mRNA (pg/μg total RNA)[b]
Intact liver	0.26 ± 0.03
Cultured PC	4.68 ± 1.22[c]
Cultured PC + NPC-conditioned media	0.99 ± 0.22[d]

[a] PC from 12 different rabbits were isolated as described in Materials and Methods and cultured for 24 h either in the presence or absence of NPC-conditioned media obtained from 12 other rabbits. A liver sample was obtained from each rabbit before beginning PC isolation. ApoA-I mRNA levels were measured using an internal standard/RNAse protection assay.
[b] Mean ± SEM.
[c] $P < 0.01$ vs. intact liver value.
[d] $P < 0.01$ vs. cultured PC value.

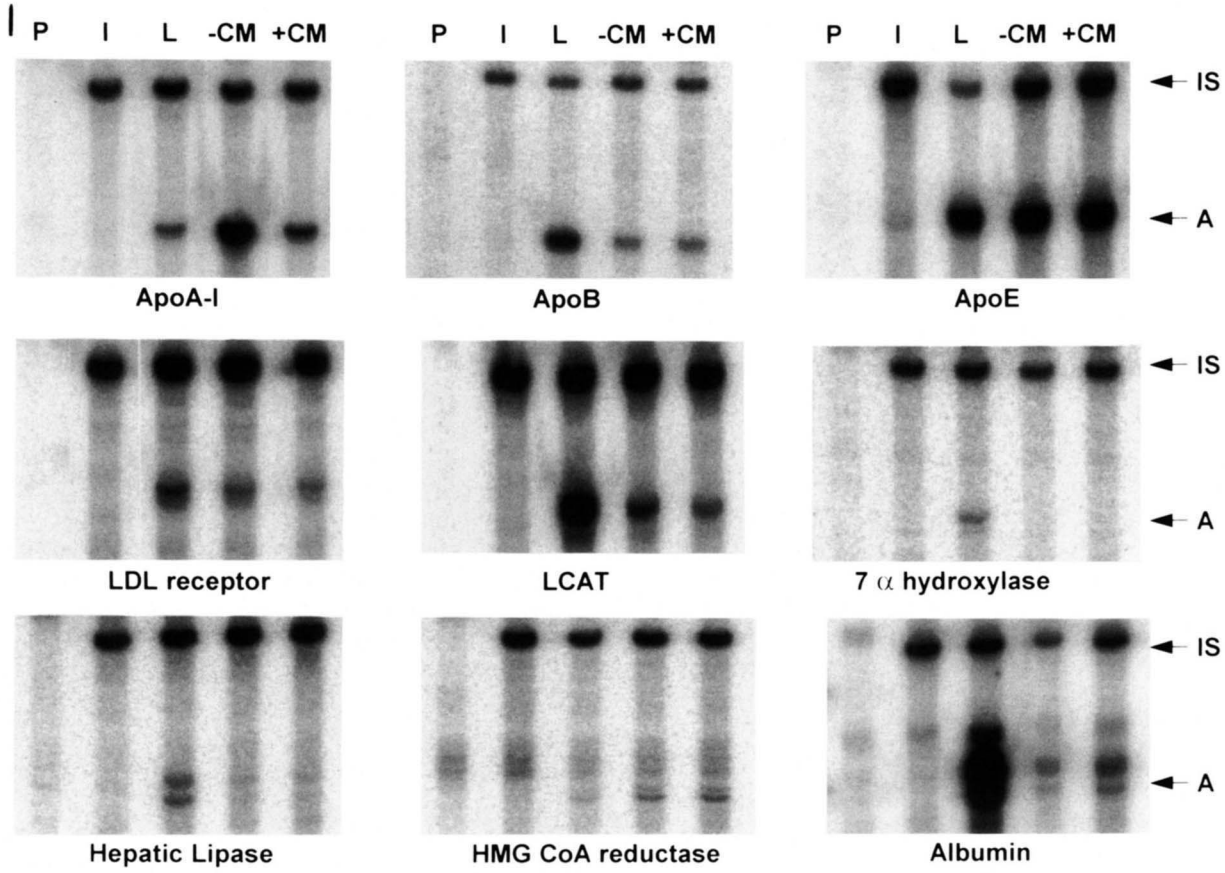
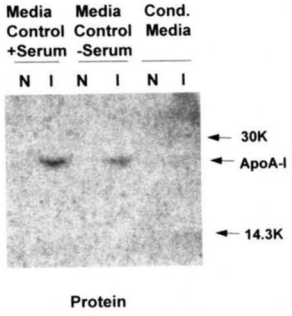


Fig. 5. Nonparenchymal cell inhibition of apoA-I mRNA elevation is specific. Primary rabbit hepatocytes were cultured for 24 h in the absence (−CM) or presence (+CM) of nonparenchymal cell conditioned media. RNA was then purified and analyzed for each of the genes shown. "P" indicates control hybridization reactions that include only the specific probe for each gene. Lane identifier "I" indicates control hybridization reactions including probe and internal standard RNA. "L" indicates liver tissue samples. "IS" and "A" adjacent to arrows mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.



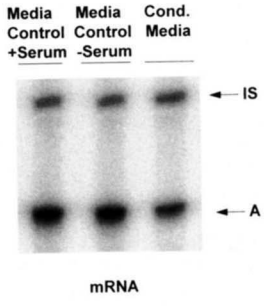


Fig. 6. Effect of nonparenchymal cell conditioned media on apoA-I synthesis and secretion in parenchymal cells. Equal trichloroacetic acid-precipitable dpm from labeled 48-h cell supernatants (control media plus or minus serum or nonparenchymal cell conditioned media) were immunoprecipitated with nonimmune sheep serum (N) or sheep anti-rabbit apoA-I serum (I) and the resulting immune complexes were resolved by gel electrophoresis and autoradiography ("Protein"). ApoA-I mRNA mass determined in a duplicate set of plates is shown in "mRNA" panel. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

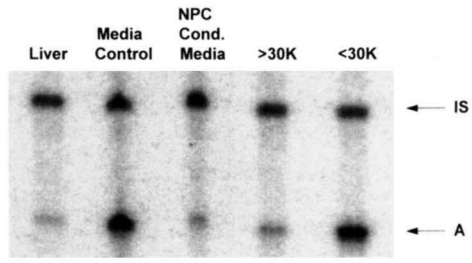


Fig. 7. Molecular weight estimate of nonparenchymal cell apoA-I mRNA inhibitory activity. Pooled nonparenchymal cell conditioned media was fractionated using pressure ultrafiltration through a 30,000 molecular weight cutoff filter. Retained and flow-through fractions were mixed in complete media to 1 × concentration for 24-h culture of freshly isolated primary hepatocytes. ApoA-I mRNA levels were measured and compared to liver, complete media, and nonparenchymal cell conditioned media controls. "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

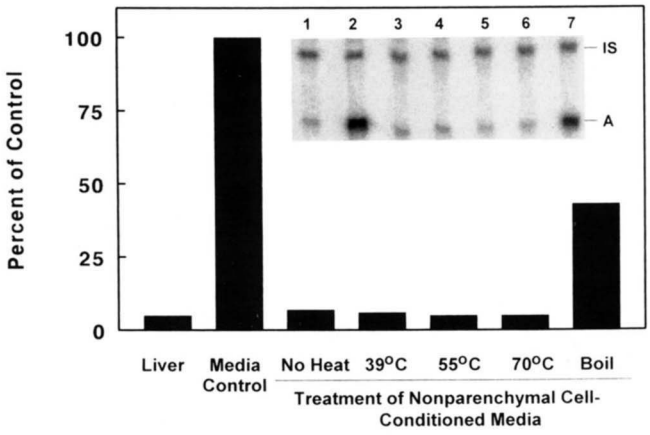


Fig. 8. Heat stability of nonparenchymal cell apoA-I mRNA inhibitory activity. Concentrated nonparenchymal cell conditioned media was incubated for 30 min at various temperatures. After the incubations the concentrates were filter-sterilized and diluted in fresh complete media and used for plating primary hepatocytes. RNA was harvested at 24 h and apoA-I mRNA content was analyzed. Inset shows primary data from protection assay from liver tissue (lane 1), media control (lane 2), no heat treatment (lane 3), 39°C (lane 4), 55°C (lane 5), 70°C (lane 6), or boiling (lane 7). "IS" and "A" mark the electrophoretic migration of probe protected by the internal standard and authentic mRNA, respectively.

for finding various nutritional, hormonal, or pharmacological states that would allow detection of elevated levels of the mRNA, a therapeutically desirable state because it could presumably lead to an increase in apoA-I plasma levels. Contrary to our expectations, placing the rabbit hepatic parenchymal cells in monolayer culture resulted in a tremendous increase in apoA-I mRNA levels compared to liver, a phenomenon not observed, in similar magnitude or direction, for other genes whose protein products are involved in lipid and lipoprotein metabolism. The slight increases observed in HMG-CoA reductase and LDL-receptor mRNAs reflect increased cholesterol synthesis and influx rates caused by the requirement of cells to maintain essential cholesterol levels. These results clearly demonstrate that rabbit hepatic parenchymal cells have the intrinsic ability to synthesize apoA-I. The potential capacity of rabbit parenchymal cells to produce apoA-I mRNA after 2 days in culture is approximately 5 pg/μg total RNA. This is less than small intestine (50 pg/μg), the major apoA-I-producing organ in the rabbit (8–10, 14). However, this capacity is far greater than that seen in vivo, where liver apoA-I mRNA levels are 200- to 500-fold less than small intestine. These data led us to inquire why the in vivo rabbit liver apoA-I mRNA phenotype (low steady state levels) was lost upon culturing the parenchymal cells.

Our data suggest that parenchymal cells in culture do not maintain the in vivo apoA-I phenotype because they no longer reside in close proximity to nonparenchymal cells (sinusoidal lining cells) as is the case in their native environment. The essential controlling element is presumably a secreted factor that is heat- and urea-stable and derived from rabbit nonparenchymal cells. Removal of this putative paracrine regulatory factor via isolation of hepatocytes from the liver results in significant and gene-specific increases of both apoA-I-specific mRNA and protein. Upon addition of nonparenchymal cell conditioned media, the apoA-I mRNA in vitro induction is attenuated. Examination of steady state mRNA levels for apoB, apoE, LDL-receptor, LCAT, 7α-hydroxylase, hepatic lipase, HMG-CoA reductase, and albumin showed that none of these gene products were similarly modulated in the presence of nonparenchymal cell media, arguing that this mechanism of control is not a generalized transcriptional or post-transcriptional regulation artifact or genotoxic effect. Other features of the nonparenchymal cell activity are that it is reversible, elicits a specific mRNA change that is concentration-dependent and leads to commensurate changes in the secreted protein encoded by the mRNA, and is relatively stable to classical denaturation treatments of heat or urea. It should be noted that conditioned media from other cell types such as fibroblasts (from a rabbit hepatic tumor) or parenchymal cells do not inhibit the accumulation of apoA-I mRNA in parenchymal cells (T. J. Rea and M. E. Pape, unpublished observation). Also, primary cultures of rabbit nonparenchymal cells do lose the ability to produce this regulatory activity after extended time in culture, suggesting loss of the differentiated hepatic phenotype in the source cells. In summary, these observations lead us to conclude that the low levels of hepatic apoA-I expression characteristic of the rabbit are the consequence of specific hepatic paracrine cell communication.

Whether a similar mechanism operates to regulate apoA-I expression in other species or tissues is presently unresolved. Castle et al. (31) have shown that monkey (*Macaca fascicularis*) hepatocytes can synthesize apoA-I mRNA ($\sim$40 pg/μg) and protein in culture. The fact that mRNA and protein secretion levels in these parenchymal cells are similar to those in intact liver suggests that an equivalent nonparenchymal cell factor(s) or a component of its signal transduction mechanism may not exist in primates (31). However, those studies were performed with cryopreserved hepatocytes and not freshly isolated cells which may compromise the cells in a way that impacts their capacity to express apoA-I. In contrast, preliminary data from experiments using cultured mouse hepatocytes from one strain (BALB/c) have also shown apoA-I mRNA elevation (K. Reue and M. Doolittle, personal communication); the restoration of the in vivo apoA-I mRNA phenotype through use of nonparenchymal cells or their conditioned media has not been attempted in that system. Experiments to evaluate circulating plasma levels of the nonparenchymal cell effector(s) and its possible effects on other tissues (e.g., small intestine apoA-I synthesis) de-

pend on purification and in vitro delivery methods still in development.

Several hormonal, pharmacological, and pathophysiological states cause changes in apoA-I mRNA levels. For instance, in a chemically induced liver regeneration model, Panduro et al. (24) showed that acute or chronic CCl_4 administration to hypothyroid rats elevated apoA-I mRNA levels 3- to 4-fold. Furthermore, triiodothyronine administration to rats elevates apoA-I mRNA levels (32); to our knowledge a similar effect of this hormone on apoA-I levels in cultured parenchymal cells has not been reported. It is possible these agents may not be acting directly on parenchymal cells to elevate apoA-I mRNA levels but, rather, indirectly by altering the expression of a nonparenchymal cell-derived factor. Indeed, CCl_4 treatment is known to dramatically alter the properties and metabolism of sinusoidal lining cells (33, 34). Future studies will need to focus on the presence, absence, and relative concentration of a nonparenchymal cell-derived apoA-I inhibitory factor in other species.

The role of hepatic nonparenchymal cells in controlling lipid metabolism is not clear. However, recent work has shown that nonparenchymal cells express LDL and scavenger receptors (35, 36), secrete apolipoproteins (37), and, furthermore, are the principle source of CETP in nonhuman primates (38). Knowledge of these functions and the culture system described here will permit studies on parenchymal–nonparenchymal cell communication and its role in lipid metabolism. Indeed, this report is the first demonstration of the existence of a paracrine system within the liver that alters the expression of genes involved in lipoprotein metabolism. We hypothesize that other genes affecting lipoprotein metabolism may display altered expression in response to secreted parenchymal or nonparenchymal cell factors.

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REFERENCES

1. Kannel, W. B., W. P. Castelli, and T. Gordon. 1979. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann. Intern. Med.* **90:** 85–91.
2. Gordon, D. J., J. L. Probstfield, R. J. Garrison, J. D. Neaton, W. P. Castelli, J. D. Knoke, D. R. J. Jacobs, S. Bangdiwala, and H. A. Tyroler. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* **79:** 8–15.
3. Maciejko, J. J., D. R. Holmes, B. A. Kottke, A. R. Zinsmeister, D. M. Dinh, and S. J. Mao. 1983. Apolipoprotein A-I as a marker of angiographically assessed coronary-artery disease. *N. Engl. J. Med.* **309:** 385–389.
4. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. *Nature.* **353:** 265–267.
5. Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams. 1988. Apolipoprotein (apo) A-I production and mRNA abundance explain plasma apoA-I and high density lipoprotein differences between two nonhuman primate species with high and low susceptibilities to diet-induced hypercholesterolemia. *J. Biol. Chem.* **263:** 5183–5189.
6. 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.
7. Rajavashisth, T. B., P. A. Dawson, D. L. Williams, J. E. Shackleford, H. Lebherz, and A. J. Lusis. 1987. Structure, evolution, and regulation of chicken apolipoprotein A-I. *J. Biol. Chem.* **262:** 7058–7065.
8. Lenich, C., P. Brecher, S. Makrides, A. Chobanian, and V. I. Zannis. 1988. Apolipoprotein gene expression in the rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues. *J. Lipid Res.* **29:** 755–764.
9. Pan, T. C., Q. L. Hao, T. T. Yamin, P. H. Dai, B. S. Chen, S. L. Chen, P. A. Kroon, and Y. S. Chao. 1987. Rabbit apolipoprotein A-I mRNA and gene. Evidence that rabbit apolipoprotein A-I is synthesized in the intestine but not in the liver. *Eur. J. Biochem.* **170:** 99–104.
10. Chao, Y. S., T. T. Yamin, G. M. Thompson, and P. A. Kroon. 1984. Tissue-specific expression of genes encoding apolipoprotein E and apolipoprotein A-I in rabbits. *J. Biol. Chem.* **259:** 5306–5309.
11. Windmueller, H. G., and A. L. Wu. 1981. Biosynthesis of plasma apolipoproteins by rat small intestine without dietary or biliary fat. *J. Biol. Chem.* **256:** 3012–3016.
12. Davidson, N. O., A. M. Magun, T. A. Brasitus, and R. M. Glickman. 1987. Intestinal apolipoprotein A-I and B-48 metabolism: effects of sustained alterations in dietary triglyceride and mucosal cholesterol flux. *J. Lipid Res.* **28:** 388–402.
13. Davidson, N. O., and R. M. Glickman. 1985. Apolipoprotein A-I synthesis in rat small intestine: regulation by dietary triglyceride and biliary lipid. *J. Lipid Res.* **26:** 368–379.
14. Rea, T. J., R. B. DeMattos, and M. E. Pape. 1993. Hepatic expression of genes regulating lipid metabolism in rabbits. *J. Lipid Res.* **34:** 1901–1910.
15. Seglen, P. O. 1976. Preparation of isolated rat liver cells. *Methods Cell Biol.* **13:** 29–83.
16. Kreamer, B. L., J. L. Staecker, N. Sawada, G. L. Sattler, M. T. Hsia, and H. C. Pitot. 1986. Use of a low-speed, iso-density percoll centrifugation method to increase the viability of isolated rat hepatocyte preparations. *In Vitro Cell Dev. Biol.* **22:** 201–211.
17. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
18. Pape, M. E., G. W. Melchior, and K. R. Marotti. 1991. mRNA quantitation by a simple and sensitive RNAse protection assay. *Genet. Anal.* **8:** 206–213.
19. Tam, S-P. 1991. Effects of gemfibrozil and ketoconazole on human apolipoprotein A-I, B and E levels in two hepatoma cell lines, HepG2 and Hep3B. *Atherosclerosis.* **91:** 51–61.
20. Varma, V. K., T. K. Smith, M. Sorci-Thomas, and W. H. Ettinger, Jr. 1992. Dexamethasone increases apolipoprotein A-I concentrations in medium and apolipoprotein A-I

mRNA abundance from HepG2 cells. *Metabolism.* **41:** 1075–1080.
21. Kaptein, A., L. Roodenburg, and H. M. Princen. 1991. Butyrate stimulates the secretion of apolipoprotein (apo) A-I and apoB-100 by the human hepatoma cell line HepG2. Induction of apoA-I mRNA with no change of apoB-100 mRNA. *Biochem. J.* **278:** 557–564.
22. Amarasuriya, R. N., A. K. Gupta, M. Civen, Y-C. Horng, T. Maeda, and M. L. Kashyap. 1992. Ethanol stimulates apolipoprotein A-I secretion by human hepatocytes: implications for a mechanism for atherosclerosis protection. *Metabolism.* **41:** 827–832.
23. Radosavljevic, M., Y. Lin-Lee, S. M. Soyal, W. Strobl, C. Seelos, A. M. Gotto, Jr., and W. Patsch. 1992. Effect of sucrose diet on expression of apolipoprotein genes A-I, C-III and A-IV in rat liver. *Atherosclerosis.* **95:** 147–156.
24. Panduro, A., Y. C. Lin-Lee, L. Chan, and D. A. Shafritz. 1990. Transcriptional and posttranscriptional regulation of apolipoprotein E, A-I, and A-II gene expression in normal rat liver and during several pathophysiologic states. *Biochemistry.* **29:** 8430–8435.
25. Apostolopoulos, J. J., G. J. Howlett, and N. Fidge. 1987. Effects of dietary cholesterol and hypothyroidism on rat apolipoprotein mRNA metabolism. *J. Lipid Res.* **28:** 642–648.
26. Seishima, M., C. L. Bisgaier, S. L. Davies, and R. M. Glickman. 1991. Regulation of hepatic apolipoprotein synthesis in the 17 α-ethinyl estradiol-treated rat. *J. Lipid Res.* **32:** 941–951.
27. Tam, S. P., T. K. Archer, and R. G. Deeley. 1985. Effects of estrogen on apolipoprotein secretion by the human hepatocarcinoma cell line, HepG2. *J. Biol. Chem.* **260:** 1670–1675.
28. Masumoto, A., S. Koga, E. Uchida, and H. Ibayashi. 1988. Effects of insulin, dexamethasone and glucagon on the production of apolipoprotein A-I in cultured rat hepatocytes. *Atherosclerosis.* **70:** 217–223.
29. Davidson, N. O., R. C. Carlos, M. J. Drewek, and T. G. Parmer. 1988. Apolipoprotein gene expression in the rat is regulated in a tissue-specific manner by thyroid hormone. *J. Lipid Res.* **29:** 1511–1522.
30. Blomhoff, R., and T. Berg. 1990. Isolation and cultivation of rat liver stellate cells. *Methods Enzymol.* **190:** 58–71.
31. Castle, C. K., M. E. Pape, K. R. Marotti, and G. W. Melchior. 1991. Secretion of pre-beta-migrating apoA-I by cynomolgus monkey hepatocytes in culture. *J. Lipid Res.* **32:** 439–447.
32. Strobl, W., N. L. Gorder, Y. C. Lin-Lee, A. M. J. Gotto, and W. Patsch. 1990. Role of thyroid hormones in apolipoprotein A-I gene expression in rat liver. *J. Clin. Invest.* **85:** 659–667.
33. Greenwel, P., M. Schwartz, M. Rosas, S. Peyrol, J-A. Grimaud, and M. Rojkind. 1991. Characterization of fat-storing cell lines derived from normal and CCl_4-cirrhotic livers: differences in the production of interleukin-6. *Lab. Invest.* **65:** 644–653.
34. Tamayo-Perez, R. 1983. Is chirrhosis of the liver experimentally produced by CCl_4 an adequate model of human cirrhosis? *Hepatology.* **3:** 112–120.
35. Kleinherenbrink-Stins, M. F., J. H. Van de Boom, D. Schouten, P. J. M. Roholl, M. N. Van der Heyde, A. Brouwer, T. J. C. V. Berkel, and D. L. Knook. 1991. Visualization of the interaction of native and modified lipoproteins with parenchymal, endothelial and Kupffer cells from human liver. *Hepatology.* **14:** 79–90.
36. Nenseter, M. S., O. Myklebost, R. Blomhoff, C. A. Drevon, A. Nilsson, K. R. Norum, and T. Berg. 1989. Low-density-lipoprotein receptors in different rabbit liver cells. *Biochem. J.* **261:** 587–593.
37. Ramadori, G., H. Rieder, F. Theiss, and K. H. Meyer zum Buschenfelde. 1989. Fat-storing (Ito) cells of rat liver synthesize and secrete apolipoproteins: comparison with hepatocytes. *Gastroenterology.* **97:** 163–172.
38. Pape, M. E., R. G. Ulrich, T. J. Rea, K. R. Marotti, and G. W. Melchior. 1991. Evidence that the nonparenchymal cells of the liver are the principal source of cholesteryl ester transfer protein in primates. *J. Biol. Chem.* **266:** 12829–12831.