Very Low Density Lipoprotein Synthesis and Secretion by Cultured Rat Hepatocytes

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Hepatocytes were obtained by collagenase perfusion of adult rat liver in situ and plated as monolayers in plastic culture dishes. [3H]Glycerol was actively incorporated into cellular triglycerides and phospholipids which were rapidly secreted into the culture medium. More than 95% of the 3H-triglyceride secreted into the medium was recovered in the very low density lipoprotein (VLDL) fraction (d < 1.006 g/ml). Newly secreted VLDL contained two major apoproteins, apoprotein B and apoprotein E. Compared to serum VLDL, newly secreted VLDL contained very low concentrations of C apoproteins and of cholesterol esters.

The incorporation of [3H]leucine into the low density lipoprotein (LDL) fraction (d = 1.02 to 1.07) was measured to evaluate the possibility that the liver directly synthesizes LDL. No radioactivity precipitable by a rabbit antiserum made against rat serum LDL was found in the d = 1.02 to 1.07 fraction in contrast, there was substantial incorporation of [3H]leucine into VLDL protein precipitable by the same antiserum. These data suggest that there is no direct synthesis of LDL by cultured rat hepatocytes.

The synthesis and secretion of VLDL by cultured hepatocytes were sensitive to several factors. Within 3 h of the addition of 10⁻⁸ M cholchicine, ³H-triglyceride secretion was inhibited by 50%. Cycloheximide (10⁻⁵ M) inhibited both ³H-phospholipid and ³H-triglyceride synthesis by 30%, whereas the secretion of newly synthesized phospholipid and triglyceride was inhibited by 55%. Hepatocytes from rats previously fed a sucrose diet secreted VLDL at twice the rate of cells that had been 3 days in culture, showing that carbohydrate feeding induces persistent changes in the biosynthetic system. Addition of orotic acid (6.4 mM) to the medium of cells obtained from sucrose-fed rats inhibited VLDL secretion by 50%, an effect prevented by the simultaneous addition of adenine (7.4 mM). These results demonstrate for the first time that orotic acid can act directly on the hepatocyte to inhibit VLDL secretion.

Triglyceride is secreted by the liver into the serum predominantly in the form of VLDL (1, 2). Serum VLDL is catabolized in a sequence of steps catalyzed by lipoprotein lipase located on the endothelial cell surface (3–5). The lipids liberated during this process are then utilized in the form of free fatty acids and monoglyceride for energy by extrahepatic cells or can be taken up, re-esterified, and stored as triglyceride by adipose tissue (6). The remaining lipoprotein particles ("remnants") are rapidly cleared by the liver (in the rat) (7, 8) or are transformed to LDL and possibly HDL particles (in man) (9). Thus, in addition to supplying lipid substrate for oxidation or storage, the synthesis and secretion of VLDL may play a central role in determining the serum concentrations of other lipoprotein particles.

The advantages of examining VLDL secretion in hepatocytes cultured as monolayers are several. First, a homogeneous population of cells can be used. Second, different experimental conditions may be singularly examined using cells obtained from one animal. Third, the environment can be chemically defined and controlled over a long time interval, allowing the study of metabolic and hormonal effects that may only be expressed after many hours. Finally, isolated liver cells do not metabolize VLDL at an appreciable rate (10). Thus, in contrast to the in vivo state, in which serum VLDL concentrations are determined by the relative rates of VLDL secretion and removal, the accumulation of VLDL in the medium of cultured hepatocytes should be dependent upon a single function, the rate of secretion. In the present study, we demonstrate the ability of adult rat hepatocytes cultured as monolayers to respond to several factors known to affect VLDL synthesis and secretion in vivo. In addition to characterizing the newly secreted VLDL particle, we also examine the effects on secretion of a known inhibitor of apoprotein synthesis (cycloheximide), inhibitors of lipoprotein secretion (colchicine and orotic acid), and the effects of dietary carbohydrate.

MATERIALS AND METHODS

FCS (heat-inactivated at 56°C for 30 min), arnizine-free Eagle's basal medium as modified by Dulbecco (11), penicillin G (5000 units/ml), streptomycin sulfate (5000 µg/ml) dissolved in 0.9% NaCl, Hanks' buffered saline, Dulbecco's phosphate-buffered saline, and trypsin in EDTA (0.05%) were all obtained from Grand Island Biological Company, Santa Clara, Calif. Before use, the medium was supplemented with 0.4 mM ornithine (Sigma Inc., St. Louis, Mo.) [³²H]Glycerol (5

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Amide gel electrophoresis (15) and SDS-urea-polyacrylamide gel electrophoresis (15) were cannulated and immediately perfused (10 ml/min) with 100 ml of modified Hanks’ buffered saline containing 1 mM MgCl₂, 10 mM Hepes buffer, and no Ca²⁺. The inferior vena cava was ligated and the right atrium was immediately opened. The perfusion lasted 5 min, after which a solution containing collagenase dissolved in Hanks’ buffered saline (100 mg in 150 ml) was infused at a rate of 10 ml/min. At the beginning of the collagenase perfusion, a catheter was sutured into the right atrium so that the perfusion medium could be recycled via a peristaltic pump. In about 15 to 20 min, the blanched liver had lost its rigid structure. It was excised and sliced into small pieces, and the pieces were placed in 10 ml of collagenase solution and shaken in a water bath for 5 min. The separated hepatocytes were drawn off slowly with a blunt-tipped pipette and immediately placed in DME medium containing PCS (20%) at 4°C. More collagenase solution was added to the medium, shaken, and the procedure was repeated twice. The dispersed hepatocytes were centrifuged at 150 x g for 13 s. Pellets were gently resuspended and washed, using the centrifugation technique, two additional times. The final preparation of hepatocytes was counted in the presence of 0.04% trypan blue using a hemocytometer. Cells were then added to DME medium containing PCS (20%), insulin (10 μg/ml), streptomycin sulfate (100 μg/ml), and penicillin G (100 units/ml). Cells were plated in 60-mm plastic culture dishes at 1 x 10⁶ cells/ml, 3 ml/dish, and placed in an incubator at 37°C in 95% air, 5% CO₂ atmosphere. After 24 h, the medium was changed to DME medium containing insulin and antibiotics but not PCS.

Isolation and Characterization of VLDL—After the indicated time period in culture, the medium was drawn off by suction and centrifuged at 1500 x g for 3 min to remove cells and debris. If necessary, the density of the medium was adjusted to 1.066 g/ml. VLDL was obtained by centrifugation as described (12). Thus, the medium was placed in polyallomer tubes and centrifuged at 7-14°C in a SW-41 rotor at 8000 x g for 5 min. The supernatant was drawn off and washed three times with the incubation buffer, 0.5 ml of NCS tissue solubilizer (Amersham, Arlington Heights, Ill.) was added, and radioactivity was assayed in PCS scintillation solution (Amersham, Arlington Heights, Ill.). Nonspecific precipitation of radioactivity was determined as substituting serum obtained from control rabbits for the rabbit anti-apo-B antiserum. An aliquot of each fraction was precipitated by trichloroacetic acid, centrifuged at 2000 x g for 5 min, and the radioactivity in the precipitate was assayed as described above.

Isolation of Hepatocytes—Cultured hepatocytes were harvested by incubating the washed cells with 2 ml of 0.05% trypsin in Versene buffer (pH 7.0) for 3 min at 37°C in a shacking incubator. DME medium containing 5% PCS (2 ml) was immediately added and the dissociated cells were washed three times with fresh culture medium, resuspended in 3 ml of Dulbecco’s phosphate-buffered saline and centrifuged again. Cell pellets were stored at −15°C overnight. After thawing, the cell samples were brought up to 1 ml with distilled water and disrupted for 1 min at maximum power using a Kontes microsonicator (Vineland, N. J.).

Lipid Extraction and Analysis—Media or sonicated cell suspensions were extracted twice with 5 volume of chloroform/methanol (2:1, v/v) (17) containing 1% glycerc. The organic phase was washed with water and then dried under reduced pressure. The recovery of [¹³C]triglyceride added as a standard was 96%. Free and esterified cholesterol concentrations were determined by gas-liquid chromatography as previously described (18). Phospholipid concentration was determined as described by Bartlett (19). An aliquot of the chloroform/methanol extract was separated into lipid classes by TLC using silica gel plates (0.2 mm, Analtech, Newark, Del.) and developed in hexane/diethyl ether/acetatic acid (85:15:1, v/v). The silica gel containing the triglyceride was extracted three times with chloroform and the chloroform was evaporated under reduced pressure. Recovery of [¹³C]triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.). The mass of triglyceride was determined by reacting the labeled glycerol with glycerol kinase coupled with pyruvate kinase and lactate dehydrogenase and assaying NAD (Calbiochem, La Jolla, Calif.).

RESULTS

Adult rat hepatocytes were obtained in excellent yield (2.85 ± 1.2 × 10⁶ cells per liver from 100- to 140-g rats; n = 8). In most preparations, more than 90% of the cells excluded 0.04% trypan blue. Occasionally, however, cells were obtained that took up the dye or failed to adhere to the culture dishes (about one preparation in 10), or both. The reason for these poor cell preparations is not known. No metabolic studies were carried out with such preparations. As early as 1 to 2 h after plating, cells lost their initial round configuration (Fig. 1A) and formed islands of spread cells (Fig. 1B). The morphology was maintained in culture for at least 7 days.

In preliminary experiments using three different cell preparations, the incorporation of [¹³C]glycerol into VLDL-[¹³C]triglyceride was measured using cells maintained in culture for 48 h and found to agree with each other within ±10%.
Secretion of VLDL by Hepatocytes

antisera (apo-B and apo-E) are both present in the same newly secreted VLDL particles.

**Synthesis of VLDL Polypeptides**—To examine further the synthesis of individual apolipoproteins, 2-day-old hepatocytes were incubated in serum-free DME medium containing [H]leucine (5 mM) for 8 h, after which the VLDL was isolated and examined by urea-polyacrylamide gel electrophoresis. The gels were first stained with Coomassie brilliant blue G-250 and the bands were scanned by densitometry. The gels were then sliced (1.5 mm) and the H in individual slices was determined. As shown in Fig. 3, the hepatocytes incorporated [H]leucine primarily into apo-E and apo-B. The total radioactivity in the band corresponding to apo-E was 50% greater than that found in the band that did not enter the gel (apo-B), and the estimated specific radioactivities of the two apolipoproteins were nearly equal. The portions of the gel containing the traces of C apoproteins contained very small quantities of radioactivity.

**Secretion of Apo-B-containing Lipoproteins**—The density distribution of apo-B-containing lipoproteins secreted by the cultured hepatocytes was examined. By means of a double antibody precipitation technique, it was found that overall incorporation of H-amino-acids into fractions precipitated by rabbit apo-B antiserum was linear, as a function of time, for at least 12 h. Subfractionation of the different lipoprotein classes by centrifugation followed by immunoprecipitation showed that all of the newly synthesized apo-B was in the

![FIG. 1. Phase contrast micrographs (X 60) of rat hepatocytes after 1 h (A) and after 3 days (B) in monolayer culture. Livers from female rats were perfused with collagenase as described under "Materials and Methods," and the dissociated hepatocytes were isolated and plated in 3 ml of DME medium containing 20% FCS at a density of 3 x 10^6 cells/dish.](http://www.jbc.org/)

**Characterization of Newly Secreted VLDL**—Newly secreted VLDL appeared similar to serum VLDL in size and shape. The range of diameters of the individual VLDL particles (425 to 1057 Å) was nearly the same as that found for serum VLDL particles concurrently examined (450 to 1150 Å). The majority of newly secreted VLDL particles (65%) had diameters in the range of 450 to 600 Å.

**Apolipoprotein Composition**—VLDL obtained from the culture medium and from rat serum were subjected to urea and urea-SDS-disc gel electrophoresis to determine the apoproteins present and their approximate molecular weights. Fig. 2 shows typical urea gels and the tentative identification of these polypeptides by reference to published data (20). Serum VLDL contained two major bands, corresponding to apo-B (at the origin) and to apo-E, and the additional bands corresponding to apo-C-III-O, apo-C-II (presumably located in the second band from the front) and apo-C-I, which is closest to the front. A small but distinct band was also found just below the origin which corresponds to apo-C-I. In contrast, newly secreted VLDL showed only two major bands, corresponding to apo-B and apo-E, and very little apo-C. A faint but definite band corresponding to either apo-C-III-O or apo-C-II was apparent in the electrophoresis gel of the newly secreted VLDL.

When analyzed by SDS-urea-10% polyacrylamide gel electrophoresis, the molecular weight of apo-E was the same in both serum and newly secreted VLDL (36,000 daltons) (data not shown).

**Immunochromatological Analysis of Apoproteins**—Antisera made against rat apo-B, apo-E, apo-A-I, and apo-C-III were tested for their ability to form precipitin bands with newly secreted VLDL by double diffusion in agarose. Newly secreted VLDL formed precipitin bands when reacted against antiserum made against rat apo-B and apo-E but not with antiserum made against apo-C-III. When apo-B and apo-E antisera were placed in adjacent wells, they both formed a single continuous precipitin band against newly secreted VLDL placed in the center well. No additional precipitin bands were observed. These data suggest that newly secreted VLDL contains immunodeterminants that are recognized by both anti-apo-B and anti-apo-E antisera. Furthermore, anti-apo-E antiserum did not cross-react with apo-B (did not form a precipitin line against rat serum LDL). These data suggest that the immunodeterminants recognized individually by their respective

![FIG. 2. Comparison of the apoprotein composition of rat serum VLDL (S) and of VLDL newly secreted by rat hepatocytes (N). Aliquots of delipidated serum VLDL (S) and of newly secreted VLDL (N) isolated by ultracentrifugation containing 50 μg of protein were subjected to 6 M urea-polyacrylamide gel electrophoresis and then stained with Coomassie blue G-250. Apoproteins are designated according to their relative rates of migration (20).](http://www.jbc.org/)
VLDL fraction and none was present in IDL, LDL, or HDL. The $d > 1.21$ fraction did not contain any radioactivity precipitable with rabbit apo-B antiserum. Of the total radioactivity which was precipitated by trichloroacetic acid in fractions with a $d < 1.21$, HDL contained 78%, VLDL contained 16%, and LDL contained 6%.

**Net Secretion of VLDL**—Hepatocytes were incubated in DME medium containing no serum, so that newly secreted VLDL could be isolated without contamination by serum-derived lipoproteins. Newly secreted VLDL was recovered from the medium in quantities sufficient to allow determinations of protein content and lipid composition (Table I). During the first 48 h in culture, the rate of VLDL triglyceride secretion was 0.20 µg/mg of cell protein/h and the rate of VLDL protein secretion was 0.04 µg/mg of cell protein/h. These rates, which were reasonably reproducible ($±20\%$ for studies using separate cell preparations), are about 30% of the rates reported in liver perfusion studies (21-24). The $d < 1.006$ g/ml fraction prepared from no-cell control dishes contained no detectable protein or lipid. After 7 days in culture, the rate of VLDL secretion fell by 50%. The cholesterol of newly secreted VLDL was mostly in the free form (Table I), whereas the cholesterol in serum VLDL is mostly in ester form (24). The content of triglyceride, phospholipid, and cholesterol in newly secreted VLDL (Table I) agreed closely with that found in VLDL obtained from liver perfusions (24, 25). The VLDL which accumulated in the medium during 7 days changed slightly in the relative concentration of its lipid components. Although the concentration of triglyceride and phospholipid showed no consistent change during the 7-day experiment, both the concentration and percentage of cholesterol esters increased. The secretion of lecithin:cholesterol acyltransferase by suspended hepatocytes has been described (26) and may explain this apparent increase in VLDL cholesterol esters with time.

**Time Course of [°H]Glycerol Incorporation into Cellular and Medium Lipids**—The incorporation of [°H]glycerol into cellular and medium triglycerides and phospholipids was linear for at least 3 h (Fig. 4). The peak of the incorporation of [°H]glycerol into cellular triglyceride preceded the peak of the [°H]glycerol incorporated into the medium. In the cells, much more [°H]glycerol was incorporated into triglycerides than into phospholipids. In contrast, in the medium, the amounts of [°H]glycerol incorporated into phospholipids and into triglycerides were approximately equal.

To examine in more detail the distribution of the [°H]glycerol lipids found in the medium, lipoprotein classes were...
TABLE II
Distribution of \(^{3}H\)glycerol incorporation into lipid classes and lipoprotein fractions in culture medium

Medium obtained from hepatocytes cultured for 3 h in serum-free DME medium in the presence of 10 \(\mu\)Ci (5 nmol) \(^{3}H\)glycerol was separated into lipoprotein fractions by ultracentrifugation. The individual lipoprotein fractions were extracted and the lipids separated by TLC as described under "Materials and Methods." Values represent the mean \pm S.D. of three individual determinations. Values in parentheses represent the percentage distribution of each individual class of lipid among the density fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Phospholipids</th>
<th>Lower glycerides</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL(^{a})</td>
<td>14 \pm 1 (29 \pm 2)</td>
<td>1.2 \pm 0.3</td>
<td>48 \pm 3 (95 \pm 6)</td>
</tr>
<tr>
<td>LDL + HDL(^{a})</td>
<td>12 \pm 2 (24 \pm 4)</td>
<td>&lt;0.2</td>
<td>2.4 \pm 0.3 (3 \pm 1)</td>
</tr>
<tr>
<td>d &gt; 1.21 g/ml</td>
<td>23 \pm 2 (47 \pm 4)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

\(^{a}\) d < 1.006 g/ml, obtained by centrifugation as described under "Materials and Methods."

\(^{b}\) d = 1.006 to 1.21 g/ml, obtained by centrifugation as described under "Materials and Methods."

TABLE III
Effect of colchicine and cycloheximide on \(^{3}H\)glycerol incorporation into lipids by cultured hepatocytes

Colchicine (10 \(\mu\)M) and cycloheximide (10 \(\mu\)M) were each separately dissolved in DME without FCS. After 1 day in culture, the medium of hepatocytes in culture was changed to fresh DME medium containing the designated inhibitor. After 14 h, \(^{3}H\)glycerol (10 \(\mu\)Ci, 5 nmol) was added. Three hours later, cells were harvested and the incorporation of \(^{3}H\)glycerol into cellular and medium lipids was determined as described under "Materials and Methods." Each value represents the mean \pm S.D.

<table>
<thead>
<tr>
<th>Inhibitor added</th>
<th>Medium</th>
<th>Cellular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglyceride</td>
<td>Phospholipid</td>
</tr>
<tr>
<td></td>
<td>cpm (^{3}H)glycerol/mg cell protein x 10(^{8})</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7.0 \pm 0.3</td>
<td>4.1 \pm 0.4</td>
</tr>
<tr>
<td>Colchicine (10 (\mu)M)</td>
<td>3.7 \pm 0.3</td>
<td>10.7 \pm 0.8</td>
</tr>
<tr>
<td>Cycloheximide (10 (\mu)M)</td>
<td>1.2 \pm 0.3</td>
<td>0.6 \pm 0.0 (^{a})</td>
</tr>
</tbody>
</table>

\(^{a}\) Designates a statistically significant difference from the control value at p < 0.01.

Effects of Cycloheximide and Colchicine—Colchicine (10 \(^{-6}\) M), a potent inhibitor of VLDL secretion and other processes dependent upon microtubular function (27), decreased the secretion of \(^{3}H\)triglyceride by 50% when the inhibitor was preincubated with the cells for 14 h (Table III). Concurrently, the incorporation of \(^{3}H\)glycerol into both cellular triglycerides and phospholipids was actually increased by colchicine, showing that it inhibited VLDL secretion and not the synthesis of the labeled lipids. Similar effects of cycloheximide were found within 3 h of its addition to the medium (data not shown). With the more extended exposure to colchicine, the cells rounded up and came off the culture dish. Cycloheximide, which inhibits both total protein and apoprotein synthesis, as well as VLDL secretion in vivo (28), inhibited both lipid synthesis and secretion. Whereas cycloheximide only partially inhibited \(^{3}H\)glycerol incorporation into both cellular phospholipid and triglyceride (30%), secretion of labeled lipids was almost totally inhibited (85%). Exposure of the cells to cycloheximide for as long as 14 h caused no observable changes in cell morphology as viewed by the phase contrast microscope.

Fig. 5. Effect of sucrose feeding (S), sucrose plus orotic acid (S + O), and sucrose plus orotic acid and adenine (S + O + A) on VLDL triglyceride secretion by cultured hepatocytes. Hepatocytes were obtained from rats fed normal chow (C) or a liquid diet containing 30% sucrose (S) for 2 days. After 24 h in culture, the medium was changed to serum-free DME medium. Orotic acid (O) (6.4 mM) and adenine (A) (7.4 mM) were then added to the culture medium of hepatocytes obtained from sucrose-fed rats. After 2 days in culture, \(^{3}H\)glycerol (10 \(\mu\)Ci; 2 nmol) was added, and 3 h later the cells were harvested. VLDL was obtained by ultracentrifugation of the medium and washed twice by ultracentrifugation at 1,000. The VLDL was extracted with chloroform/methanol (2:1, v/v) and the lipids were separated by TLC. A portion of the triglyceride was assayed for radioactivity and another portion was eluted from the TLC plate and assayed for triglyceride mass using an enzyme assay (Calbiochem, La Jolla, Calif.). Each value represents the mean \pm S.E. of three individual determinations.
increased 2-fold ($p < 0.005$) (data not shown). Thus, it can be 
concluded that the effect of erotic acid is a direct effect on 
hepatic VLDL secretion. When added in combination with 
 erotic acid, adenine (7.5 mM) prevented the erotic acid inhi-
bition of VLDL secretion. Two additional experiments using 
different cell preparations gave similar results.

**DISCUSSION**

VLDL secretion is dependent upon a highly complex proc-
ess involving the synthesis of specific lipids and apoproteins 
and the association of these species into a particle which is 
transported in a membrane-bound vesicle to the hepatocyte 
surface and discharged into the sinusoidal space (31). It is not 
 surprising that this highly differentiated function, which is 
specific for hepatic and intestinal cells, is lost in transformed 
cells and in fetal cells (32). Previous attempts to examine 
VLDL secretion using isolated rat liver cells have generally 
been confined to single cell suspensions (33, 34) having short 
term viability. Recently, Lamb et al. (35) reported that adult 
rat hepatocytes in monolayer culture synthesized and secreted 
triglyceride, but they were unable to detect secretion of apo-
proteins. The medium composition and the culture conditions 
used by Lamb et al. differed considerably from those used 
in the present study. Also, they employed cells obtained from 
rats that had been partially hepatectomized, a process which 
stimulates hepatocyte replication. Letturt and Weinsten (32) 
have shown that VLDL levels in the rat are linked to sup-
pression of hepatocyte proliferation and preliminary results 
have indicated that replicating hepatocytes secrete VLDL at 
very low rates compared to nonproliferating hepatocytes.2 Our 
results show that, under the present culture conditions, adult 
rat hepatocytes maintain the capacity to synthesize and se-
crete VLDL but do not replicate, as shown by a constant 
amount of DNA/culture dish and a low rate of [3H]thymidine 
incorporation into DNA.3 The studies described above utilize 
rat hepatocytes which are maintained in an arginine-free, 
ornithine-supplemented medium. Leffert and co-workers have 
shown that this selective medium suppresses the growth of 
nonparenchymal liver cells and allows maintenance of liver-
specific protein synthesis and urea cycle function (36–38).

Adult rat hepatocytes secrete a VLDL particle that is 
similar in most respects to serum VLDL, but there are some 
differences. The average size and shape of newly secreted 
VLDL as determined by negative-staining electron micro-
copy is similar to that found in rat serum. Although the 
apoproteins of newly secreted VLDL and serum VLDL are 
qualitatively similar, the apoprotein compositions differ sig-
ificantly (Fig. 2). The newly secreted VLDL particles contain 
predominantly apo-E and apo-B but only very small amounts 
of other apoproteins. The failure of antisera made against rat 
serum apo-C-III-3 and apo-A-1 to form precipitin bands 
between newly secreted VLDL and intestinal 
secreted by the intestine. While both newly secreted VLDL 
particles are relatively deficient in apo-C proteins, intestinal 
(42). Thus, if apo-E found in newly secreted hepatic VLDL is 
important for VLDL structure, this role is apparently assumed 
by apo-A-I and/or apo-A-IV in intestinal VLDL.

Perfused livers of the monkey (43) and swine (44) secrete lipid 
and protein in the density ranges 1.019 to 1.063 and 1.019 
to 1.090, respectively. Although direct experimental evidence 
is lacking, kinetic studies in man and rat have been interpreted 
to suggest that some LDL is synthesized directly by the liver 
(45, 46). We examined this question by measuring [3H]leucine 
incorporation into protein precipitable by antisera prepared 
against rat serum LDL. Our data clearly show that essentially 
all of the [3H]apo B is found in the VLDL density range (d < 
1.006 g/ml). If hepatocyte cultures described in this report 
are representative of the in vivo state, it is likely that LDL is 
derived from VLDL catabolism, as previously suggested (9).

Since no appreciable triglyceride hydrolase activity was found 
above a pH of 5 in the culture medium of hepatocytes cultured 
for 3 days, it is likely that these cells do not contain the 
enzymes necessary for the conversion of VLDL to LDL. It is 
possible, however, that physiological conditions may exist 
which induce the direct synthesis of LDL via the liver (e.g. 
familial hypercholesterolemia (15)).

Feeding rats a sucrose diet prior to culturing their hepato-
cyes increased the secretion of both VLDL-H-triglycerides 
and total triglycerides (mass) after the cells had been in 
culture for 3 days (Fig. 5). These data suggest that the 
nutritional state of the animal from which the hepatocytes 
are obtained has profound long lasting effects on VLDL secretion 
even after the cells are placed in culture. Similar conclusions 
were reported by Tarlow and co-workers using avian liver 
cells (47) and Broener and Van Golde using rat liver cells (48). 
It is interesting to note that carbohydrate loading stimulates 
lipogenesis irrespective of the medium insulin concentration

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2 J. C. Williams and D. B. Weinstein, unpublished observations.

3 R. A. Davis, unpublished observations.
but not lipid synthesis (49, 50). Furthermore, these data suggest that erotic acid can act directly upon the liver via a mechanism that is blocked by adenine.

In conclusion, the results show that hepatocytes can be cultured under conditions that allow VLDL synthesis and secretion to be maintained for many days at rates approaching in vivo. In addition to synthesizing and secreting HDL, bile acids, and albumin,4 under basal culture conditions can be made in order to stimulate these functions in culture and in vivo may help us to understand what factors may regulate these functions in vivo.

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