Bile duct cells: a novel in vitro model for the study of lipid metabolism and bile acid production

MONIKA ZOLTOWSKA, EDGARD E. DELVIN, KHAZAL PARADIS, ERNEST SEIDMAN, AND EMILE LEVY
Departments of Nutrition, Biochemistry, and Pediatrics, Centre de Recherche, Hôpital Ste-Justine, Université de Montréal, Montreal, Quebec, Canada H3T 1C5

Zoltowska, Monika, Edgard E. Delvin, Khazal Paradis, Ernest Seidman, and Emile Levy. Bile duct cells: a novel in vitro model for the study of lipid metabolism and bile acid production. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G407–G414, 1999.—Immortalized bile duct cells (BDC), derived from transgenic mice harboring the SV40 thermosensitive immortalizing mutant gene ts458, were utilized to investigate the role of the biliary epithelium in lipid and sterol metabolism. This cell model closely resembles the in vivo situation because it expresses the specific phenotypic characteristics of the biliary epithelium. BDC can modify the composition of bile by secreting a variety of lipids, including phospholipids, triglycerides, and cholesterol esters. This cell model also serves as a delivery system for the regulatory enzymes and conversion factors that promote and control the metabolic relationships and remodeling of plasma lipoproteins. Furthermore, the liver also serves as a delivery system for the regulatory enzymes and conversion factors that promote and control the metabolic relationships and remodeling of plasma lipoproteins.

The liver plays a central role in the metabolism of plasma lipids and lipoproteins, serving as the major site of synthesis of several apoprotein components and various lipid species. It also secretes nascent lipoproteins (very-low-density lipoproteins and high-density lipoproteins) and degrades chylomicron remnants, as well as low- and high-density lipoproteins, after their uptake. Furthermore, the liver also serves as a delivery system for the regulatory enzymes and conversion factors that promote and control the metabolic relationships and remodeling of plasma lipoproteins. Finally, hepatic biliary cholesterol secretion and bile acid synthesis constitute the main routes of cholesterol elimination from the body. Disturbances of plasma lipid and lipoprotein composition frequently occur in patients with chronic cholestatic liver disease. Despite the association between primary biliary cirrhosis and other cholestatic syndromes with major lipid and lipoprotein derangements, little is known about the contribution of the biliary epithelium to the metabolic disturbances. Although they make up only 3–5% of the overall population of liver cells, bile duct cells (BDC) provide a large surface area for exchange between blood and bile. They play a key physiological role in the formation of bile, producing as much as 40% of the daily volume. BDC, in close proximity to the hepatic arterioles that serve as their vascular supply, also display absorptive and secretory capabilities. Furthermore, increasing evidence has been put forth indicating that BDC can modify the composition of bile by secreting water, protein, and bicarbonate and reabsorbing glucose, glutamate, and anions. Bile is a complex mixture of organic compounds, studies on biliary epithelium reported to date have been largely restricted to bile flow and ion permeability. It is noteworthy that bile flow is the result of plasma/bile transport of bile acids and other solutes, which create osmotic gradients that stimulate bile formation.

Progress in understanding the cellular and molecular basis of biliary epithelium metabolism and transport of sterols and lipoproteins has lagged behind studies on hepatocytes, largely because of technical problems in isolating pure BDC. However, the recent availability of immortalized BDC, originating from H-2Kb-ts458 transgenic mice, allows for more rigorous studies of the biology and function of the biliary epithelium. In the present study, we utilized this cell model to examine the synthesis of neutral lipid and phospholipid classes, as well as the production and conjugation of bile acids.

THE LIVER PLAYS A CENTRAL ROLE in the metabolism of plasma lipids and lipoproteins, serving as the major site of synthesis of several apoprotein components and various lipid species. It also secretes nascent lipoproteins (very-low-density lipoproteins and high-density lipoproteins) and degrades chylomicron remnants, as well as low- and high-density lipoproteins, after their uptake. Furthermore, the liver also serves as a delivery system for the regulatory enzymes and conversion factors that promote and control the metabolic relationships and remodeling of plasma lipoproteins. Finally, hepatic biliary cholesterol secretion and bile acid synthesis constitute the main routes of cholesterol elimination from the body. Disturbances of plasma lipid and lipoprotein composition frequently occur in patients with chronic cholestatic liver disease. Despite the association between primary biliary cirrhosis and other cholestatic syndromes with major lipid and lipoprotein derangements, little is known about the contribution of the biliary epithelium to the metabolic disturbances. Although they make up only 3–5% of the overall population of liver cells, bile duct cells (BDC) provide a large surface area for exchange between blood and bile. They play a key physiological role in the formation of bile, producing as much as 40% of the daily volume. BDC, in close proximity to the hepatic arterioles that serve as their vascular supply, also display absorptive and secretory capabilities. Furthermore, increasing evidence has been put forth indicating that BDC can modify the composition of bile by secreting water, protein, and bicarbonate and reabsorbing glucose, glutamate, and anions. Bile is a complex mixture of organic compounds, studies on biliary epithelium reported to date have been largely restricted to bile flow and ion permeability. It is noteworthy that bile flow is the result of plasma/bile transport of bile acids and other solutes, which create osmotic gradients that stimulate bile formation.

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METHODS

Cell culture. The immortalized BDC line, originating from the transgenic mouse harboring the SV40 thermosensitive mutant gene ts458, was established as previously described (22). Cells were allowed to grow on Matrigel in 75-cm² ventilated flasks (Corning Costar, Cambridge, MA). The culture medium consisted of a 50:50 mix of Dulbecco’s modified Eagle’s medium with L-glutamine and D-glucose-Ham’s nutrient mixture F-12 with L-glutamine (0.1 mmol/l), minimal essential medium nonessential amino acid solution, D-glucose (5.4 g/l), and HEPES (10 mmol) buffer adjusted at pH 7.40. The medium was supplemented with 100 µg/l penicillin G and 100 µg/ml streptomycin sulfate (both from GIBCO, Grand Island, NY); 10 µg/ml epidermal growth factor, 5 ng/ml each of insulin and transferrin, and 5 µg/ml selenium (all from Collaborative Biomedical Products, Bedford, MA); and 32 ng/ml thyroxin, 10 ng/ml prostaglandin E₁, 40 ng/ml hydrocortisone, and 10 µg/ml mouse recombinant interferon-γ (all from Boehringer Mannheim). Cells were grown at 33°C and were used for experimental studies after confluence (7–10 days). Their viability was assessed by trypan blue exclusion.

Immunofluorescence. Standard immunofluorescence microscopy techniques were used to detect cytokeratin-19 (CK-19), albumin, and macrophage F4/80 antigen. Briefly, cells were washed with PBS at pH 7.40, fixed in cold acetone for 10 min, and air dried. After nonspecific antibody binding was blocked, the slides were incubated with primary mouse anti-CK-19 (Amersham, Oakville, ON, Canada), the macrophage-specific antibody F4/80, or anti-albumin (Cedarlane Laboratories, Hornby, ON, Canada). This was followed by incubation with fluorescein isothiocyanate-labeled sheep anti-mouse immunoglobulin G2b. Slides were washed with PBS, mounted, and photographed.

Electron microscopy. Cells with apparent ductlike structures were fixed in glutaraldehyde, embedded in Epon, and then sectioned using routine methods for electron microscopy (23).

Lipid synthesis. [¹⁴C]oleic acid (specific activity 53.9 mCi/mmol; Amersham, Oakville, PQ, Canada) complexed with albumin (25) was added to the medium (final specific activity 1,000,000 dpm/mol). Lipids were extracted from aliquots of cell homogenates and their respective incubation media with chloroform-methanol (2:1, vol/vol) (25). Tracer amounts of

Fig. 1. Immortalized bile duct cells (BDC) express cytokeratin-19 (CK-19) and characteristic features of biliary epithelium. A: cell suspension prepared from cultured BDC (5–8 passages) after adherence. Mouse anti-CK-19 followed by fluorescein isothiocyanate-labeled sheep anti-mouse immunoglobulin G2b revealed CK-19 (original magnification, ×67). B: negative control for anti-CK-19 staining (original magnification, ×67). C: BDC display ductlike structure formation. Cells were grown on Matrigel in 75-cm² ventilated flasks. After 5–6 days in culture, clumps of cells were clearly seen to have branched, ductlike structures. Observations were made with inverted microscope (original magnification, ×67). D: ultrastructural features of immortalized BDC. Ductlike structures formed by BDC were aspirated with pipette, fixed in glutaraldehyde, embedded in Epon, and prepared for electron microscopy. Well-formed microvilli (M) projected from apical region of cell into central lumen (L). Occluding-type junctions (TJ) were present between cells (original magnification, ×10,000).
lipid standards were added to the samples before separation of individual lipid classes by unidimensional thin-layer chromatography (TLC) (silica gel, Eastman-Kodak, Rochester, NY) as described previously (25). The apolar solvent system was hexane-diethylether-glacial acetic acid (80:23.3: vol/vol/vol) and the polar solvent was chloroform-methanol-water-acetic acid (65:25:4:1, vol/vol/vol/vol). After scraping, the radioactive spots corresponding to migration of bile acid standards were visualized by iodine vapor, scraped, and counted.

Enzyme activity assays. Microsomal activity of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and acyl-CoA: cholesterol acyltransferase (ACAT) was determined as previously described (21).

Statistics. Statistical evaluation of the results was performed by the Student's two-tailed t-test.

**RESULTS**

Morphology and immunofluorescence studies. Morphological properties characteristic of bile duct epithelial cells were observed in our BDC line. The majority of BDC consisted of homogeneous populations of small round cells, readily distinguished from hepatocytes by their size and morphology. The epithelial origin of the immortalized cell line was confirmed by immunofluorescent microscopy technique showing the presence of CK-19 (Fig. 1, A and B). Negative staining using specific antibodies against F4/80 and albumin excluded contamination by macrophages and hepatocytes, respectively. After the first 24-h period in culture, BDC formed small islands on Matrigel, a basement membrane gel complex. Thereafter, they developed branched, ductlike structures (Fig. 1C). Electron microscopy revealed well-formed microvilli and apical tight intercellular junctions (Fig. 1D).

Lipid synthesis and secretion. To determine whether biliary epithelial cells had the ability to synthesize and secrete newly formed lipids, BDC were incubated with [14C]oleic acid. The incorporation of this radioactive substrate into BDC was linear over time for up to 20 h (results not shown). We observed substantial incorpora-

**Table 1. Composition of lipids synthesized by immortalized BDC**

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<th></th>
<th>TG</th>
<th>PL + MG</th>
<th>CE</th>
<th>FC</th>
<th>DG</th>
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<tr>
<td>Cells</td>
<td>60.48 ± 1.63</td>
<td>29.50 ± 1.44</td>
<td>8.06 ± 0.20</td>
<td>1.05 ± 0.12</td>
<td>0.91 ± 0.09</td>
</tr>
<tr>
<td>Media</td>
<td>68.51 ± 1.27</td>
<td>22.77 ± 1.15</td>
<td>4.88 ± 0.17</td>
<td>2.08 ± 0.24</td>
<td>1.77 ± 0.12</td>
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Values are expressed as % of total [14C]-labeled lipid distribution and represent means ± SE of 5 experiments. Bile duct cells (BDC) were incubated with [14C]oleic acid substrate for 20 h. Lipids of cell homogenates and media were then extracted with chloroform-methanol (21), isolated by thin-layer chromatography (TLC), and quantitated as described in METHODS. PL, phospholipids; MG, monoacylglycerol; DG, diacylglycerol; FC, free cholesterol; FFA, free fatty acids; TG, triacylglycerol; CE, cholesteryl ester. *P < 0.005, †P < 0.01, media vs. cells.
tion into cellular triglycerides, phospholipids, and cholesteryl esters (Fig. 2). In all experiments, the amount of lipids was higher in cells than in the media, suggestive of a limited secretory capacity of BDC. Table 1 depicts the composition of total lipids analyzed by TLC. Triglycerides were the predominant lipids, followed by phospholipids and cholesteryl esters. The same profile was observed for both cells and media. Although the medium was slightly enriched in triacylglycerol, diacylglycerol, and free cholesterol, it had a low content of phospholipids plus monoacylglycerol and cholesterol ester.

Phospholipid profile. The BDC were also found to be capable of producing all major phospholipid classes. The absolute amount of phospholipids synthesized was higher in cells than in media, the latter accounting for <5% of cell phospholipids (Table 2). Phosphatidylcholine was the predominant form of 14C-labeled lipids elaborated. Major differences were noted in phospholipid composition between the cellular compartment and the media (Fig. 3). The percentage of sphingomyelin, phosphatidylserine, and phosphatidylinositol was higher in the medium, whereas the proportion of phosphatidylcholine and phosphatidylethanolamine was preponderant within the cells.

[14C]acetate incorporation into cholesterol. The BDC cholesterol biogenesis was assessed using [14C]acetate. As can be seen in Fig. 4, BDC incorporated substantial amounts of [14C]acetate into cholesterol and cholesteryl ester. These findings confirm the data (Table 1) obtained with [14C]oleic acid and suggest the presence, in BDC, of HMG-CoA reductase and ACAT, the two key enzymes involved in cholesterol metabolism.

Bile acid synthesis. BDC were evaluated for their ability to synthesize bile acids in the presence of [14C]acetate (Table 3). Several types of bile acids were produced, including taurocholate, tauroursodeoxycholate, glycocholate, lithocholate, and cholic acid (Fig. 5). Similar results were obtained using [14C]cholesterol as substrate (results not shown). The composition of the bile acids in the cells was quite different from their distribution in the culture medium. Intracellularly, a preponderance of taurocholate, tauroursodeoxycholate, and lithocholate was found, whereas glycocholate and glycochenocholate were dominant in the medium. Overall, the total incorporation of [14C]acetate into bile acids in

<table>
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<th>Table 2. Immortalized BDC incorporation of [14C]oleic acid into phospholipid classes</th>
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<td></td>
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<tr>
<td>Cells</td>
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<tr>
<td>Media</td>
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Values are expressed in nmol/mg protein × 10⁻³ and represent means ± SE of 7 experiments. Phospholipids from cell homogenates and media were extracted, isolated by TLC, and quantitated. *P < 0.01, †P < 0.005, media vs. cells.
the medium consistently exceeded fivefold that in the
cells, indicating an active bile acid secretory capacity.

Enzyme activity. We also determined the activity of
the three sterol enzymes that regulate hepatic intracel-
lar cholesterol homeostasis. The activity of HMG-
CoA reductase, cholesterol 7α-hydroxylase, and ACAT
was detected in microsomes isolated from BDC (Fig. 6).
The mean activities measured (in pmol·min⁻¹·mg pro-
tein⁻¹) were 53.0 ± 6.7 for HMG-CoA reductase, 15.5 ±
0.5 for cholesterol 7α-hydroxylase, and 201.7 ± 10.2 for
ACAT.

**DISCUSSION**

Until recently, study of the cell biology and function
of biliary epithelial cells has been hampered by their
inaccessibility. Attempts to isolate BDC have shed light
on many properties of BDC (1, 2, 12, 18, 30, 35, 51, 52).
However, none of these techniques yield pure popula-
tions of homogeneous BDC in sufficient quantity. With
one exception (35), they do not recreate the tubular
orientation of polarized BDC in vitro. Strategies have
thus been designed to develop immortalized BDC clones
that would mimic the in vivo situation as closely as
possible. In this study, we confirm that immortalized
BDC display well-differentiated features of mature
biliary epithelium, with definite cellular polarization
and formation of ductlike structures, well-developed
apical microvilli, and tight junctions. The immortalized
BDC also express cytokeratin-19, a phenotypic marker
normally found in BDC. This cell line thus provides us
with the opportunity to verify the emerging concept
that intrahepatic biliary epithelial cells are actively
involved in lipid and bile acid metabolism.

In the present study, we demonstrate the ability of
BDC to incorporate fatty acids for lipid esterification.
The mechanism of cellular uptake of these lipophilic
compounds is not yet elucidated. Fatty acids may enter
cells by passive diffusion or may be absorbed via an
energy-independent, facilitated diffusion mechanism
(6, 7, 36). Recent studies have also suggested that a
saturable process mediated by specific binding sites on
the cell surface is involved in the uptake of the albumin-
ligand complex (36). The relative importance of these
potential routes depends on the tissue and organ
involved and the concentration and properties of the
ligand (5, 7, 36). Additional work is needed to define the
uptake and translocation of fatty acids to the endoplas-
mic reticulum for lipid esterification.

Triglycerides are the dominant class of lipids elabo-
rated by BDC when using [14C]oleic acid as precursor.
In the liver, after acylation in the rough endoplasmic
reticulum, the majority of fatty acids are incorporated
into triglycerides via the phosphatidic acid pathway
(10). The conversion of phosphatidate to 1,2-diacylgly-
cerol by phosphatidate phosphatase is the rate-limiting
reaction of triglyceride biosynthesis. Our data suggest
that BDC possess this important anabolic step. Further-
more, the high de novo-formed phospholipid content
points to the active intracellular participation of the
enzymes required for their synthesis.

In our experiments, the medium lipid content ap-
proaches only 7% of intracellular production, indicating
a limited lipid export capacity of BDC. In this respect,
BDC do not contribute a significant movement of locally
manufactured lipids into bile, in contrast to hepatocytes.
However, it is possible that lipid reabsptive
mechanisms are also operative in BDC.

Our results provide evidence for the capacity of BDC
to synthesize and secrete bile acids that, qualitatively,
are similar to those produced by hepatocytes. They

**Table 3. Bile acids synthesized by immortalized BDC**

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<th></th>
<th>Total</th>
<th>Taurocholate</th>
<th>Taurocholinate</th>
<th>Taurochenodeoxycholate</th>
<th>Glycocholate</th>
<th>Glycochenodeoxycholate</th>
<th>Cholic Acid</th>
<th>Chenodeoxycholic Acid/Glycocholic Acid</th>
<th>Lithocholic Acid</th>
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<tr>
<td>Cells</td>
<td>11.29 ± 1.92</td>
<td>8.58 ± 1.34</td>
<td>0.69 ± 0.15</td>
<td>0.21 ± 0.02</td>
<td>0.31 ± 0.04</td>
<td>0.28 ± 0.06</td>
<td>0.29 ± 0.05</td>
<td>0.84 ± 0.21*</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>Media</td>
<td>61.53 ± 6.62*</td>
<td>13.75 ± 1.12†</td>
<td>2.32 ± 0.17*</td>
<td>3.67 ± 0.74*</td>
<td>12.89 ± 2.43*</td>
<td>25.06 ± 2.86*</td>
<td>2.63 ± 0.21*</td>
<td>0.37 ± 0.04</td>
<td>0.37 ± 0.04</td>
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</table>

Values are expressed as nmol/mg cell protein × 10⁻⁴ and represent means ± SE of 6 experiments. BDC were incubated with [14C]acetate
substrate for 20 h. Bile acids of cell homogenates and media were then extracted with H₂O/MeOH, isolated by TLC, and quantitated. *P <
0.05, †P < 0.01, media vs. cells. The mean activities measured (in pmol·min⁻¹·mg pro-
tein⁻¹) were 53.0 ± 6.7 for HMG-CoA reductase, 15.5 ±
0.5 for cholesterol 7α-hydroxylase, and 201.7 ± 10.2 for
ACAT.

![Fig. 5. Composition of bile acids synthesized by immortalized BDC. Bile acids were extracted from BDC and cell culture medium. Results, expressed as % of total bile acids, represent means ± SE of 5 experiments. Chenodeoxy, chenodeoxycholic acid; glycolitho, glyco-
lithocholate. *P < 0.02, **P < 0.005, media vs. cells.](http://ajpgi.physiology.org/)

Viewed from http://ajpgi.physiology.org/ by 10.220.33.2 on March 31, 2017
differ, however, in their relative proportions. Indeed, in our model, newly synthesized bile acids mostly consist of lithocholic acid and its tauro- and glycoconjugates (83%). Cholic and chenodeoxycholic acid derivatives account for 11 and 6%, respectively. Javitt (17) have clearly established that there are two main pathways for bile acid synthesis. The first, which utilizes the classical 7α-hydroxylation pathway, yields cholic and chenodeoxycholic acids, which account for 95% of the bile acids and salts synthesized by Hep G2 liver cells in culture (15). It must be stressed at this point that 7α-hydroxylase activity has been measured in our cells (Fig. 6). The fact that its specific activity is lower than that observed in liver microsomes in other models (19) supports our [14C]acetate incorporation results. We may thus surmise that this pathway is a minor one in BDC. The C-26 hydroxylation of cholesterol is the preferential pathway for the biosynthesis of lithocholic acid (15). It can also be derived from the 7α-dehydroxylation of chenodeoxycholic acid by the intestinal bacterial flora (16). Because our culture model is devoid of such bacterial contamination and because the enzyme responsible for C-26 hydroxylation of cholesterol is present in a number of epithelial cells (37), it can legitimately be invoked as a major pathway in our model.

Under physiological conditions, the coordination of the microsomal enzymes HMG-CoA reductase, cholesterol 7α-hydroxylase, and ACAT is closely associated with the maintenance of liver cholesterol homeostasis (3). HMG-CoA reductase has been demonstrated to be the rate-limiting enzyme in cholesterol biosynthesis (39). Cholesterol 7α-hydroxylase, a specific microsomal cytochrome P-450 isoenzyme, is the initial and rate-determining enzyme in the bile acid biosynthesis pathway (31, 42). Based on the failure to observe detectable activity of the cytochrome P-450 system (28, 29), it was assumed that the biliary epithelium does not possess the de novo sterol-synthesizing enzymes, which have not been tested in these studies. However, our data unequivocally show that labeled bile acids are formed from a radioactive precursor and that BDC microsomes contain HMG-CoA reductase and cholesterol 7α-hydroxylase activities. Furthermore, our results demonstrated the presence of microsomal ACAT, which is not part of the cytochrome P-450 system. It is therefore tempting to speculate that the biliary epithelium, having the capacity to elaborate and conjugate bile acids, may modify the composition of bile acids secreted. It is important to emphasize that our BDC model is devoid of any hepatocytes and macrophages, potential sources of contamination. These BDC have furthermore been clearly characterized with regard to their enzyme profiles and do not contain albumin, an essential characteristic of hepatocytes.

ACAT is the enzyme responsible for the acylation of cholesterol to cholesterol esters, a transformation that strongly influences hepatic excretion of cholesterol (44). Activity of this enzyme is detected in BDC, suggesting a potential role in the biliary epithelium. Activation of ACAT would effectively result in an increment of cholesteryl ester, with deficient conversion to bile acids.

In conclusion, evidence has been presented that BDC synthesize various lipid classes albeit with a limited capacity of exporting them. The biogenesis and secretion of sterols, including bile acids, suggest an active role in the modification of biliary content by the biliary epithelium. This BDC line represents an experimental model to study biliary epithelial cell biology under normal and pathophysiological conditions.

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Address for reprint requests: E. Levy, Gastroenterology-Nutrition Unit, Hôpital Ste-Justine, 3175 Côte Ste-Catherine, Montreal, Quebec, Canada H3T 1C5.

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