Bile Acid Secretion by Cultured Rat Hepatocytes
REGULATION BY CHOLESTEROL AVAILABILITY*

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Roger A. Davis‡‡, Paul M. Hyde‡, Jui-Chang W. Kuan†‡, Monica Malone-McNeal‡, and Jane Archambault-Schernsnyder‡

From the Departments of ‡Physiology and †Biochemistry, Louisiana State University Medical School, New Orleans, Louisiana 70112

A sensitive gas-liquid chromatographic technique employing electron capture detection of capillary column separated fluoro-substituted bile acid derivatives allowed us to examine regulation of bile acid secretion by cultured rat hepatocytes. In serum-free medium, the rate of secretion of cholic and β-muricholic acids was constant for 96 h. Since the amount of cell-associated bile acids were similar at 0 and 72 h of the experiment, bile acid secretion was due to de novo synthesis. Under basal conditions (no additions), the secretion of cholic and β-muricholic acids was nearly equal. The amount of chenodeoxycholic acid secreted was negligible. The net secretion rate of total bile acids was 8 nmol/g of liver/h, which is 20–50% of in vivo estimates. Within 18 h of adding up to 100 µM taurochenodeoxycholic acid, essentially all was converted to β-muricholic (~80%) and α-muricholic acids. Thus, the capacity of cultured hepatocytes to 6-hydroxylate is sufficient to account for the absence of chenodeoxycholic acid and the presence of β-muricholic acid in the culture medium. To examine if cholesterol availability plays a role in regulating bile acid secretion, three different methods were used to alter its availability: 1) changing the rate of cholesterol biosynthesis, i.e. addition of mevazol and d < 1.02 g/ml lipoproteins obtained from cholesterol-fed rats decreased de novo cholesterol synthesis from [14C]acetate (68–95%), increased [14C]cholesterol esterification (5-10-fold), increased hepatocyte cholesterol ester concentrations (2-24-fold), and increased bile acid secretion (2-10-fold). In contrast, mevinolin inhibited cholesterol biosynthesis (~60%), inhibited [14C]cholesterol esterification (~50%), slightly decreased hepatocyte cholesterol ester concentrations (~25%), and inhibited bile acid secretion (~40%). These results show that hepatocyte cholesterol concentrations and rates of bile acid secretion vary in parallel to these aforementioned effectors. Moreover, the results show for the first time a direct effect between hepatocyte uptake of lipoproteins and bile acid secretion, which imparts to the liver the capacity to maintain cholesterol homeostasis, are coupled to a unique polar cellular architecture. The parenchymal cell of the liver has at least two distinct surfaces: sinusoidal and canalicular. The almost complete segregation of the bile canaliculus from the blood plasma provides an anatomical outlet through which cholesterol (in the form of biliary cholesterol and bile acids) is diverted for elimination from the body. Under normal circumstances, bile acids are circulated many times between the intestinal tract and the liver, i.e. the enterohepatic circulation. Each cycle through the intestine causes a net loss of about 5–10% of the bile acid pool, which is compensated by de novo synthesis (1). The return of bile acid via the enterohepatic circulation is thought to play a major role in regulating bile acid synthesis, via negative feedback control on 7α-hydroxylase (2, 3). Interruption of the enterohepatic circulation rapidly results in increased bile acid synthesis (4, 5).

The complexity of the enterohepatic circulation has impeded attempts to measure directly bile acid synthesis and examine its regulation in vivo. Quantitation of biliary bile acid secretion does not necessarily reflect rates of de novo synthesis, since only a variable portion of biliary bile acids are newly synthesized.

Although many anatomical features present in vivo are altered when they are plated on a plastic matrix, the cultured hepatocytes afford a direct approach to examining how bile acid synthesis is regulated. By ablation of the enterohepatic circulation, the gross level of control is lost. The inherent advantage provided by the hepatocyte culture model is that additional modes of regulating bile acid synthesis may be more easily exposed. The disadvantage of the hepatocyte culture model (as well as other culture models) is the potential loss of processes or factors which may exist in the complete in vivo system. These differences from in vivo models which may be uncovered by culture models can be by themselves valuable toward our understanding of the intact organism.

In vivo studies using several species show that interruption of the enterohepatic circulation by feeding bile acid sequestrants increases 125I-LDL turnover (6, 7) and decreases serum LDL concentrations (6, 7). In addition, bile acid sequestrant-treated animals have an increased expression of a hepatic receptor which recognizes apo-B and apo-E (7, 8). These data indirectly suggest that bile acid synthesis and lipoprotein degradation may somehow be linked.

With the proviso that a hepatocyte in culture may behave somewhat differently than in vivo, we have utilized the cul-

The liver is the major organ site of both the synthesis and degradation of plasma cholesterol. These metabolic processes,

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‡ Established Investigator of the American Heart Association, to whom all correspondence should be addressed.

1 The abbreviations used are: LDL, low density lipoproteins; GLC, gas-liquid chromatography; DME, Dulbecco's modified Eagle's medium; PBS, phosphate buffered saline; VLDL, very low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; apo-B, apolipoprotein B.
tured rat hepatocyte model to examine the relationship between cholesterol availability and bile acid synthesis. To obtain accurate qualitative and quantitative analysis of the small quantities of bile acids secreted by a plate of hepatocytes, a sensitive GLC assay was developed. The results show that bile acid secretion by cultured hepatocytes is sensitive to changes in cholesterol availability induced through changes in cholesterol biosynthesis as well as via uptake of the cholesterol associated with specific lipoprotein particles.

**EXPERIMENTAL PROCEDURES**

**Materials**—Medium, antibiotics, collagenase, and other culture supplies were obtained from suppliers as previously described (9). [24-14C]Taurocholate (48 mCi/mmol) and [4-2H]mevalonolactone (8 mCi/mmol) were obtained from Amersham (Arlington, Heights, IL). Trifluoroacetic anhydride and hexafluoroisopropyl alcohol were obtained from PCR Research, Inc. (Gainesville, FL). Nordeoxycholic acid was obtained from Steraloids (Wilton, NH). Cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid were obtained from Sigma (St. Louis, MO). Purity was greater than 98% as determined by GLC. Cholylglycine hydrolase was obtained from Sigma (St. Louis, MO).

**Preparation of Hepatocytes**—Sprague-Dawley rats were fed diets consisting of chow (control) and chow + 20% olive oil + 2% cholesterol as described (9). For most experiments, rats weighing 150-200 g were used. Because of the long-term cholesterol-feeding regimen (8 weeks), in this experiment (Table II and Fig. 4), both control and cholesterol-fed rats weighed 300-350 g, prior to killing. Hepatocytes were obtained via collagenase digestion using recently published methods (9). Cells were plated in Dulbecco's modified Eagle's medium + 20% calf serum on 100-mm plastic dishes. Each dish was inoculated with 9 ml of medium containing 11.7 × 10⁶ cells. After 4 h, cells had attached to the plastic and spread to form "monolayers" and the culture medium was changed to serum-free medium (5 ml/plate) plus insulin and antibiotics as described (9). At the time indicated (in legends), the medium was drawn off via suction and stored at -20°C for bile acid analysis. Cells were scraped off the dish using a rubber policeman, washed with phosphate-buffered saline, and stored at -20°C.

**Cell Lipid Studies**—After adding &stigmasterol (GLC-internal standard) cells were extracted using chloroform-methanol (2:1, v/v) (10) as described (9). Cholesterol concentrations were determined using a previously described GLC method (11). Cholesterol ester concentrations were calculated as the difference between free and total cholesterol and are expressed as mass of free cholesterol. In the experiments in which the incorporation of [2-14C]acetate into cholesteryl ester was measured, 2 h prior to harvest (see tables for exact time), [2-14C]acetate (3 μCi; final concentration, 0.1 mm) was added to the culture medium. After a 2-h incubation, lipids were extracted as described above and then separated using TLC using methods previously reported (9, 12).

**Cell protein content of each plate was determined by the Lowry method (13).**

**Bile Acid Analysis**—Nordeoxycholic acid (2 μg/ml culture medium) and [24-14C]taurocholate were added to culture medium as a GLC internal standard, and a recovery standard, respectively. Bile acids were extracted from the culture medium using a Sep-Pak C-18 column (25 m × 0.2 mm i.d.). Helium was used as the carrier gas at a flow rate of 1 ml/min, the column temperature was 240°C (isotherm), and the injector was at 250°C with a 100:1 split ratio. The detector temperature was 300°C and Ne (29 ml/min) was used as the make-up gas. A Hewlett-Packard integrator 3390 was used to determine retention times and detector response. Bile acids were quantitated using peak area ratios (nordeoxycholic as the internal standard). Relationships between area ratios and weight ratios of all standards were linear (as determined by least squares analysis) with a correlation coefficient >0.992.

**Isolation of Serum Lipoproteins**—Rats were fed chow (control) or chow + 20% olive oil + 2% cholesterol as described (9). Blood was obtained via the aorta and centrifuged at 2000 rpm for 30 min to obtain serum. Serum lipoprotein fractions were obtained by ultracentrifugation as described (15). Lipoprotein fractions were dialyzed against phosphate-buffered saline (4 × 4 liters).

Apolipoprotein content was determined by Lowry et al. (13) (protein content), electrophromassay (apo-B, apo-E, and apo-A-I quantitations) were as described (15), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed total apolipoprotein composition (16). Lipids were extracted and quantitated as described (15).

**Statistical Analysis**—All data are reported as mean ± S.D. Student's t test was used to determine statistical differences. A P value of 0.05 (double-tailed) was considered significant. Linear least squares analysis was performed using a standard program from Hewlett-Packard. Retention times of GLC peaks are all reported in minutes from time of injection.

**RESULTS**

**Bile Acid Secretion: Qualitative and Quantitative Analysis**—For all experiments, hepatocytes were initially plated using medium + 20% calf serum for 4 h after which it was changed to serum-free medium. Previous studies show that the calf serum is necessary to allow the cells to adhere to the plastic and spread (17) (4 h is sufficient time for cells to attach to the plates).

Preliminary studies were necessary to develop the isolation, derivatization, and GLC quantitation of bile acids. The method as described was optimized to give essentially complete recoveries (using [24-14C]taurocholate added as a standard; see "Experimental Procedures"). We originally used N₂ as a carrier gas. By changing to He (less viscosity, inert gas), flow rates could be increased and sensitivity was improved by 4-fold. A typical GLC of bile acid standards is shown in Fig. 1A.

The accumulation of bile acids in the culture medium was determined at 0, 12, 24, 48, and 96 h (after changing the culture medium to serum-free medium). A GLC analysis of

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2 The procedure for quantitative hydrolysis of conjugated bile acids was originally developed by William E. Highsmith, Jr. who kindly shared this information with us.
nordeoxycholic acid (8.59 min) cholic acid (11.86 min), P-muricholic acid; and 13.01, chenodeoxycholic acid.

tography using a C-18 column as described under "Experimental Procedures." The chromatogram shown was obtained as described using a capillary column SE-54 and electron capture detection. The following retention times (in minutes) correspond to authentic bile acid standards: 8.09, α-muricholic acid; 8.59, nordeoxycholic acid (internal standard); 11.82, cholic acid; 12.55, β-muricholic acid; and 13.01, chenodeoxycholic acid. Bile acids which accumulated in the serum-free cultured medium in which hepatocytes were incubated for 24 h were extracted by reverse-phase column chromatography using a C-18 column as described under "Experimental Procedures." The bile acids conjugates were hydrolyzed with cholinesterase hydrolase and then derivatized as in A. The presence of nordeoxycholic acid (8.59 min) cholic acid (11.86 min), β-muricholic acid (12.54 min), and an as yet unidentified peak (12.90 min) is shown by GLC as described. Note the apparent absence of chenodeoxycholic acid (13.00 min).

**Fig. 1.** GLC analysis of derivatized bile acid standards (A) and bile acids secreted by cultured rat hepatocytes (B). A, bile acid standards were derivatized with trifluoroacetic anhydride (—OH) and hexafluoroisopropyl alcohol (carboxyl) as described under "Experimental Procedures." The chromatogram shown was obtained as described using a capillary column SE-54 and electron capture detection. The following retention times (in minutes) correspond to authentic bile acid standards: 8.09, α-muricholic acid; 8.59, nordeoxycholic acid (internal standard); 11.82, cholic acid; 12.55, β-muricholic acid; and 13.01, chenodeoxycholic acid. B, bile acids which accumulated in the serum-free cultured medium in which hepatocytes were incubated for 24 h were extracted by reverse-phase column chromatography using a C-18 column as described under "Experimental Procedures." The bile acids conjugates were hydrolyzed with cholinesterase hydrolase and then derivatized as in A. The presence of nordeoxycholic acid (8.59 min) cholic acid (11.86 min), β-muricholic acid (12.54 min), and an as yet unidentified peak (12.90 min) is shown by GLC as described. Note the apparent absence of chenodeoxycholic acid (13.00 min).

**Fig. 2.** Time course of the accumulation of cholic acid (A) and β-muricholic acid (B) in the culture medium of rat hepatocytes. Hepatocytes (obtained by collagenase digestion) were first plated in medium ± 20% calf serum. After 4 h, the culture medium was changed to serum-free medium. The medium was drawn off at the times indicated, nordeoxycholic acid was added as an internal standard, and the bile acids were extracted, deconjugated, derivatized, and quantitated by GLC as described in Fig. 1. The rates of accumulation of cholic acid (R = 0.97; slope = 4.7 × 10^{-3} µg/mg of cell protein/h) and β-muricholic acid (R = 0.99; slope = 2.4 × 10^{-3} µg/mg of cell protein/h) were determined by linear least squares analysis. Values are the mean of duplicate determinations.

**Fig. 3.** GLC analysis of medium containing 100 µM taurochenodeoxycholic acid. A, no incubation. B, incubation with cultured hepatocytes for 18 h. Medium containing 100 µM taurochenodeoxycholic acid was placed on cultured hepatocytes and immediately removed (A) or was removed after 18 h (B). The bile acids were quantitated by GLC as described in Fig. 1. Unincubated medium contained only chenodeoxycholic acid (13.00 min); the peak at 8.58 min is nordeoxycholic acid (internal standard). Incubated medium shows almost a complete disappearance of chenodeoxycholic acid (13.00 min) and the appearance of α-muricholic acid (8.10 min), cholic acid (11.84 min), β-muricholic acid (12.55 min), and two unidentified peaks at 12.89 and 14.38 min.
words, the capacity of cultured hepatocytes to 6-hydroxylate chenodeoxycholic acid is sufficient to account for the absence of chenodeoxycholic acid and the presence of β-muricholic acid in the culture medium.

**Effect of Mevalonic Acid and Mevinolin on Bile Acid Secretion**—The major impetus for these studies was to examine the relationship between hepatic cholesterol availability and bile acid secretion. By using the ability to precisely control the environment of a hepatocyte in a defined medium, the cholesterol content could be specifically varied and the effects on bile acid secretion could be determined. Previous experience showed that addition of mevalonic acid to the culture medium increased hepatocyte cholesterol availability, causing dramatic effects upon the secretion of VLDL core lipids (9). Mevinolin, on the other hand, has been shown to inhibit 3-hydroxy-3-methylglutaryl-CoA reductase via competitive binding to the enzyme (18). A result of mevinolin is decreased cholesterol biosynthesis and decreased cellular cholesterol availability. We therefore examined if altering cholesterol availability by increasing (mevalonic acid) or decreasing (mevinolin) cholesterol synthesis would cause similar changes in bile acid secretion.

Although via different mechanisms (9, 18), both mevalonic acid (10 mM) and mevinolin (50 μg/ml) inhibited the incorporation of [14C]acetate into cholesterol (Table I). Neither agent significantly altered hepatocyte-free cholesterol concentrations. However, mevalonic acid increased cholesterol ester concentration by 7-fold. In contrast, mevinolin slightly decreased cholesterol ester concentration, but this decrease was not statistically significant. In three separate experiments (hepatocyte preparations), the mean cholesterol ester concentration was always less with mevinolin, but the difference was not statistically significant. Similar changes in the esterification of de novo synthesized [14C]cholesterol were observed (Table I), i.e. mevalonic acid increased and mevinolin decreased [14C]cholesterol esterification. Mevalonic acid, which increased hepatocyte cholesterol concentration, also significantly (p < 0.05) stimulated the secretion of both cholic acid (+30%) and β-muricholic acid (+75%). In contrast, mevinolin significantly (p < 0.05) decreased the secretion of cholic acid (−20%) and β-muricholic acid (−40%).

**Effect of Cholesterol Feeding on Bile Acid Secretion**—Another approach to alter hepatocyte cholesterol availability was also examined, i.e. cholesterol feeding. We have previously shown that feeding rats a 20% olive oil + 2% cholesterol + chow diet dramatically increases the cholesterol concentration of hepatocytes (9). We therefore examined if hepatocytes obtained from cholesterol-fed rats would exhibit, in culture, changes in bile acid secretion.

Consistent with previous results (9), hepatocytes obtained from cholesterol-fed rats exhibited an almost complete inhibition of the incorporation of [14C]acetate into cholesterol (Table II). There was also a significant increase in the concentrations of both free (+40%) and esterified (+46-fold) cholesterol. Moreover, hepatocytes from cholesterol-fed rats exhibited a greater rate of secretion of both cholic acid (+2-fold) and β-muricholic acid (+10-fold) (Fig. 4). The increased rate of both cholic acid and β-muricholic acid displayed by hep-

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**TABLE I**

**Effect of mevalonic acid and mevinolin on hepatocyte cholesterol synthesis, concentrations, and secretion of bile acids**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+ Mevalonic Acid</th>
<th>+ Mevinolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Acetate</td>
<td>2308 ± 280*</td>
<td>27 ± 6*</td>
<td>900 ± 150*</td>
</tr>
<tr>
<td>[14C]Cholesterol</td>
<td>7 ± 1</td>
<td>96 ± 7*</td>
<td>3 ± 1*</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>10 ± 1</td>
<td>11 ± 2</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Concentration (μg/mg cell protein)*</td>
<td>2.4 ± 0.3</td>
<td>16 ± 2*</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Cholesterol ester concentration (μg/mg cell protein)</td>
<td>0.141 ± 0.006</td>
<td>0.184 ± 0.003*</td>
<td>0.117 ± 0.003*</td>
</tr>
<tr>
<td>Cholic acid secretion (μg/mg cell protein)*</td>
<td>0.12 ± 0.01</td>
<td>0.21 ± 0.04*</td>
<td>0.07 ± 0.02*</td>
</tr>
<tr>
<td>β-Muricholic acid secretion (μg/mg cell protein)*</td>
<td>0.02 ± 0.001</td>
<td>0.03 ± 0.002*</td>
<td>0.01 ± 0.001*</td>
</tr>
</tbody>
</table>

* Cells were extracted and the incorporation of [14C]acetate into cholesterol was determined after TLC.

* All values are the mean ± S.D. of three individual plates of cells.

* Denotes significant difference from control value at p < 0.05.

* The per cent [14C]cholesterol esterified was determined after TLC separation of free and esterified cholesterol.

* Free cholesterol and cholesterol ester concentrations were determined by GLC and are expressed as micrograms of free cholesterol.

* Bile acid secretion was determined by GLC as described in Fig. 1.
Hepatocytes from control rats were plated and cultured as described in Table I. Twelve h after changing the medium to serum-free DME, [14C]acetate was added. Two h later, cells and medium were harvested and analyzed as described in Table I. Lipoprotein fractions were obtained by ultracentrifugation as described under "Experimental Procedures." The following cuts were made: VLDL + IDL, d < 1.02 g/ml; LDL, d = 1.02-1.05 g/ml; and HDL, d = 1.05-1.21 g/ml. Lipoprotein fractions were added to DME in the following protein concentrations which corresponds to normal serum concentrations: VLDL + IDL, 100 μg/ml; LDL, 50 μg/ml and HDL, 500 μg/ml.

**Table III**

<table>
<thead>
<tr>
<th>Lipoprotein Fraction</th>
<th>Control</th>
<th>+ VLDL and IDL</th>
<th>+ LDL</th>
<th>+ HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C] Acetate → cholesterol (cpm/mg cell protein)</td>
<td>2223 ± 306</td>
<td>762 ± 83</td>
<td>2160 ± 126</td>
<td>2015 ± 30</td>
</tr>
<tr>
<td>[14C] Cholesterol esterified (%)</td>
<td>12 ± 1</td>
<td>54 ± 1</td>
<td>13 ± 2</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Free cholesterol concentration (μg/mg cell protein)</td>
<td>13 ± 1</td>
<td>54 ± 0.6</td>
<td>15 ± 1</td>
<td>9.4 ± 0.4</td>
</tr>
<tr>
<td>Cholesterol ester concentration (μg/mg cell protein)</td>
<td>3.8 ± 0.4</td>
<td>5.9 ± 0.6</td>
<td>2.9 ± 0.3</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Cholic acid secretion (μg/mg cell protein)</td>
<td>0.066 ± 0.009</td>
<td>0.081 ± 0.009</td>
<td>0.067 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>β-Muricholic acid secretion (μg/mg cell protein)</td>
<td>0.060 ± 0.002</td>
<td>0.11 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

* Denotes significant difference from control value at p < 0.05; values represent mean ± S.D. of three individual plates of cells.

The two major bile acids secreted by cultured rat hepatocytes were cholic and β-muricholic acid. Both bile acids were secreted at a constant rate for 96 h (Fig. 2). This finding indicates that, with respect to bile acid metabolism, the cultured hepatocytes maintained this highly specific liver function throughout the duration of the experiments. Additional studies show the intracellular concentrations of both cholic acid and β-muricholic acid was unchanged between the time of starting the experiments and after 72 h (the longest time point examined). These data indicate that bile acid secretion was due to synthesis and is not caused by leakage due to cell lysis or death. Moreover, we have previously shown that these cells maintain rates of albumin and lipoprotein synthesis and secretion which are similar to those found in perfused livers (9, 17). These data combined with the findings that cells maintain the ability to exclude (>90%) trypan blue throughout the course of our experiments (data not shown) suggest that the integrity of our preparation is maintained.

Qualitatively, compared to rat bile (26), hepatocytes secrete a greater proportion of β-muricholic acid and less chenodeoxycholic acid. Under basal conditions, hepatocytes secreted slightly more cholic acid compared to β-muricholic. However, since β-muricholic acid secretion was more responsive to both stimulation and inhibition, the composition of bile acids secreted by hepatocytes was variable.

The absence of detectable amounts of chenodeoxycholic acid in the culture medium is likely to be due to its efficient 6-hydroxylation (27, 28) by the cultured hepatocytes. This hypothesis was examined directly by adding taurochenodeoxycholic acid to the culture medium. Within 18 h, essentially all of the chenodeoxycholic acid was transformed, mainly to β-muricholic acid (Fig. 3). This large capacity to 6-hydroxylate chenodeoxycholic acid exhibited by cultured hepatocytes is sufficient to account for the absence of chenodeoxycholic acid and the presence of β-muricholic acid in the culture medium. In the rat, 6-hydroxylation has been shown to be cytochrome P-450 dependent and to be activated by phenobarbital administration (29).

Under basal conditions, the total secretion rate of bile acids (cholic + β-muricholic acid) was estimated to be 6 nmol/g of liver/h, which is 20-50% of values reported for freshly isolated hepatocytes and in vivo estimates (see Ref. 25 for a table of

**DISCUSSION**

Because of the small quantities of bile acids secreted by cultured hepatocytes (each plate contains about 1% of the liver), a sensitive quantitative assay was developed. It was reported that 3α-hydroxy steroid dehydrogenase analysis gives an inaccurate overestimation of bile acids secreted by isolated hepatocytes (24, 25). Both these reports urge the use of GLC for such analysis (24, 25). The GLC technique described in this report has the proven capacity to detect 10⁻⁹ g of bile acid (±5% variation; data not shown). This assay might have additional use for the analysis of serum bile acids used in a clinical liver function test.
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Rates. This rate is a minimal estimate, since as yet unidentified GLC peaks have not been quantitated. Moreover, as shown in the results, addition of cholesterol precursors and cholesterol-feeding and cholesterol-enriched lipoproteins to the culture medium increases the net rate of bile acid secretion by up to 10-fold (Fig. 4).

Current concepts suggest that bile acids regulate their own synthesis via negative feedback inhibition of 7α-hydroxylase (1). The data showing that bile acid secretion by cultured rat hepatocytes is linear for 96 h (Fig. 2) might be interpreted to suggest that bile acids have not accumulated in the culture medium in quantities sufficient to inhibit 7α-hydroxylase. However, analysis of the data in Fig. 3B clearly shows that cholic acid (retention time, 11.54 min) was secreted even in the presence of 100 μM taurochenodeoxycholate. In addition, quantitation of the amount of cholic acid secreted in the presence (Fig. 3B) and absence (Fig. 1B) of 100 μM taurochenodeoxycholate clearly shows no inhibition of cholic acid secretion (data not shown). Since essentially all of the added taurochenodeoxycholic acid was metabolized to α- and β-muricholic acids (Fig. 3B), the hepatocytes clearly took up, metabolized, and secreted the bile acids. These data suggest that even at a concentration of taurochenodeoxycholate which is higher than that found in portal blood (30, 31) the secretion of cholic acid by cultured rat hepatocytes was not inhibited.

In recent studies, we found that different bile acids at concentrations as high as 2.5 mM do not affect bile acid synthesis, i.e., the incorporation of [14C]cholesterol into 14C-labeled bile acids. These data indicate that bile acids do not have a direct inhibitory effect on bile acid synthesis and secretion by cultured rat hepatocytes.

The data of this study suggest that substrate availability, i.e. hepatocyte cholesterol concentrations, plays a role in regulating bile acid secretion. It is likely that whereas hepatocyte-free cholesteryl is an essential membrane component, cholesteryl esters are a storage form. The results showing that hepatocyte cholesterol ester concentrations are quite variable, whereas free cholesterol concentrations remain relatively constant (Tables I-III), support this proposal. It is not, therefore, unreasonable to assume that cholesterol esters represent a pool which can be activated (by hydrolysis) for bile acid synthesis. Under basal conditions, about 5% of the cholesterol ester pool would be turned over to account for basal net rate of bile acid secretion exhibited by cultured hepatocytes. However, the net effect of bile acid secretion on the turnover of the hepatocyte cholesterol pool is likely to be considerably greater. Biliary cholesterol secretion is driven by bile acid secretion (32, 33). Thus, the requirements which the hepatocyte cholesterol pool must satisfy for bile acid secretion include that which is utilized as a substrate for bile acid synthesis and that which is secreted as a component of biliary bile acids. These data suggest that bile acid secretion can have a substantial impact on the hepatocyte cholesterol pool. If adaptable to changes in cholesterol availability, bile acid secretion could maintain cholesterol homeostasis.

The ability to effect specific changes in cholesterol availability in the hepatocyte culture model allowed us to examine this latter possibility. The data show that as a result of altering the hepatocyte cholesterol pool, there are concomitant changes in bile acid secretion. Three methods were used to increase cholesterol availability: 1) changing the rate of cholesterol biosynthesis via adding mevalonic acid to the culture medium; 2) feeding a cholesterol-rich diet; and 3) adding


lipoprotein fractions obtained from serum. In all three approaches, similar results were obtained, i.e. [14C]cholesterol synthesis from [14C]acetate was inhibited, esterification of de novo synthesized [14C]cholesterol was increased, cholesterol ester concentrations were increased, and there was a significant stimulation of bile acid secretion. Conversely, in an attempt to decrease cholesterol availability, hepatocytes were treated with mevinolin, a drug which competitively blocks 3-hydroxy-3-methylglutaryl-CoA reductase (18). Although statistically significant changes in hepatocyte cholesterol concentrations were not obtained with mevinolin, there was a significant inhibition of [14C]acetate incorporation into cholesterol and bile acid secretion (Table I).

In several species including both rats (34) and humans (35), cholesterol-enriched diets increase bile acid excretion. In these studies, we found that hepatocytes obtained from cholesterol-fed rats exhibit an increased rate of bile acid secretion which is similar in magnitude to that obtained by sterol balance studies (34). These data show that the hepatocyte culture model possesses the capacity to express in culture changes in bile acid secretion which are of similar magnitude to those observed in vivo. However, changes in bile acid secretion obtained via in vitro effects (i.e. mevalonic acid, mevinolin, and lipoproteins) were smaller than those obtained in vivo via the cholesterol-rich diet. Although the in vitro-induced changes were small, they were consistently found and differences were statistically significant. It is possible that more time is needed for effectors to maximally alter bile acid secretion. This has been previously shown to be the case for dietary cholesterol stimulation of bile acid excretion in vivo (34). It is also possible that additional factors (not present in the isolated hepatocyte system) act in concert to regulate bile acid synthesis and secretion in vivo. The hepatocyte culture model should provide an ideal system to examine these possibilities.

Recently, Shefer et al. (36) showed that delipidated micromesomes obtained from cholesterol-fed rats exhibit unaltered activity of 7α-hydroxylase. These authors proposed that cholesterol feeding increases bile acid synthesis by increasing the cholesterol substrate pool (36). The results of our study are in complete agreement with this hypothesis. However, it is as yet unknown if cholesterol availability affects the activity of 7α-hydroxylase in these cultured hepatocytes.

Our studies show for the first time that serum lipoproteins can stimulate hepatic bile acid secretion (Table III). The results showing that the d < 1.02 g/ml lipoprotein fraction obtained from cholesterol-fed rats caused a concomitant increase in cholesterol ester concentrations, increased the esterification of de novo synthesized [14C]cholesterol, and decreased the synthesis of de novo synthesized [14C]cholesterol are consistent with this lipoprotein being taken up and degraded by the liver in a manner similar to that described for chylomicron remnants (21-23). Since other lipoprotein fractions from the same group of rats (Table III) as well as all lipoprotein fractions obtained from control rats had no effect on cholesterol-bile acid metabolism, the effects initiated by the d < 1.02 g/ml fraction from cholesterol-fed rats were specific.

Adult animals express hepatic receptors which recognize apo-E (8). Recognition, uptake, and degradation of lipoproteins via the apo-E receptor could account for our findings. Analysis of the d < 1.02 g/ml fraction obtained from cholesterol-fed rats suggests the presence of cholesterol ester-rich chylomicron remnants. Additional studies are needed to characterize the types of lipoproteins which influence bile acid synthesis and the mechanisms responsible for their uptake by hepatocytes.
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