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 cholesterol esters.

The incorporation of [3H]leucine into the very 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 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^-5 M colchicine, [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 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. 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), aramine-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 (0.02%) 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.) [2-3H]Glycerol (5

* The abbreviations used are: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; apo, apolipoprotein; FCS, fetal calf serum; DME, medium, Dulbecco’s modified Eagle’s medium; SDS, sodium dodecyl sulfate; I-rays, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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§ To whom reprint requests should be directed.
amide gel electrophoresis (15) and SDS-urea-polyacrylamide gel electrophoresis were performed using rabbit and goat antibodies described by Ouchterlony (14), using rabbit and goat antibodies to rat VLDL and LDL but not against HDL and, on occasion, against apo-B. All radiolabeled and unlabelled standards had a standard deviation of less than 10%. In experiments in which L-[^4,5-^H]leucine was added, the silica gel containing the phospholipid (origin) and triglyceride was scraped into vials and its radioactivity assayed in a toluene scintillation mixture containing 5% Bio-Solv (Beckman, Fullerton, Calif.). All data are reported as mean ± S.D. Significance of group differences was determined by Student’s t test, using two-tailed p values.

RESULTS

Adult rat hepatocytes were obtained in excellent yield (2.85 ± 1.2 × 10^6 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 [1^4C]gycerol into VLDL-[1^4C]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 [\(^{3}H\)leucine (5 mM) for 8 h, after which the VLDL was isolated and examined by urea-polyacrylamide gel electrophoresis. The gels were then stained with Coomassie brilliant blue G-250 and the bands were scanned by densitometry. The gels were then sliced (1.5 mm) and the \(^{3}H\) in individual slices was determined. As shown in Fig. 3, the hepatocytes incorporated \(^{3}H\)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 \(^{3}H\)-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

**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.

**Apoprotein 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-0, apo-C-II (presumably located in the second band from the front) and apo-C-III-3, 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-0 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).

**Immunoochemical 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 antisera made against rat apo-B and apo-E but not with antisera 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
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 DMEM 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 $\mu$g/mg of cell protein/h and the rate of VLDL protein secretion was 0.04 $\mu$g/mg of cell protein/h. These rates, which were reasonably reproducible ($\pm$20% for studies using separate cell preparations), are about 30% of the rates reported in liver perfusion studies (21–24). 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.

![Graph](http://www.jbc.org/)

**Fig. 4.** Incorporation of $[\text{H}]$glycerol into glycerol lipids by hepatocytes. $[2-\text{H}]$Glycerol (10 $\mu$Ci; 2 nmol) was added to hepatocytes which were cultured for 3 days in serum-free DMEM medium. At the indicated times, cells were separated from medium and each was extracted with chloroform/methanol (2:1, v/v). Phospholipids and triglycerides were separated by TLC and the radioactivity was measured. Each point represents the mean of three determinations. (Legend: $\oplus$, cellular triglyceride; $\Delta$, medium triglyceride; $\bigcirc$, cellular phospholipid; $\bullet$, medium phospholipid.)

**Table I**

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Free cholesterol</th>
<th>Ester cholesterol*</th>
<th>Cholesterol esterified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$g/mg protein</td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>4886</td>
<td>839</td>
<td>655</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>4705</td>
<td>836</td>
<td>806</td>
<td>237</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>5035</td>
<td>893</td>
<td>914</td>
<td>442</td>
<td>33</td>
</tr>
</tbody>
</table>

* Ester cholesterol values represent the amount of cholesterol present in esterified form.
Individual lipoprotein fractions were extracted and the lipids 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 ± S.D. of three individual determinations. Values in parentheses represent the percentage distribution of each individual class of lipid among the density fractions.

\[
\begin{align*}
\text{VLDL}^a &: 14 ± 1 \ (29 ± 2) \quad 1.2 ± 0.3 \quad 45 ± 3 \ (95 ± 6) \\
\text{LDL + HDL}^a &: 12 ± 2 \ (24 ± 4) <0.2 \quad 2.4 ± 0.3 \ (3 ± 1) \\
d > 1.21 \text{ g/ml} &: 23 ± 2 \ (47 ± 4) <0.1 \quad <0.1
\end{align*}
\]

\[a \ d < 1.006 \text{ g/ml, obtained by centrifugation as described under “Materials and Methods.”}
\]

\[b \ d = 1.006 \text{ to 1.21 g/ml, obtained by centrifugation as described under “Materials and Methods.”}
\]

**Table II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Phospholipids</th>
<th>Triacylglycerides</th>
<th>Lower glycerides</th>
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</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>14 ± 1 (29 ± 2)</td>
<td>1.2 ± 0.3</td>
<td>45 ± 3 (95 ± 6)</td>
</tr>
<tr>
<td>LDL + HDL</td>
<td>12 ± 2 (24 ± 4)</td>
<td>&lt;0.2</td>
<td>2.4 ± 0.3 (3 ± 1)</td>
</tr>
<tr>
<td>d &gt; 1.21 g/ml</td>
<td>23 ± 2 (47 ± 4)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

**Effects of Cycloheximide and Colchicine—Colchicine (10 μM) and cycloheximide (10 μM) 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, [2-14C]glycerol (10 μCi, 9 nmol) was added. Three hours later, cells were harvested and the incorporation of [14C]glycerol into cellular and medium lipids was determined as described under “Materials and Methods.” Each value represents the mean ± S.D.**

**Table III**

<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>None</td>
<td>7.0 ± 0.3</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Colchicine 5 μM</td>
<td>3.7 ± 0.3</td>
<td>10.7 ± 0.8</td>
</tr>
<tr>
<td>Cycloheximide 10 μM</td>
<td>1.2 ± 0.3</td>
<td>0.6 ± 0.01</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 μM), a potent inhibitor of VLDL secretion and other processes dependent upon microtubular function (27), decreased the secretion of 3H-triglyceride by 50% when the inhibitor was preincubated with the cells for 14 h (Table III). Concurrently, the incorporation of [3H]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 observed 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 [3H]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.

Assay for VLDL Lipolysis—Addition of Triton WR-1339 (1% w/w), a known inhibitor of serum lipolytic enzymes (29), did not alter [14C]glycerol incorporation into medium and cellular lipids (data not shown). In the medium, no significant lipase activity was found above pH 5, assessed as described elsewhere (30). A small but detectable activity was found at pH 5 in the medium obtained from 3-day-old hepatocytes (less than 0.5 nmol of triglyceride hydrolyzed/h/mg of cell protein). However, in cell homogenates, there was substantial triglyceride hydrolyase activity at pH 5 (28 nmol/h/mg of protein) and lower activities at pH 7 (6 nmol/h/mg of protein) and pH 9 (0.9 nmol/h/mg of protein). Thus, most of the detectable triglyceride lipase present in the cultured hepatocytes is probably of lysosomal origin.

Effects of Sucrose Feeding, Orotic Acid, and Adenine on VLDL Secretion—Hepatocytes obtained from sucrose-fed rats showed a marked increase in the rate of VLDL secretion. Both the incorporation of [14C]glycerol into VLDL triglyceride and the net secretion of VLDL triglyceride were increased—by 4-fold (p < 0.001) and 1.6-fold (p < 0.01), respectively (Fig. 5). This stimulatory effect of dietary sucrose on VLDL secretion was found in hepatocytes that had been in culture for as long as 3 days, demonstrating that the nutritional state of the donor animal has long lasting effects on the hepatocytes in culture.

Orotic acid (6.5 mM) reduced both the incorporation of [14C]glycerol into VLDL triglyceride and the net secretion of VLDL triglyceride (Fig. 5). The incorporation of [14C]glycerol into cellular phospholipid was unaffected by orotic acid, and its incorporation into cellular triglyceride was actually increased. Most of the secreted triglyceride (>95%) was in the VLDL fraction. In contrast, about 50% of medium 14C-phospholipids was recovered in the fraction of density >1.21 g/ml. Thus, about half of the 14C-phospholipid secreted by the hepatocytes was not associated with lipoproteins. It is possible that some of the phospholipid secreted by the hepatocytes may correspond to that which is secreted via the biliary system in the intact liver.

Effects of Colchicine and Cycloheximide—Colchicine (10 μM), a potent inhibitor of VLDL secretion and other processes dependent upon microtubular function (27), decreased the secretion of 3H-triglyceride by 50% when the inhibitor was preincubated with the cells for 14 h (Table III). Concurrently, the incorporation of [3H]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 observed 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 [3H]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.

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**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 (O) 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, [14C]glycerol (10 μCi; 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 1.006. The VLDL was extracted with chloroform/methanol (2:1, v/v) and the lipids were assayed for triglyceride mass using an enzyme assay (Calbiochem, La Jolla, Calif.). Each value represents the mean ± S.E. of three individual determinations.
creased 2-fold ($p < 0.005$) (data not shown). Thus, it can be concluded that the effect of orotic acid is a direct effect on hepatic VLDL secretion. When added in combination with orotic acid, adenine (7.5 mM) prevented the orotic acid inhibition of VLDL secretion. Two additional experiments using different cell preparations gave similar results.

**DISCUSSION**

VLDL secretion is dependent upon a highly complex process 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 apoproteins. 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. Leffert and Weinstein (32) have shown that VLDL levels in the rat are linked to suppression of hepatocyte proliferation and preliminary results have indicated that replicating hepatocytes secrete VLDL at very low rates compared to nonproliferating hepatocytes. Our results show that, under the present culture conditions, adult rat hepatocytes maintain the capacity to synthesize and secrete 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. 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 microscopy 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 significantly (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 with newly secreted VLDL suggests that these apoproteins are present at very low levels, if at all. Since the same isolation techniques yielded VLDL from rat serum which did contain apo-C, it seems unlikely that the apo-C peptides were selectively lost from newly secreted VLDL during isolation. Reduced quantities of apo-C proteins have been found in VLDL obtained in liver perfusions (23, 24) and from freshly isolated hepatocytes (33). Furthermore, kinetic analysis of tracer studies has shown that apo-C proteins are more likely to be secreted with nascent HDL than with nascent VLDL (189). Apo-C proteins have been found to be secreted on nascent HDL disc-like particles by the perfused liver (24). However, as in the case of newly secreted VLDL, the nascent HDL disc particles also contain lower concentrations of apo-C proteins than do rat serum HDL particles (24).

Since newly secreted VLDL contains reduced concentrations of apo-C proteins compared to serum VLDL, it is likely that the apo-C proteins are conserved in the serum. In the rat, the half-life of apo-C proteins is much greater than that of VLDL apo-B (40). Since apo-C proteins are transferred to HDL during the catabolism of VLDL to HDL, the rapid uptake and degradation of IDL by the liver of the rat (7, 8) probably plays no appreciable role in apo-C protein catabolism. Thus, the small but detectable quantities of apo-C proteins found on newly secreted VLDL (this study) and nascent HDL (24) may adequately account for the steady state levels of apo-C proteins present in rat serum.

The lipid composition of newly secreted VLDL is generally similar to that of VLDL secreted in liver perfusions (24, 25). However, newly secreted VLDL appears to have a greatly reduced concentration of cholesterol esters compared to serum VLDL. Only 13% of the cholesterol is esterified (Table I). A reduced concentration of cholesterol esters in VLDL secreted by perfused rat livers has been reported by several laboratories (24, 25). As opposed to free cholesterol, which interacts with lipoprotein phospholipids at the water interface, cholesterol esters are hydrophobic and are forced within the inner liquid core of VLDL (41). Furthermore, since crystalline cholesterol esters are dissolved by the liquid core of triglyceride, which makes up the overwhelming bulk of the hydrophobic lipids of VLDL, it is likely that cholesterol esters play a minor role (if any) in maintaining the structure of VLDL.

There are interesting distinctions between the apoprotein composition of VLDL secreted by the liver and that of VLDL secreted by the intestine. While both newly secreted VLDL particles are relatively deficient in apo-C proteins, intestinal VLDL contains apo-B, apo-A-I, and apo-A-IV and no apo-E (42). Thus, if apo-E found in newly secreted hepatic VLDL is important for VLDL structure, this role is apparently assumed by apo-A-1 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 antiserum prepared against rat serum LDL. Our data clearly show that essentially all of the $[^3H]$apo B is found in the VLDL density range (δ < 1.069 g/ml). If hepatocyte cultures described in this report are representative of the in vitro 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 through the liver (e.g., familial hypercholesterolemia (145)).

Feeding rats a sucrose diet prior to culturing their hepatocytes increased the secretion of both VLDL-$[^3H]$-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 Broemer 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.
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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
those seen in vivo. In addition to synthesizing and secreting
VLDL, these cultured hepatocytes synthesize and secrete
in the absence of added serum, these specific hepatic functions
which were equal for both groups of rats). The additional
finding that erotic acid inhibits the secretion but not the
synthesis of VLDL triglyceride agrees with the previous finding
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