# Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: pathophysiology of biliary lipid secretion[1]

**David Q-H. Wang,* Frank Lammert,[2,*] Beverly Paigen,[†] and Martin C. Carey[3,*]**

Department of Medicine, Gastroenterology Division, Brigham and Women's Hospital, Harvard Medical School and Harvard Digestive Diseases Center,* Boston, MA 02115, and The Jackson Laboratory,[†] Bar Harbor, ME 04609

**Abstract** **The inbred C57L strain but not the AKR strain of mice carry *Lith* genes that determine cholesterol gallstone susceptibility. When C57L mice are fed a lithogenic diet containing 15% fat, 1% cholesterol, and 0.5% cholic acid, gallbladder bile displays rapid cholesterol supersaturation, mucin gel accumulation, increases in hydrophobic bile salts, and rapid phase separation of solid and liquid crystals, all of which contribute to the high cholesterol gallstone prevalence rates (**D. Q-H. Wang, B. Paigen, and M. C. Carey. ***J. Lipid Res.* **1997.** 38: **1395–1411). We have now determined the hepatic secretion rates of biliary lipids in fasting male and female C57L and AKR mice and the intercross (C57L × AKR)$F_1$ before and at frequent intervals during feeding the lithogenic diet for 56 days. Bile flow and biliary lipid secretion rates were measured in the first hour of an acute bile fistula and circulating bile salt pool sizes were determined by the "washout" technique after cholecystectomy. Compared with AKR mice, we found that *i*) C57L and $F_1$ mice on chow displayed significantly higher secretion rates of all biliary lipids, and larger bile salt pool sizes, as well as higher bile salt-dependent and bile salt-independent flow rates; *ii*) the lithogenic diet further increased biliary cholesterol and lecithin outputs, but bile salt outputs remained constant. Biliary coupling of cholesterol to lecithin increased approximately 30%, setting the biophysical conditions necessary for cholesterol phase separation in the gallbladder; and *iii*) no gender differences in lipid secretion rates were noted but male mice exhibited significantly more hydrophobic bile salt pools than females. We conclude that in gallstone-susceptible mice, *Lith* genes determine increased outputs of all biliary lipids but promote cholesterol hypersecretion disproportionately to lecithin and bile salt outputs thereby inducing lithogenic bile formation.**—Wang, D. Q-H., F. Lammert, B. Paigen, and M. C. Carey. **Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: pathophysiology of biliary lipid secretion. *J. Lipid Res.* 1999.** 40: **2066–2079.**

**Supplementary key words** gallstones • enterohepatic circulation • genetics • phase diagrams • pool size • bile salt species • bile flow • dominant trait • microscopy

The molecular origins of altered secretion rates of biliary lipids in human cholesterol (Ch) cholelithiasis remain unknown (1, 2). Although Ch supersaturation, accumulation of mucin gel, and rapid Ch crystallization in gallbladder bile are invariable (3–9), relative Ch hypersecretion into hepatic bile may or may not be accompanied by normal, high, or low secretion rates of biliary bile salts (BS) or lecithin (Lec) (5, 7, 10–13). Whether defects exist in hepatic lipid regulatory enzymes, responsible for Ch and BS biosynthesis, is controversial (14–17). Recently, we reported that Ch gallstone formation in mice is genetically determined, and a major *Lith* (gallstone) gene maps to mouse Chromosome 2 (18). Furthermore, phenotypic studies of gallbladder bile (19) and hepatic lipid-regulatory enzymes (20) in inbred mice fed a lithogenic diet containing 15% dairy fat, 1% Ch, and 0.5% cholic acid (21) revealed several key differences between gallstone-susceptible and resistant strains. Susceptible mice display early Ch supersaturation, accumulation of mucin gel, and larger gallbladders as well as higher gallstone prevalence rates compared to resistant mice (19). In both male and female gallstone-susceptible mice, activity of hepatic HMG-CoA reductase controlling Ch biosynthesis failed to downregulate, and Ch 7α-hydroxylase and sterol 27-hydroxylase, both regulatory enzymes in BS biosynthesis, became profoundly suppressed on the lithogenic diet (18, 20, accompanying paper). Because these different responses in mouse strains may be secondary to primary dysfunctions

Abbreviations: BS, bile salt; Ch, cholesterol; CSI, cholesterol saturation index; HI, hydrophobicity index; HPLC, high performance liquid chromatography; Lec, lecithin; TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; T-β-MC, tauro-β-muricholate; T-ω-MC, tauro-ω-muricholate; TUDC, tauroursodeoxycholate.

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[2] Present address: Department of Internal Medicine III, University of Technology at Aachen, Germany.

[3] To whom correspondence should be addressed.

encoded by *Lith* genes, it became essential to unravel the nature of the hepatic phenotypes in freshly secreted hepatic bile of Ch gallstone susceptible and resistant mice. Therefore, we investigated the biliary lipid secretion rates before and during lithogenic diet feeding for 56 days in inbred mice carrying either the susceptible (C57L strain) or resistant (AKR strain) *Lith* alleles, and the heterozygous (C57L × AKR) $F_1$ progeny. Our findings indicate that in gallstone-susceptible mice, there is absolute and relative Ch hypersecretion leading to elevated Ch saturation indexes (CSI) in the face of up-regulated BS and Lec secretion rates. These data provide important clues for exploring further the roles of *Lith* genes in pathogenesis of Ch cholelithiasis as well as for investigating the individual *Lith* gene phenotypes.

## MATERIALS AND METHODS

### Chemicals

For high performance liquid chromatographic (HPLC) analyses, all reagents were HPLC grade and purchased from Fisher Scientific Co. (Fair Lawn, NJ). BS standards were obtained from Sigma Chemical Co. and CalBiochem-Behring (San Diego, CA), with the exceptions of the taurine conjugates of β- and ω-muricholates (3α, 6β, 7β-trihydroxy-5β-cholanoate (T-β-MC) and 3α, 6α, 7β-trihydroxy-5β-cholanoate (T-ω-MC), respectively) which were provided generously by Mr. H. Sugata of the Tokyo Tanabe Co., Tokyo, Japan. Purity of individual BS by HPLC was >98% (22, 23). All other chemicals and solvents were American Chemical Society (ACS) or reagent grade quality (Fisher Scientific Co., Medford, MA).

### Animals and diets

Male and female C57L/J, AKR/J, and (C57L × AKR)$F_1$ mice, 4–6 wks old, were bred at the Jackson Laboratory, Bar Harbor, ME. Inbred C57L mice are homozygous for susceptible *Lith* alleles; AKR mice are homozygous for the resistant alleles; and (C57L × AKR)$F_1$ mice are the heterozygous progeny (18, 19). All animals were maintained in a temperature-controlled (22 ± 1°C) room with 12-h day–night cycles (6 am–6 pm). Mice were allowed to adapt to the environment for at least 2 wks prior to feeding the lithogenic diet and were provided free access to water and Purina laboratory chow which contains traces (<0.02%) of Ch (21) (The Mouse Diet no. 1401, S. Hanky Road, St. Louis, MO). Once animals achieved 8 wks of age, they were fed a semisynthetic lithogenic diet containing 1 g Ch, 0.5 g cholic acid, 15 g butter fat, 2 g corn oil, 50 g sucrose, 20 g casein, and essential vitamins and minerals (24). Surgery was performed at 9 am in groups of mice (n = 5 each) before (day 0) and during feeding of the lithogenic diet (7, 14, 28, and 56 days). All experiments were executed according to accepted criteria for the care and experimental use of laboratory animal and euthanasia was consistent with recommendations of the American Veterinary Medical Association. All protocols were approved by the Institutional Animal Care and Use Committee of Harvard University and The Jackson Laboratory.

### Cannulation of the common bile duct, collection of hepatic bile, and measurement of the circulating BS pool

Animals were fasted overnight but had free access to water. Mice were weighed and anesthetized with an i.p. injection of 35 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL). Laparotomy was performed through an upper midline incision under sterile conditions. After the liver and the gallbladder were exposed, the lower end of the common bile duct was ligated and the common bile duct was cannulated below the entrance of the cystic duct. The PE-10 polyethylene catheter had an I.D. of 0.28 mm and an O.D. of 0.61 mm (Becton Dickinson Primary Care Diagnostics, Becton Dickinson and Company, Sparks, MD) sufficiently large to prevent cholestasis and suitably short to minimize dead space. After successful catheterization, the cystic duct was doubly ligated and cholecystectomy was performed. Hepatic bile was collected by gravity every 15 min for the first h, and then every h for 8–12 h. After measurement of volume and microscopic studies of fresh biles, all samples were frozen and stored at −20°C for further lipid analyses (see below). For measurement of the circulating BS pool sizes, 8-h biliary "washout" studies were performed according to the methods of Eriksson (25) and Dowling, Mack, and Small (26, 27). During surgery and bile collection, mouse body temperature was maintained at 37 ± 0.5°C with a heating lamp and monitored with a thermometer. Animals were kept lightly anesthetized every 1.5 h with an i.p. injection of 17 mg/kg pentobarbital, and were given a small amount (~25 μL) of 0.9% NaCl every 2 h via the abdominal cavity to maintain hydration. Hepatic bile volumes were determined by weighing the bile samples (18, 19).

### Microscopic studies

Immediately after collection, 5 μL of fresh hepatic biles from the first 30-min drainage period was examined microscopically according to previous methods (19, 22, 28). Hepatic bile was dropped onto a glass slide at room temperature (22 ± 1°C). The sample was observed initially without a cover slip using a polarizing light microscope (Photomicroscope III; Carl Zeiss Inc., Thornwood, NY) at ×400 and ×800 magnifications. The bile sample was then compressed with a cover glass and re-examined using phase contrast optics. Separated phases, when present, were defined according to previous criteria (19, 22, 28).

### Lipid analyses

Biliary phospholipids were determined as inorganic phosphorus by the method of Bartlett (29). In a validatory study, six hepatic bile samples from C57L and AKR mice were analyzed by chemical determination of lipid phosphorus (29) and the enzymatic measurement of choline-containing phospholipids (30), respectively. Our pilot data showed that the values for lipid phosphorus determined chemically (29) were ~5% higher than those for lipid choline measured enzymatically (30). Furthermore, as there was good agreement between two methods with a correlation coefficient of 0.94 ($P < 0.001$), non-lipid phosphorus in mouse hepatic bile did not influence appreciably the results by Bartlett's method (29). Total BS and individual BS concentrations were measured by HPLC according to the methods of Rossi, Converse, and Hofmann (31). Biliary Ch was determined by HPLC (19, 32). The CSIs of hepatic bile were calculated from the critical tables (33) adjusted for the appropriate total lipid concentration. Because with 0.5% cholic acid feeding the BS pool became mainly taurocholate, the calculation of CSI is valid except on chow (day 0). Relative biliary lipid compositions were plotted on condensed phase diagrams according to the appropriate mean total lipid concentrations of hepatic bile according to mouse gender and strain. Phase boundaries and crystallization pathways were extrapolated from model bile systems developed for taurocholate (TC) at 37°C (3, 22). Hydrophobicity indexes (HI) of hepatic bile were calculated according to Heuman's method (34).

### Statistical methods

All data are expressed as means ± SD. Statistically significant differences among C57L, AKR, and $F_1$ mice were assessed by Student's *t*-test or by Mann-Whitney U tests. Analyses were performed with a *SuperANOVA* software (Abacus Concepts, Inc., Berkeley, CA). By linear regression analyses, parameters significantly

associated with bile flow rates and BS secretion rates were further assessed by a stepwise multiple regression analysis to identify the independence of the association. Statistical significance was defined as a two-tailed probability of less than 0.05.

## RESULTS

### Microscopic observations of hepatic biles

Fresh hepatic biles of mice before (0 time) and during feeding the lithogenic diet were invariably clear to the unaided eye and light yellow in color. Careful microscopic examination by polarizing light microscopy revealed no evidence of mucin gel, solid Ch crystals, or gallstones. Occasionally, sparse quantities of small non-birefringent liquid crystals were detected at high magnification (×800).

### Bile flow rates

As a consequence of the operative procedure which ensured complete interruption of the enterohepatic circulation of BS, there were significant ($P < 0.05$) time-dependent reductions in bile flow from 90 ± 9 μL/min/kg (first h) to 60 ± 4 μL/min/kg (eighth h) in male C57L mice, from 87 ± 12 μL/min/kg to 58 ± 5 μL/min/kg in male $F_1$ mice, and from 41 ± 3 μL/min/kg to 18 ± 4 μL/min/kg in male AKR mice. The ranges of bile flow in male C57L mice (88–127 μL/min/kg during the first 30 min) were similar to those in male $F_1$ mice (78–123 μL/min/kg) but significantly higher ($P < 0.01$) than in male AKR mice (40–59 μL/min/kg). Among C57L, $F_1$, and AKR mice, we did not observe any gender differences in bile flow nor any significant influence of the lithogenic diet.

**Figure 1** summarizes the relationships of bile flow to BS secretion rates from the "washout" data. The linear slopes of the relationships define BS-dependent bile flow, which are not significantly different between strains or gender. They are 28 ± 5 μL (bile $H_2O$)/μmol BS (males) and 26 ± 6 μL/μmol (females) for C57L mice; 29 ± 6 μL/μmol (males) and 27 ± 7 μL/μmol (females) for $F_1$ mice; and 31 ± 7 μL/μmol (males) and 33 ± 6 μL/μmol (females) for AKR mice, respectively. The extrapolated intercepts with the ordinates estimate the BS-independent apparent choleretic activity which has canalicular and/or ductular components (35). The extrapolated *y*-intercepts display similar values between C57L males and females (328 ± 97 μL/h/100 g and 311 ± 78 μL/h/100 g) and between $F_1$ males and females (301 ± 89 μL/h/100 g and 298 ± 86 μL/h/100 g), and both are significantly greater ($P < 0.01$) than the values for AKR mice (143 ± 57 μL/h/100 g for males and 125 ± 63 μL/h/100 g for females).

### Biliary lipid secretion rates and lipid compositions of hepatic bile

**Figure 2** displays biliary Ch, Lec, and BS outputs during the first h of biliary washout as functions of days on the

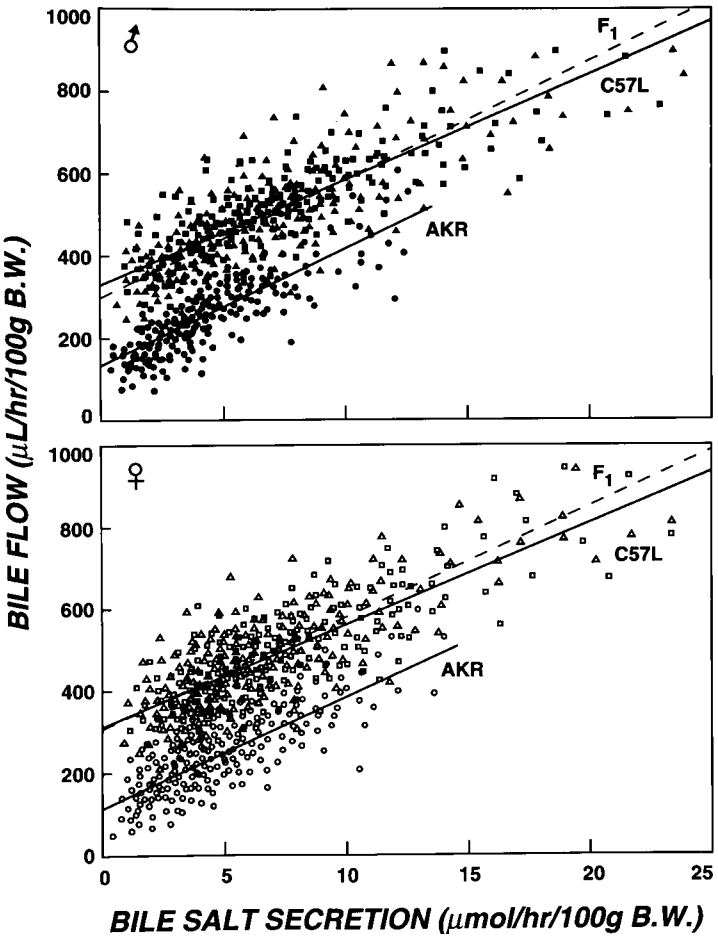


**Fig. 1.** Bile flow as functions of BS output in inbred mice before and during feeding the lithogenic diet (56 days). Each point represents bile flow and BS output measurements in the same sample during 8-h periods of biliary "washout". No significant differences exist in the slopes of any of the regression lines for C57L, $F_1$, and AKR mice of each gender. This indicates that the BS-dependent apparent choleretic activity is similar in the three mouse strains. Upon extrapolation of the regression lines to the ordinate, the *y*-intercepts are similar between C57L and $F_1$ mice and significantly larger ($P < 0.01$) than in AKR mice, suggesting that BS-independent flow is significantly greater ($P < 0.01$) in C57L and $F_1$ mice than in AKR mice. Equations for canalicular BS-dependent flow are $y = 328(\pm 97) + 28(\pm 5)x$ ($r = 0.91$; $P < 0.001$) and $y = 311(\pm 78) + 26(\pm 6)x$ ($r = 0.86$; $P < 0.001$) for male and female C57L mice; $y = 301(\pm 89) + 29(\pm 6)x$ ($r = 0.88$; $P < 0.001$) and $y = 298\ (\pm 86) + 27(\pm 7)x$ ($r = 0.90$; $P < 0.001$) for male and female $F_1$ mice; and $y = 143(\pm 57) + 31(\pm 7)x$ ($r = 0.89$; $P < 0.001$) and $y = 125(\pm 63) + 33\ (\pm 6)x$ ($r = 0.91$; $P < 0.001$) for male and female AKR mice, respectively. Symbol ■ represents male C57L; □ female C57L; ▲ male $F_1$; △ female $F_1$; ● male AKR; and ○ female AKR mice. See text for further description.

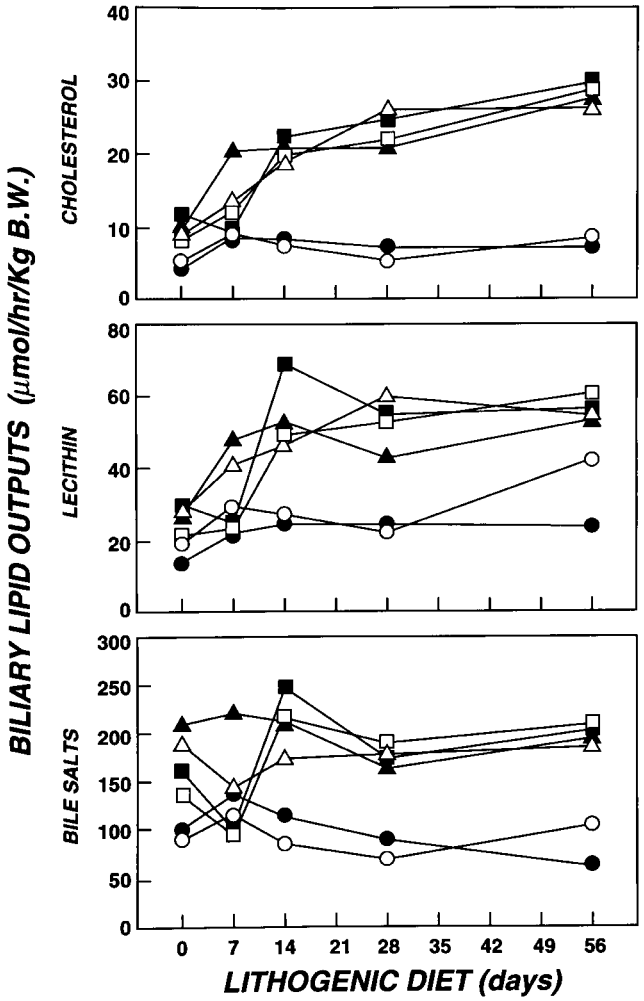


**Fig. 2.** Total outputs (μmol/h/kg B.W.) of Ch, Lec, and BS during the first h of biliary secretion before (day 0) and during feeding the lithogenic diet. In all data sets, $F_1$ mice display biliary lipid outputs similar to C57L mice and both are significantly higher ($P < 0.01$) than in AKR mice. Compared with secretion rates on chow (day 0), total Ch and Lec outputs in C57L and $F_1$ mice increase significantly ($P < 0.01$) during the lithogenic diet feeding period. However, the lithogenic diet did not influence BS secretion rates significantly in any strain of mice. Symbol ■ represents male C57L; □ female C57L; ▲ male $F_1$; △ female $F_1$; ● male AKR; and ○ female AKR mice. See text for further description.

lithogenic diet. Biliary lipid outputs are identical between C57L and $F_1$ mice and both are significantly higher ($P < 0.01$) than AKR mice with no gender effect. Ch outputs (Fig. 2, top panel) at day 0 in C57L (10.2 ± 4.0 μmol/h/kg) and $F_1$ mice (9.7 ± 2.6 μmol/h/kg) are similar and significantly ($P < 0.01$) higher than in AKR mice (5.0 ± 1.3 μmol/h/kg). Similarly, Lec and BS outputs (Fig. 2, middle and bottom panels, respectively) at day 0 are significantly higher ($P < 0.05$) in C57L mice (Lec = 25.6 ± 9.3 μmol/h/kg; BS = 146.7 ± 52.6 μmol/h/kg), and $F_1$ mice (Lec = 29.1 ± 4.0 μmol/h/kg; BS = 200.2 ± 78.2 μmol/h/kg) than in AKR mice (Lec = 16.9 ± 5.7 μmol/h/kg; BS = 97.1 ± 21.7 μmol/h/kg). Both Ch and Lec outputs increase significantly ($P < 0.01$) on the lithogenic diet in C57L (Ch = 29.7 ± 5.2 μmol/h/kg; Lec = 57.2 ± 15.7 μmol/h/kg) and $F_1$ mice (Ch = 27.7 ± 6.8 μmol/h/kg; Lec = 54.8 ± 12.0 μmol/h/kg). However, AKR mice on the lithogenic diet display only slight increases in biliary Ch (8.3 ± 2.4 μmol/h/kg) and Lec (33.0 ± 14.1 μmol/h/kg) outputs compared with chow. Outputs of BS (Fig. 2, bottom panel) vary slightly in all strains and genders during lithogenic diet feeding, but were not significantly different from those at day 0.

**Figure 3** shows the dependencies of Ch and Lec outputs on BS outputs utilizing data from the first h of biliary lipid secretion, i.e., immediately after interruption of the enterohepatic circulation. Curvilinear relationships are observed in all cases when Ch (Fig. 3, top panel) and Lec (Fig. 3, bottom panel) secretion rates are plotted against BS secretion rates. It is clear that the curvilinear relationships for $F_1$ mice are similar to those for C57L mice; however, Ch and Lec secretion rates are 2- to 3-fold lower in AKR mice with no gender differences. The shapes of these relationships are in agreement with what has been reported for rats (36), mice (37), humans (38), and a variety of other mammals (39).

**Table 1** lists biliary lipid compositions of hepatic biles from C57L, $F_1$, and AKR mice during the first h of interrupted enterohepatic circulation while on chow (day 0) and at four time intervals on the lithogenic diet. Plotted in **Fig. 4** (left panel) are the relative lipid compositions of "individual" hepatic biles and on the right panel mean ± SD relative lipid compositions (n = 5 per mouse group) on condensed phase diagrams with appropriate micellar phase boundaries (total lipid concentration = 2.5 g/dL, see Table 1) (3, 22) and Ch crystallization pathways for taurocholate-rich biles (19, 22, 28). In all cases (Table 1 and Fig. 4) progressive increases in the relative Ch as well as Lec concentrations, and decreases in relative BS concentrations of hepatic biles, occurred. When plotted on phase diagrams there are progressive shifts upward and to the right in relative compositions most notably for C57L and $F_1$ mice (Fig. 4). All relative lipid compositions (Fig. 4) plot above the appropriate micellar zone and because these are dilute hepatic biles, they plot in crystallization pathway denoted region E (22). If such dilute biles were allowed to reach equilibrium, they would be composed of liquid crystals and saturated micelles but not solid crystals as shown for model systems in region E (Fig. 4) (22). Occasionally we detected sparse numbers of small liquid crystals in fresh samples under microscopic examination (×800 magnification), and when observed over several days, these did not form solid Ch monohydrate crystals (22).

Mean CSIs of hepatic biles were >1 on chow (day 0) and increased during feeding the lithogenic diet, and were indifferent to gender (Table 1). At 56 days, CSIs of C57L (2.0 ± 0.3) and $F_1$ biles (2.0 ± 0.4) increased significantly ($P < 0.01$), whereas AKR biles showed only slight increases (1.2 ± 0.3). Furthermore, in C57L and $F_1$ mice, the Ch/Lec ratios during the first h of biliary secretion increased significantly ($P < 0.05$) from 0.41 ± 0.12 (day 0) to 0.54 ± 0.11 (56 days), and from 0.34 ± 0.06 (day 0) to 0.51 ± 0.12 (56 days), respectively. However, in biles of

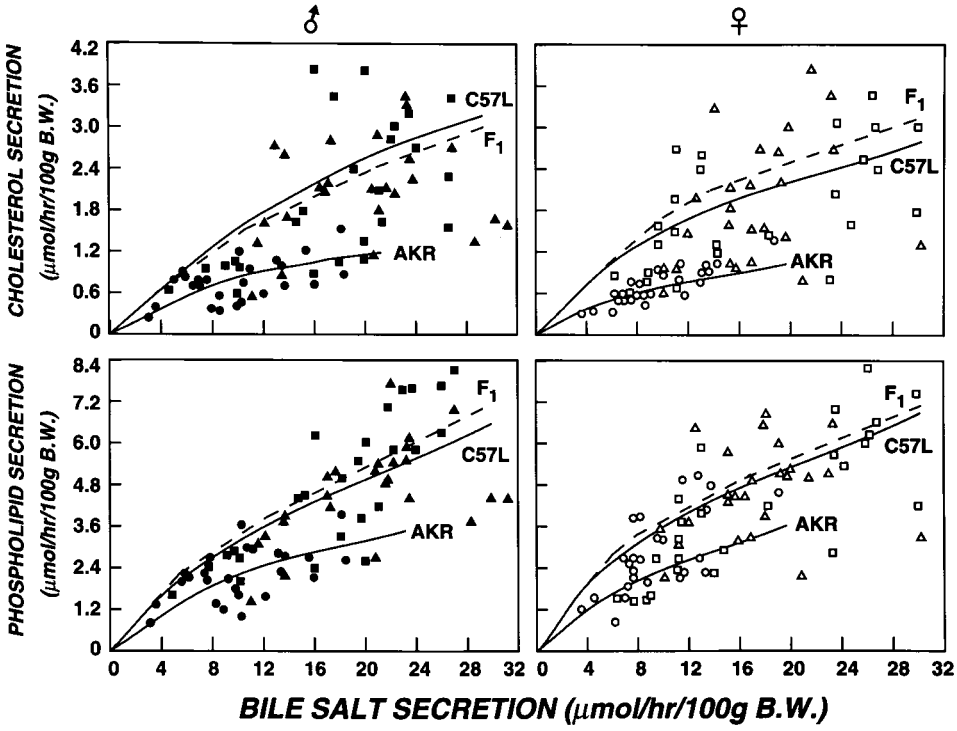


**Fig. 3.** Dependencies of biliary Ch (top panels) and Lec outputs (bottom panels) on biliary BS outputs. Individual data for Ch and Lec output rates in all mice over the entire experimental period are plotted against BS output rates for the same time intervals. The curvilinear relationships show that Lec secretion rate is ~2-fold higher than Ch secretion rate. $F_1$ mice display Ch and Lec secretion rates similar to C57L mice, and outputs are ~2- to 3-fold higher than in AKR mice. No gender differences are observed. Symbol ■ represents male C57L; □ female C57L; ▲ male $F_1$; △ female $F_1$; ● male AKR; and ○ female AKR mice.

AKR mice, the Ch/Lec ratios decreased slightly from 0.31 ± 0.08 (day 0) to 0.27 ± 0.07 (56 days). Moreover, the Ch/BS ratios that reflect the linkage of Ch to BS secretion increased significantly in C57L and $F_1$ mice (0.06 at day 0 to 0.15 at day 56), but increased non-significantly in AKR mice (0.05 at day 0 to 0.10 at day 56). There were no significant differences in Lec/BS ratios among C57L, $F_1$, and AKR mice from beginning to end of the feeding period (0.17–0.18 at day 0 vs. 0.29–0.38 at day 56). Furthermore, no gender differences were noted in the Ch/Lec, Ch/BS, and Lec/BS coupling ratios.

### Circulating BS pool sizes

After complete interruption of the enterohepatic circulation (see Methods), external biliary drainage in each mouse gave a "washout curve" as evidenced by measurement of BS secretion rates. **Figure 5** (top panel) shows an average washout curve for male C57L mice (n = 5) ingesting chow on day 0. After interruption of the enterohepatic circulation, BS secretion rates are unaltered during the first h, hence our 1-h analysis (Figs. 1 and 2) provides BS secretion during simulation of an intact enterohepatic circulation (25–27, 40). Over the next 2 h (Fig. 5, top panel), BS secretion rate falls markedly to a low point which persists for 4 h. Thereafter, with continued biliary drainage, BS secretion rate increases to levels 1.5-fold greater than the low point. The mass of BS secreted from the time of interrupting the enterohepatic circulation until the low point is reached represents the circulating BS pool which was "washed out" (40). Figure 5 (top panel) also shows that the low point in the washout curve represents the basal rate of BS synthesis, and the subsequent increase in the BS secretion represents a compensatory increase in de novo hepatic BS synthesis in response to loss of feedback inhibition by BS (40).

Mean BS compositions during 12-h biliary washout in 10 male C57L mice are shown in Fig. 5 (bottom panel). Because of the interrupted enterohepatic circulation, secondary, i.e., bacterially modified BS, which normally returns to the liver via portal blood after intestinal absorption, are not made and therefore do not return to the liver for secretion into bile (40). As shown in Fig. 5 (bottom panel), all BS levels reach a low point at 3–5 h indicating that both primary and secondary BS in the circulating BS pool had been "washed-out." Then, over the subsequent 8–12 h, BS concentrations increase slightly, showing that there is hepatic compensation from de novo BS synthesis. As only the concentrations of TC, T-β-MC, and TCDC become elevated, these newly synthesized BS are the primary BS in hepatic bile. Over the same washout time period (e.g., 8–12 h), TDC, T-ω-MC, and TUDC became undetectable in fistula biles. Our results verified that TC, T-β-MC, and TCDC are primary BS, and TDC, T-ω-MC, and TUDC are secondary BS in mice (41).

**Figure 6** (top panels) plots the circulating BS pool sizes

TABLE 1. Biliary lipid compositions of hepatic biles

| | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | Mole%Ch | Mole%Lec | Mole%BS | Lec/(Lec+BS) | [TL](g/dl) | CSI[a] | Mole%Ch | Mole%Lec | Mole%BS | Lec/(Lec+BS) | [TL](g/dl) | CSI |
| C57L | | | | | | | | | | | | |
| 0 | 6.36 ± 1.95 | 14.71 ± 3.64 | 78.93 ± 5.31 | 0.16 ± 0.04 | 2.11 ± 0.37 | 1.61 ± 0.37 | 6.04 ± 2.15 | 13.59 ± 2.11 | 80.37 ± 3.24 | 0.14 ± 0.02 | 1.73 ± 0.56 | 1.81 ± 0.74 |
| 7 | 7.70 ± 2.28 | 20.16 ± 3.47 | 72.14 ± 5.67 | 0.22 ± 0.04 | 1.17 ± 0.43 | 1.77 ± 0.39 | 9.10 ± 2.15 | 17.35 ± 1.52 | 73.55 ± 3.54 | 0.19 ± 0.02 | 1.32 ± 0.14 | 2.30 ± 0.37 |
| 14 | 6.75 ± 1.98 | 20.60 ± 1.95 | 72.65 ± 2.80 | 0.22 ± 0.02 | 1.86 ± 0.29 | 1.38 ± 0.36 | 7.30 ± 1.72 | 18.10 ± 1.53 | 74.59 ± 2.98 | 0.20 ± 0.02 | 2.09 ± 0.36 | 1.58 ± 0.31 |
| 28 | 9.72 ± 2.73 | 22.34 ± 0.76 | 67.94 ± 3.40 | 0.25 ± 0.02 | 1.80 ± 0.55 | 1.83 ± 0.40 | 8.65 ± 1.98 | 21.25 ± 2.24 | 70.10 ± 3.43 | 0.23 ± 0.03 | 1.74 ± 0.50 | 1.74 ± 0.44 |
| 56 | 10.06 ± 2.57[b] | 18.18 ± 1.66 | 71.76 ± 4.04 | 0.20 ± 0.02 | 2.69 ± 0.74 | 1.99 ± 0.33[b] | 10.80 ± 3.13[b] | 20.49 ± 2.50[b] | 68.71 ± 4.90 | 0.23 ± 0.03 | 2.10 ± 0.45 | 2.06 ± 0.50 |
| $F_1$ | | | | | | | | | | | | |
| 0 | 4.16 ± 0.98 | 11.93 ± 0.95 | 83.91 ± 1.57 | 0.13 ± 0.01 | 2.62 ± 0.61 | 1.19 ± 0.31 | 4.37 ± 0.77 | 13.36 ± 3.54 | 80.28 ± 4.27 | 0.14 ± 0.04 | 1.77 ± 0.92 | 1.33 ± 0.19 |
| 7 | 7.06 ± 1.41 | 16.84 ± 3.37 | 76.10 ± 4.46 | 0.18 ± 0.04 | 2.50 ± 0.54 | 1.56 ± 0.27 | 6.55 ± 1.34 | 21.01 ± 3.18 | 72.44 ± 3.35 | 0.22 ± 0.03 | 1.99 ± 0.42 | 1.32 ± 0.30 |
| 14 | 7.42 ± 0.97 | 18.74 ± 1.35 | 73.84 ± 2.01 | 0.20 ± 0.02 | 2.17 ± 0.25 | 1.56 ± 0.21 | 7.79 ± 2.00 | 19.56 ± 2.01 | 72.65 ± 2.04 | 0.21 ± 0.02 | 2.21 ± 0.26 | 1.58 ± 0.42 |
| 28 | 9.23 ± 2.09 | 18.83 ± 1.46 | 71.95 ± 2.60 | 0.21 ± 0.01 | 2.30 ± 0.39 | 1.86 ± 0.34 | 9.66 ± 1.43 | 23.25 ± 4.72 | 67.09 ± 5.17 | 0.26 ± 0.05 | 1.96 ± 0.34 | 1.80 ± 0.26 |
| 56 | 10.06 ± 0.78[b] | 20.02 ± 2.86[b] | 69.92 ± 2.36 | 0.22 ± 0.03 | 2.28 ± 0.19 | 1.99 ± 0.34[c] | 10.24 ± 1.38[b] | 20.68 ± 3.62[b] | 69.08 ± 2.86 | 0.23 ± 0.04 | 2.23 ± 0.26 | 2.01 ± 0.54[b] |
| AKR | | | | | | | | | | | | |
| 0 | 3.77 ± 0.34 | 11.87 ± 1.97 | 84.36 ± 1.81 | 0.12 ± 0.02 | 2.55 ± 0.74 | 1.10 ± 0.20 | 4.63 ± 0.51 | 16.60 ± 4.57 | 78.77 ± 4.67 | 0.17 ± 0.05 | 1.86 ± 0.40 | 1.19 ± 0.28[d] |
| 7 | 5.22 ± 1.01 | 14.87 ± 4.07 | 79.92 ± 4.74 | 0.16 ± 0.04 | 2.61 ± 0.80 | 1.27 ± 0.15 | 5.14 ± 0.77 | 19.68 ± 6.12 | 75.17 ± 6.31 | 0.21 ± 0.06 | 2.12 ± 0.53 | 1.12 ± 0.26[d] |
| 14 | 5.67 ± 1.12 | 17.46 ± 3.86 | 76.87 ± 4.72 | 0.19 ± 0.04 | 3.63 ± 0.74 | 1.13 ± 0.17 | 5.99 ± 0.75 | 22.78 ± 2.14 | 71.23 ± 2.57 | 0.24 ± 0.02 | 2.58 ± 0.59 | 1.06 ± 0.10 |
| 28 | 6.45 ± 1.23 | 20.56 ± 2.04 | 72.99 ± 3.14 | 0.22 ± 0.02 | 2.69 ± 0.41 | 1.21 ± 0.15[d] | 5.93 ± 5.76[d] | 23.90 ± 0.91 | 70.35 ± 0.90 | 0.25 ± 0.01 | 1.76 ± 0.52 | 1.09 ± 0.10[d] |
| 56 | 8.10 ± 1.19 | 24.15 ± 1.45 | 67.75 ± 2.44 | 0.26 ± 0.02 | 2.34 ± 0.64 | 1.39 ± 0.22[d] | 5.77 ± 0.62[d] | 27.25 ± 2.59 | 66.98 ± 3.04 | 0.29 ± 0.03 | 1.75 ± 0.14 | 1.00 ± 0.09[e] |

Values were determined from five hepatic biles (the first hour of biliary secretion) per group at each time point. Abbreviations: Ch, cholesterol; Lec, lecithin; BS, bile salt; [TL], total lipid concentration; CSI, cholesterol saturation index.

[a] The CSI values of five hepatic biles were calculated from the critical tables (33).

[b] $P < 0.05$, compared to day 0.

[c] $P < 0.01$, compared to day 0.

[d] $P < 0.05$, compared to C57L and $F_1$ strains.

[e] $P < 0.01$, compared to C57L and $F_1$ strains.

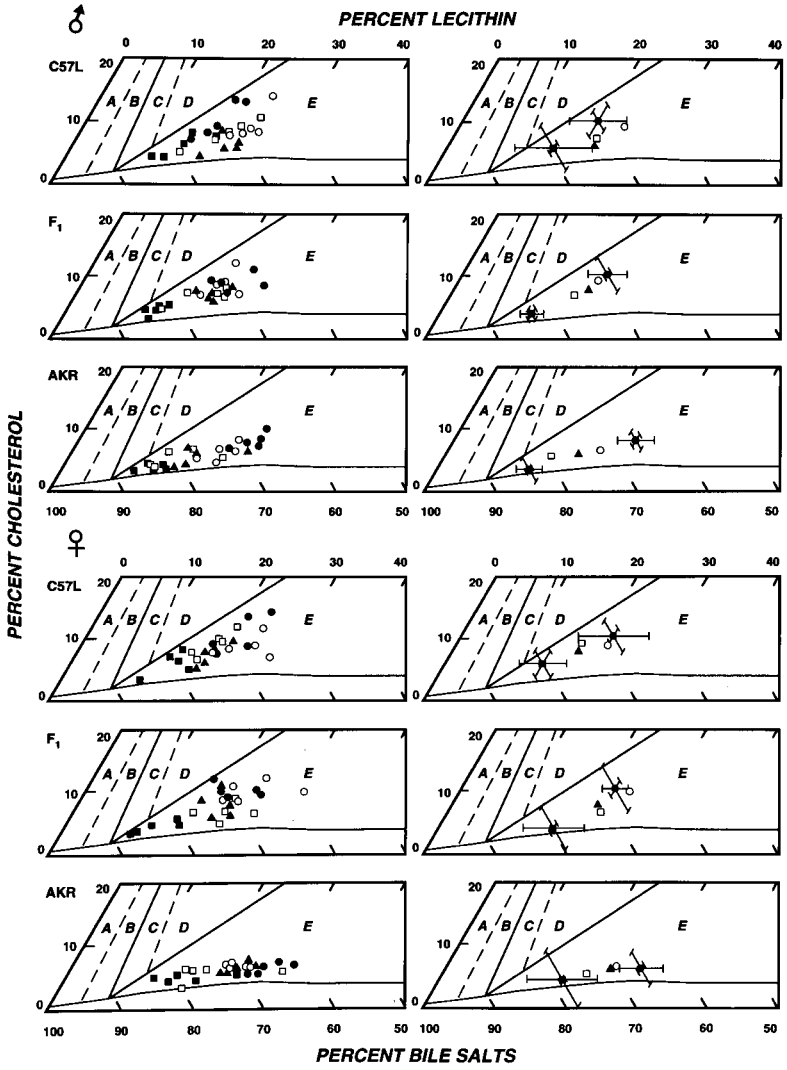


**Fig. 4.** Lipid compositions (moles per 100 moles) of individual (5 mice at each time point) hepatic biles (left panels) and mean (right panels, n = 5 for each group) relative lipid compositions (moles per 100 moles) at five time points plotted on condensed phase diagrams according to the average total lipid concentration (2.5 g/dL) and predominantly TC (3, 22) in the biles. The micellar zone is enclosed by a solid curved line above which two solid and two dashed lines divide the phase diagrams into regions A to E with different crystallization sequences (see ref. 22). The relative lipid compositions of all hepatic biles are located in region E where at theoretical equilibrium they would be composed of liquid crystals and Ch-saturated micelles but not solid Ch crystals. The relative biliary lipid compositions shift progressively upwards and to the right, most notably in C57L and $F_1$ mice with length of time on the lithogenic diet. Symbol ■ represents day 0; □ 7 days; ▲ 14 days; ○ 28 days; and ● 56 days on the lithogenic diet. See text for further description.

for each mouse strain (n = 5), and we found neither a gender nor time difference. Although BS pool sizes are identical between C57L (3.1 ± 0.4 μmol) and $F_1$ mice (2.7 ± 0.4 μmol), they are significantly larger ($P < 0.05$) in gallstone-susceptible strains than in AKR mice (1.7 ± 0.3 μmol). Possibly because the lithogenic diet contained 0.5% cholic acid (see Methods), BS pool sizes increase slightly but not significantly in all mouse groups during study. At 56 days, the circulating BS pool sizes are 3.6 ± 0.5 μmol in C57L and 3.3 ± 0.6 μmol in $F_1$ mice, and 2.1 ± 0.4 μmol in AKR mice. Using the gallbladder BS pool data from our previous phenotypic paper on gallbladder bile (19), we calculated total BS pool size (i.e., the circulating BS pool size plus the BS pool in the gallbladder) which is also displayed in Fig. 6 (bottom panels). These values are 4.7 μmol in C57L, 4.8 μmol in $F_1$, and 2.7 μmol in AKR mice on chow, respectively. It is notable that $F_1$ mice display total BS pool sizes similar to C57L mice and both have significantly ($P < 0.05$) larger total BS pool sizes than AKR mice. We noted no gender or lithogenic diet influences on total BS pool sizes among the three strains of mice (Fig. 6).

### Molecular species of BS in hepatic bile

**Table 2** lists the distribution of BS species in hepatic biles of mice during the first h of biliary secretion before (day 0)

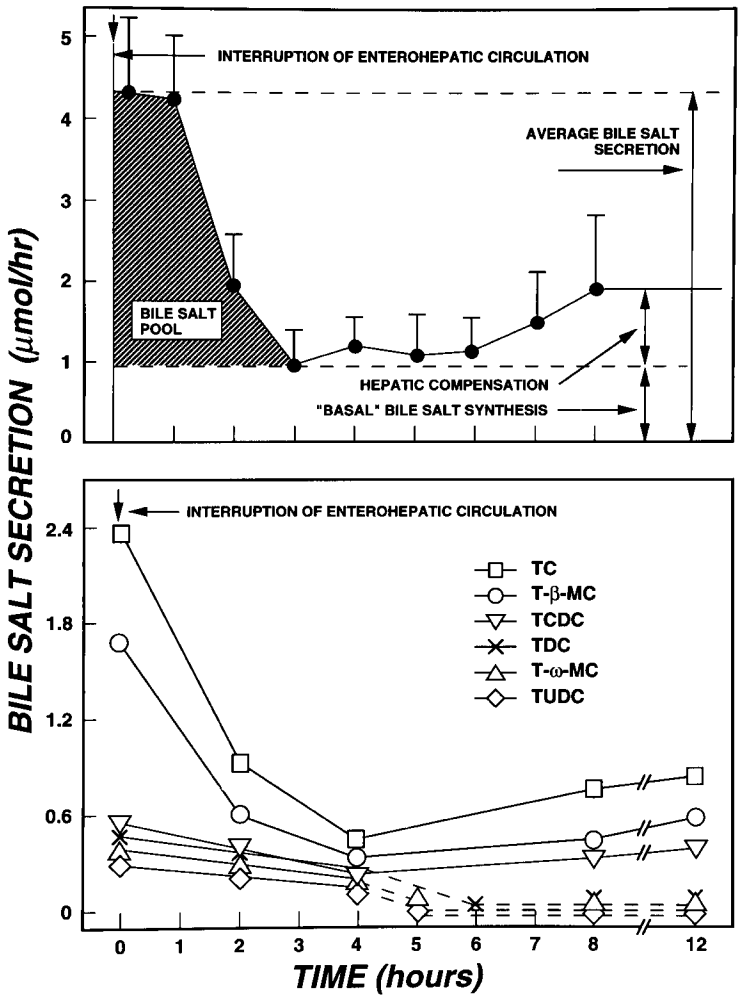


**Fig. 5.** Top panel shows washout curves for male C57L mice (n = 5) on chow (day 0). After cannulation of the common bile duct (100% interruption of the enterohepatic circulation), BS secretion rate falls over the subsequent 2 h and reaches a low point at 3 h. The shaded area shows the direct measurement of BS pool sizes. The low points (dashed line) at 3 h represents "basal" BS synthesis in the liver. When the BS secretion increases again, this indicates that newly synthesized BS are appearing. Subsequently, BS secretion rate reaches a new steady state due to hepatic compensation. Bottom panel exhibits distinctions between the "primary" and "secondary" components of the six major murine BS in male C57L mice (n = 10) by monitoring changes in their concentrations during a 12-h "washout" period. The hepatic bile contains TC, T-β-MC, TCDC, TDC, T-ω-MC, and TUDC. After 4-h biliary washout, the concentration of all BS decreases. With passage of time, the secretion of the newly synthesized BS shows that only TC, T-β-MC, and TCDC increases and these are primary, i.e., hepatic synthesized BS. As TDC, T-ω-MC, and TUDC are not detected after 4 h, they are secondary BS in the mouse. See text for further description.

and at various times during feeding of the lithogenic diet. HPLC analysis of individual BS revealed that all are taurine-conjugated and display similar distributions in hepatic biles. Before feeding the lithogenic diet, the predominant BS are taurocholate (TC) (range 44.3-57.9%), and tauro-β-muricholate (T-β-MC) (range 34.2–45.3%). Present in smaller concentrations are tauro-ω-muricholate (T-ω-MC) (1.0–3.0%), tauroursodeoxycholate (TUDC) (1.3–4.7%), taurochenodeoxycholate (TCDC) (0.4–1.2%), and taurodeoxycholate (TDC) (2.1–6.6%). Upon feeding the lithogenic diet, TC increases markedly to about 73% at day 7 in C57L and $F_1$ mice, and then decreases a few percent remaining at a high (60%) level thereafter. T-β-MC decreases markedly to 10% at day 7, and remains at this low level for 56 days. In the gallstone-susceptible strains, both TCDC and TDC increase sharply at 7 days reaching a combined ~18% for the remainder of the feeding period; however, both T-ω-MC and TUDC remain unchanged. There are gender differences in the ratios of T-β-MC to TCDC in gallstone-susceptible C57L and $F_1$ mice in that after 7 days conversion of chenodeoxycholate (the natural precursor) to T-β-MC is less efficient in males compared with females. When fed the lithogenic diet for 7 days, AKR mice also show a TC increase to 62% and a decrease in T-β-MC to 23% with both remaining constant for the duration of the experiment. However, in AKR mice, the lithogenic diet produces almost no change in the pattern of minor BS. Furthermore, we find that among the three strains of mice on chow, biliary hydrophilicity indexes (HI) are similar (−0.26 to −0.37), but at 56 days of lithogenic diet feeding, biliary HI increase significantly ($P < 0.05$) in C57L (+0.11 ± 0.01) and $F_1$ mice (+0.13 ± 0.00) with males showing markedly higher values than females. In contrast, biliary HI values remain unchanged in AKR mice (−0.20 ± 0.02) over the 56-day feeding period.

## DISCUSSION

### *Lith* genes and hepatic bile phenotypes

The key hepatic defect in human Ch cholelithiasis is hypersecretion of Ch at the hepatocyte canalicular level leading to the formation of lithogenic bile (42). Because BS and Lec are necessary for Ch solubilization in bile (3, 4), lithogenic bile can be formed by relative excess Ch in relation to normal, high, or low amounts of these solubilizing agents. We established (18) that Ch gallstone formation in mice is genetically controlled so it became impor-

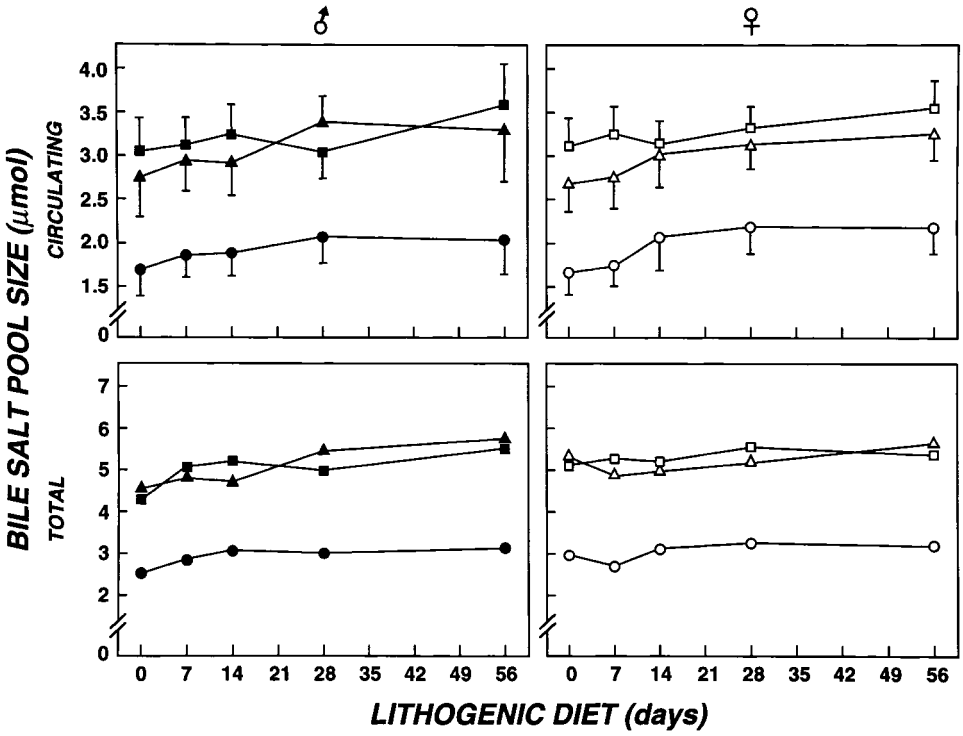


**Fig. 6.** The circulating BS pool sizes by washout (top panels) and total (i.e., the circulating BS pool sizes plus the gallbladder BS pool) (bottom panels) as functions of mouse strain, gender, and days on the lithogenic diet. The circulating and total BS pool sizes are similar between C57L and $F_1$ mice, and significantly larger ($P < 0.05$) than in AKR mice, as well as increasing slightly (N.S.) with feeding of the lithogenic diet. There are no gender differences among the three mouse strains. Symbol ■ represents male C57L; □ female C57L; ▲ male $F_1$; △ female $F_1$; ● male AKR; and ○ female AKR mice. See text for further description.

tant to explore how *Lith* genes influence hepatic secretion of biliary lipids. The most significant findings in the present study are three. *i*) Lithogenic hepatic biles precede the development of Ch gallstones in gallstone-susceptible mice, which supports the concept that changes in the hepatic secretion of biliary lipids are the primary precursors of Ch supersaturated bile in the gallbladder and Ch gallstone formation. Our results are in agreement with other investigators' observations on hepatic biles from Ch gallstone patients (4–7, 38, 39), as well as mice (43–46), prairie dogs (47–49), and hamsters (50, 51) fed a Ch gallstone-inducing diet. *ii*) The lithogenic diet (15% dairy fat, 1% Ch, and 0.5% cholic acid) markedly increased biliary Ch secretion which was accompanied by an increase in Lec secretion and unchanged BS secretion in all strains of mice (Fig. 2). However, compared to gallstone-resistant mice, susceptible mice secreted approximately double the amount of BS even in the basal state and a larger amount of Ch in relation to Lec, which increased on the lithogenic diet resulting in significant increases in Ch/Lec coupling ratios in bile. Therefore, our results suggest that *Lith* genes induce both relative and absolute biliary Ch hypersecretion upon feeding the lithogenic diet. *iii*) As $F_1$ mice are heterozygous for *Lith* alleles and the phenotypes of hepatic bile mimicked the C57L parent (Figs. 1–4 and 6), this confirms again that susceptibility to Ch gallstones in mice is a dominant trait (18, 19).

**Pathophysiological characterization of hepatic biles**

Biliary Ch and Lec secretion rates (40) are considered to be closely linked to BS secretion rates and to be enhanced by BS. Linkage coefficients (10) have been proposed to relate biliary lipid secretion rates meaningfully to physical–chemical events at the canalicular level. The Ch/Lec ratios of bile therefore, in principle, should indicate the Ch/Lec ratios of the vesicles whose secretion is induced by BS at the canaliculus (52). In the present study, the most notable aspect of biliary lipid secretion is that compared with gallstone-resistant AKR mice, susceptible C57L and $F_1$ mice display much higher Ch/Lec ratios. Furthermore, during lithogenic diet feeding, there were significant increases in Ch/Lec ratios from 0.38 (day 0) to 0.53 (day 56) in C57L and $F_1$ mice, which paralleled the elevated CSIs in bile. In contrast, AKR mice did not display any increase in Ch/Lec ratio (0.31 at day 0 vs. 0.27 at day 56). Clearly, susceptible mice secrete more Ch molecules into bile at canalicular membrane levels, which is the major factor leading to the formation of lithogenic bile. Our results are in agreement with the National Cooperative Gallstone Study where Hofmann et al. (53) observed that there was a high Ch/Lec ratio of 0.45 in bile of Ch gallstone patients compared with 0.34 in control subjects. This suggests that in Ch gallstone patients as well as mice, nascent phospholipid vesicles at the canalicular level (52) may be enriched approximately ~30% with Ch compared to controls.

TABLE 2. Percent bile salt species in hepatic biles

| | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day | TC | T-β-MC | TCDC | T-ω-MC | TUDC | TDC | HI[a] | TC | T-β-MC | TCDC | T-ω-MC | TUDC | TDC | HI |
| C57L | | | | | | | | | | | | | | |
| 0 | 49.7 ± 6.7 | 42.2 ± 5.7 | 0.6 ± 0.3 | 1.4 ± 0.6 | 2.6 ± 1.6 | 3.4 ± 1.3 | −0.33 ± 0.05 | 44.3 ± 2.8 | 45.3 ± 4.1 | 0.7 ± 0.2 | 3.0 ± 1.7 | 1.9 ± 0.7 | 4.8 ± 1.4 | −0.36 ± 0.04 |
| 7 | 74.3 ± 7.3[b] | 2.9 ± 0.2[b] | 11.1 ± 3.4[b] | 1.7 ± 0.4 | 3.2 ± 1.0 | 6.9 ± 3.3 | +0.04 ± 0.03[c] | 76.0 ± 6.5[b] | 16.1 ± 5.7[b] | 0.6 ± 0.1 | 1.1 ± 0.6 | 1.4 ± 0.7 | 4.8 ± 9.1[c] | −0.11 ± 0.00 |
| 14 | 58.7 ± 3.1[c] | 3.9 ± 0.4[b] | 14.0 ± 1.4[b] | 2.9 ± 1.6 | 2.9 ± 0.6 | 17.6 ± 3.4[b] | +0.10 ± 0.01[b] | 65.8 ± 8.2[b] | 14.7 ± 5.4[b] | 2.2 ± 3.7 | 0.9 ± 0.2 | 2.0 ± 1.0 | 14.4 ± 9.0 | −0.04 ± 0.02 |
| 28 | 69.6 ± 4.0[c] | 2.6 ± 1.9[b] | 13.4 ± 0.9[b] | 2.4 ± 0.7 | 2.1 ± 1.5 | 10.0 ± 1.3[c] | +0.07 ± 0.02[c] | 69.5 ± 5.5[b] | 10.8 ± 2.0[b] | 3.8 ± 0.6 | 0.9 ± 0.6 | 1.3 ± 0.8 | 13.7 ± 4.3 | −0.00 ± 0.00 |
| 56 | 57.2 ± 3.1[c] | 6.1 ± 1.4[b] | 22.6 ± 1.2[b] | 1.0 ± 1.0 | 1.8 ± 0.3 | 11.3 ± 0.7[c] | +0.11 ± 0.01[b] | 64.2 ± 5.5[b] | 7.9 ± 2.4[b] | 11.5 ± 3.9[b] | 0.8 ± 0.2 | 1.5 ± 0.7 | 14.2 ± 4.8[c] | +0.06 ± 0.02[c] |
| $F_1$ | | | | | | | | | | | | | | |
| 0 | 50.5 ± 1.7 | 36.1 ± 2.6 | 0.6 ± 0.2 | 1.6 ± 0.5 | 4.7 ± 0.4 | 6.6 ± 1.2 | −0.27 ± 0.02 | 57.9 ± 4.7 | 34.2 ± 2.3 | 1.2 ± 0.4 | 2.0 ± 2.1 | 1.3 ± 0.3 | 3.4 ± 1.7 | −0.26 ± 0.02 |
| 7 | 72.1 ± 4.9[b] | 10.6 ± 4.0 | 6.4 ± 2.4 | 1.8 ± 1.6 | 4.0 ± 0.4 | 5.1 ± 3.3 | −0.06 ± 0.02 | 76.1 ± 5.5[b] | 12.3 ± 1.6[c] | 1.4 ± 0.8 | 1.5 ± 1.0 | 1.6 ± 0.4 | 7.2 ± 4.2 | −0.07 ± 0.01 |
| 14 | 73.1 ± 6.2[b] | 6.5 ± 2.0[b] | 11.0 ± 4.6[b] | 0.8 ± 1.3 | 2.5 ± 0.6 | 6.1 ± 3.1 | +0.02 ± 0.01[c] | 70.2 ± 1.7[b] | 10.5 ± 1.8[c] | 6.5 ± 0.5[c] | 0.9 ± 0.3 | 2.2 ± 0.9 | 9.6 ± 2.2 | −0.01 ± 0.01 |
| 28 | 69.9 ± 1.3[b] | 5.5 ± 2.0[b] | 12.1 ± 1.2[b] | 1.3 ± 0.8 | 2.3 ± 0.4 | 8.9 ± 2.2 | +0.04 ± 0.01[c] | 72.4 ± 3.5[b] | 9.2 ± 6.1[c] | 5.5 ± 1.9 | 1.1 ± 1.0 | 1.5 ± 0.9 | 9.4 ± 3.5 | −0.01 ± 0.03 |
| 56 | 56.7 ± 2.4[c] | 4.6 ± 1.9[b] | 25.7 ± 1.3[b] | 0.3 ± 0.1 | 2.0 ± 1.0 | 10.8 ± 1.6[c] | +0.13 ± 0.00[b] | 70.3 ± 2.4[b] | 9.3 ± 2.1[b] | 7.5 ± 1.1[c] | 1.4 ± 1.0 | 1.8 ± 0.6 | 9.8 ± 1.4[c] | +0.00 ± 0.01[c] |
| AKR | | | | | | | | | | | | | | |
| 0 | 46.7 ± 4.8 | 43.3 ± 3.0 | 0.4 ± 0.2 | 2.7 ± 3.4 | 4.7 ± 2.8 | 2.1 ± 2.5 | −0.37 ± 0.05 | 56.2 ± 4.4 | 38.1 ± 4.1 | 1.1 ± 0.3 | 1.0 ± 1.3 | 1.5 ± 0.5 | 2.2 ± 1.2 | −0.30 ± 0.04 |
| 7 | 64.0 ± 6.0 | 21.5 ± 3.6[c,d] | 0.7 ± 0.5[e] | 2.7 ± 2.3 | 6.3 ± 4.1 | 4.7 ± 3.4 | −0.19 ± 0.04 | 60.3 ± 5.7 | 27.0 ± 2.9[d] | 2.0 ± 0.7 | 2.9 ± 2.4 | 2.0 ± 0.3 | 5.8 ± 2.4 | −0.20 ± 0.03 |
| 14 | 62.9 ± 4.0 | 24.7 ± 3.0[c,e] | 0.4 ± 0.1[e] | 3.1 ± 1.3 | 3.7 ± 1.2 | 5.4 ± 3.6[b] | −0.20 ± 0.02[d] | 60.5 ± 9.9 | 28.0 ± 5.6[d] | 2.3 ± 1.5 | 4.3 ± 3.4 | 2.3 ± 0.6 | 2.5 ± 4.0 | −0.24 ± 0.04 |
| 28 | 60.4 ± 9.9 | 25.6 ± 7.5[c,e] | 0.8 ± 0.3[e] | 3.1 ± 0.4 | 5.0 ± 2.4 | 5.2 ± 4.1 | −0.21 ± 0.05[e] | 56.4 ± 6.2 | 26.2 ± 0.5[d] | 3.9 ± 2.1 | 5.4 ± 2.1 | 3.0 ± 0.8 | 5.2 ± 3.7 | −0.21 ± 0.01[d] |
| 56 | 59.9 ± 4.1 | 22.9 ± 3.3[c,e] | 1.0 ± 0.8[e] | 3.9 ± 1.6 | 5.8 ± 1.0 | 6.4 ± 3.9 | −0.20 ± 0.02[e] | 66.7 ± 2.2 | 22.8 ± 4.5[d] | 2.6 ± 1.6[e] | 4.9 ± 2.5 | 2.0 ± 0.4 | 1.0 ± 0.9[e] | −0.21 ± 0.05[d] |

Values were determined from five hepatic biles (the first hour of biliary secretion) per mouse group at each time point. Abbreviations: T-ω-MC, tauro-ω-muricholate; T-β-MC, tauro-β-muricholate; TUDC, tauroursodeoxycholate; TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; HI, hydrophobicity index.

[a] The HI values of five hepatic biles were calculated (34).

[b] $P < 0.01$, compared to day 0.

[c] $P < 0.05$, compared to day 0.

[d] $P < 0.05$, compared to C57L and $F_1$ strains.

[e] $P < 0.01$, compared to C57L and $F_1$ strains.

Canalicular bile formation has been traditionally divided into two components (54, 55): BS-dependent flow, defined as the slope of the line relating canalicular bile flow to BS output (Fig. 1), and BS-independent flow, attributed to the active secretion of inorganic electrolytes and other solutes, defined as the extrapolated *y*-intercept of this line (Fig. 1). Our results showed that BS-dependent flow was similar in all three strains of mice as the major BS patterns were the same (Table 2). In contrast, BS-independent flow was significantly greater in C57L and $F_1$ mice than in AKR mice (Fig. 2). The higher BS-independent bile flow in gallstone-susceptible mice could be due to genetic differences (56) in rate-limiting canalicular transport proteins like the canalicular multispecific organic anion transporter (cMOAT) that may mediate up to 50% of BS-independent bile flow (57).

It is interesting to note that Ch crystallization was never detected in dilute mouse hepatic biles (total lipid concentration ~2.5 g/dL) despite high CSI values (range 1.00–2.30, see Table 1). This is because biliary compositions of dilute hepatic biles plotted in region E of the crystallization pathways in the phase diagram (Fig. 4), which is a zone containing no solid crystals at equilibrium, but only stable liquid crystals and saturated micelles (22). Conversely, in concentrated mouse gallbladder biles (range 7.4–13.7 g/dL, see ref. 19), liquid crystals, Ch crystals, and gallstones separate from supersaturated bile despite much lower CSI values (range 1.00–1.53, see ref. 19). The explanation for this stems from the complete mapping of Ch crystallization pathways in model systems (22) which demonstrate that with increases in total lipid concentration, all boundaries of the crystallization pathways (Fig. 4) shift to the right, i.e., to higher Lec contents. Although concomitantly the micellar zone becomes larger and hence CSIs fall, the relative biliary compositions move from region E (Fig. 4) into regions B, C, and D where solid Ch crystallization occurs rapidly (22). It is notable that administration of amiloride, a blocker of sodium/$H^+$ exchange, greatly reduces Ch gallstone prevalence by preventing increases in total lipid concentration in gallbladder bile (58).

Although mice were fed 0.5% cholic acid as part of the lithogenic diet for 56 days, the circulating BS pool sizes (Fig. 6, top panel) measured by the washout technique (25–27) did not change significantly compared with data from chow-fed mice (day 0). In a systematic study of hepatic enzyme activities in mice (20), we observed that in response to the lithogenic diet, all mice displayed lower activities of Ch 7α-hydroxylase and sterol 27-hydroxylase, two regulatory enzymes in BS biosynthesis. We also found that the circulating BS pool sizes in mice were similar before and after gallstone formation, which was consistent with human studies showing that there were no differences in the circulating BS pool sizes between Ch gallstone patients and controls (5, 59). Although it was suggested earlier that BS pool sizes were diminished in Ch gallstone patients compared with controls, other investigations subsequently have indicated that this might be caused by incomplete gallbladder emptying (59) in Ch gallstone patients. Using our previous data on gallbladder size and BS concentration in the same mouse strains (19), we calculated total BS pool sizes (Fig. 6, bottom panel) and found that C57L and $F_1$ mice displayed similar total BS pool sizes and both were significantly ($P < 0.05$) larger than those of AKR mice. We did not observe any gender differences in total BS pool sizes among the three strains of mice or any significant influences of the lithogenic diet.

Biliary BS compositions (Table 2) were greatly altered with feeding the lithogenic diet, and the changes in mouse hepatic bile were similar to those observed in gallbladder bile (19). This indicates, as anticipated, that the liver and the colonic flora but not the gallbladder are responsible for the changes in BS compositions. Furthermore, hydrophobic BS (TC, TCDC, and TDC) increased markedly in C57L and $F_1$ mice fed the lithogenic diet, whereas hydrophilic BS (T-β-MC and TUDC) decreased. The most likely reason for the sharp increase in TDC in males and females between days 7 and 14 (Table 2) is that with onset of lithogenic changes in the gallbladder (19), most secreted TC bypasses the gallbladder and is metabolized to deoxycholate by the anaerobic flora. Upon return to the mouse liver it is fully conjugated with taurine but incompletely rehydroxylated. The elevation of TCDC at day 7 in male but not in female mice does not have a simple explanation. Table 2 shows that the sum of TCDC plus T-β-MC at each time point is approximately the same in male and female gallstone-susceptible mice after 7 days except at 56 days in males. However, as T-β-MC is biosynthesized from chenodeoxycholate in mouse liver, the low ratio of T-β-MC to TCDC in male mice suggests that some component of the lithogenic diet (cholic acid?) interferes with both the microsomal 7β-epimerase and 6β-hydroxylase steps, the onset of which occurs later in females compared to males (Table 2). In contrast, in AKR mice, TCDC and TDC did not increase and T-β-MC remained at a high level during lithogenic diet feeding. These changes of BS species significantly increased HI values in C57L and $F_1$ mice but not in AKR mice. This may explain, in part, why C57L and $F_1$ mice show significantly higher intestinal Ch absorption (21, 60) and more rapid Ch phase transitions in bile (21) compared to AKR mice. Another finding in the present study is that T-β-MC, TC, and TCDC can be detected by HPLC in the mouse hepatic bile after "washout" for 8–12 h, whereas TUDC, TDC, and T-ω-MC disappeared after 4 h (Fig. 5, bottom panel). This verifies that T-β-MC, TC, and TCDC are primary BS, and TUDC, TDC, and T-ω-MC are secondary BS as observed in conventional compared with germ-free rodents (41).

### Mechanisms of lithogenic bile formation as inferred from biliary secretory phenotypes

Earlier studies suggested that many factors could participate in hypersecretion of biliary Ch during gallstone formation in genetically gallstone-susceptible C57L mice. For example, *i*) Ch absorption efficiency correlates significantly and positively with Ch gallstone prevalence (60) and is significantly higher in the gallstone-susceptible C57L strain (37 ± 7%) compared with the resistant strain AKR (24 ± 8%) (21, 60). *ii*) We have also observed (18, 20) that gallstone-

resistant AKR mice fed the lithogenic diet down-regulated HMG-CoA reductase, but susceptible C57L mice did not, indicating that de novo Ch synthesis may, in part, influence the formation of Ch-supersaturated hepatic bile. *iii*) Several studies (61–63) have shown that Ch from HDL is preferentially secreted into bile relative to Ch from other lipoproteins. Recent preliminary findings showed that the HDL receptor Sr-b1 is up-regulated in C57L mice (64) compared to AKR mice, suggesting that Sr-b1 may transfer Ch from HDL to the hepatocyte inducing a substantial increase in biliary Ch secretion (65). *iv*) Other observations (66, 67) have implicated sterol carrier protein 2 (Scp2) in hepatocellular trafficking of biliary Ch, and found that elevated Scp2 levels may be associated with biliary Ch hypersecretion. This suggests that Scp2 may contribute to biliary Ch hypersecretion by ferrying newly synthesized Ch and that hepatocellular trafficking of Ch may be enhanced (67). Although these factors could provide important sources of excess Ch molecules for secretion into hepatic bile, thereby inducing its Ch supersaturation, their structural genes (68) do not map to mouse chromosome 2 and therefore, all are excluded as candidate genes for *Lith1* (18).

The present studies are consistent with the possibility that *Lith* genes might encode lipid transporters in the canalicular membrane that could transfer lipid molecules into hepatic bile. It has been suggested that megalin (*Gp330*) (68, 69), a member of the *Ldlr* gene family, or the sister of P-glycoprotein (*Spgp*) (70), a member of the *Mdr* gene family (71), might be possible candidate genes. Both of them (68–70) map to the same region as the *Lith1* gene on mouse chromosome 2 (18). An in vitro study (72) has suggested that Spgp is a canalicular BS export pump of mammalian liver and mediates ATP-dependent transport for the canalicular secretion of BS into bile. Also, our preliminary results (Lammert, F., D. Q-H. Wang, D. E. Cohen, J. D. Schuetz, M. C. Carey, and B. Paigen, unpublished observations) showed that mRNA and protein levels of Spgp were significantly higher ($P < 0.05$) in C57L mice than in AKR mice. These findings could explain why C57L mice have BS hypersecretion compared to AKR mice. Therefore, it is crucial to further investigate in vivo functions of *Spgp* and *Gp330* on biliary lipid secretion.

In summary, the pathophysiological phenotypes of *Lith* genes as inferred from the present study suggest that an increased secretion of all biliary lipids but relative and absolute hypersecretion of Ch are major factors for development of supersaturated bile in gallstone-susceptible mice. The phenotypes therefore provide important clues for exploring the mechanisms of excess secretion of biliary Ch by the liver. Also, in agreement with our previous genetic analysis (18), this study suggests that the pathophysiological changes in hepatic biles of C57L and $F_1$ mice are determined by multiple *Lith* genes, each of which contributes to the observed phenotypes. This work therefore provides a basic framework for investigating how individual *Lith* genes induce biliary lipid hypersecretion and the other lithogenic abnormalities associated with Ch lithogenicity in bile of the inbred mouse.

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## REFERENCES

1. Afdhal, N. H., and M. C. Carey. 1995. New perspectives on gallstone pathogenesis and prevention. *J. Ir. Coll. Phys. Surg.* **24:** 267–278.
2. Apstein, M. D., and M. C. Carey. 1996. Pathogenesis of cholesterol gallstones: a parsimonious hypothesis. *Eur. J. Clin. Invest.* **32:** 343–352.
3. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
4. Admirand, W. H., and D. M. Small. 1968. The physiochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* **47:** 1043–1052.
5. Northfield, T. C., and A. F. Hofmann. 1975. Biliary lipid output during three meals and an overnight fast. I. Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects. *Gut.* **16:**1–17.
6. Nilsell, K., B. Angelin, L. Liljeqvist, and K. Einarsson. 1985. Biliary lipid output and bile acid kinetics in cholesterol gallstone disease. *Gastroenterology.* **89:** 287–293.
7. Valdivieso, V., R. Palma, F. Nervi, C. Covarrubias, C. Severin, and C. Antezana. 1979. Secretion of biliary lipids in young Chilean women with cholesterol gallstones. *Gut.* **20:** 997–1000.
8. Shiffman, M. L., H. J. Sugerman, J. M. Kellum, and E. W. Moore. 1992. Changes in gallbladder bile composition following gallstone formation and weight reduction. *Gastroenterology.* **103:** 214–221.
9. Holan, K. R., R. T. Holzbach, R. E. Hermann, A. M. Cooperman, and W. T. Claffey. 1979. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology.* **77:** 611–617.
10. Hofmann, A. F. 1990. Bile acid secretion, bile flow and biliary lipid secretion in humans. *Hepatology.* **12:**12S–25S.
11. Carey, M. C. 1989. Formation of cholesterol gallstones: The new paradigms. *In* Trends in Bile Acid Research. G. Paumgartner, A. Stiehl, and W. Gerok, editors. Kluwer, Dordrecht. 259–281.
12. Grundy, S. M. 1985. Factors affecting biliary lipid composition. *In* Gallstones. S. Cohen, and R. D. Soloway, editors. Churchill Livingstone, New York. 45–71.
13. Einarsson, K., and B. Angelin. 1988. Pathogenesis of cholesterol gallstone disease: the secretory defect. *In* Bile Acids in Health and Disease. T. Northfield, R. Jazrawi, and P. Zentler-Munro, editors. Kluwer, Dordrecht. 99–116.
14. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
15. Ahlberg, J., B. Angelin, and K. Einarsson. 1981. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and biliary lipid composition in man: relation to cholesterol gallstone disease and effect of cholic acid and chenodeoxycholic acid treatment. *J. Lipid Res.* **22:** 410–422.
16. Reihnér, E., B. Angelin, I. Björkhem, and K. Einarsson. 1991. Hepatic cholesterol metabolism in cholesterol gallstone disease. *J. Lipid Res.* **32:** 469–475.

17. Kern, F., Jr. 1994. Effects of dietary cholesterol on cholesterol and bile acid homeostasis in patients with cholesterol gallstones. *J. Clin. Invest.* **93:** 1186–1194.
18. Khanuja, B., Y-C. Cheah, M. Hunt, P. M. Nishina, D. Q-H. Wang, H. W. Chen, J. T. Billheimer, M. C. Carey, and B. Paigen. 1995. *Lith1*, a major gene affecting cholesterol gallstone formation among inbred strains of mice. *Proc. Natl. Acad. Sci. USA.* **92:** 7729–7733.
19. Wang, D. Q-H., B. Paigen, and M. C. Carey. 1997. Phenotypic characterization of *Lith* genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: physical–chemistry of gallbladder bile. *J. Lipid Res.* **38:** 1395–1411.
20. Lammert, F., D. Q-H. Wang, B. Paigen, and M. C. Carey. 1999. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: integrated activities of hepatic lipid regulatory enzymes. *J. Lipid Res.* **40:** 2080–2090.
21. Wang, D. Q-H., F. Lammert, D. E. Cohen, B. Paigen, and M. C. Carey. 1999. Cholic acid aids absorption, biliary secretion and phase transitions of cholesterol in murine cholelithogenesis. *Am. J. Physiol.* **275:** G751–G760.
22. Wang, D. Q-H., and M. C. Carey. 1996. Complete mapping of crystallization pathways during cholesterol precipitation from model bile: influence of physical-chemical variables of pathophysiologic relevance and identification of a stable liquid crystalline state in cold, dilute and hydrophilic bile salt-containing systems. *J. Lipid Res.* **37:** 606–630.
23. Cohen, D. E., and M. R. Leonard. 1995. Immobilized artificial membrane chromatography: a rapid and accurate HPLC method for predicting bile salt–membrane interactions. *J. Lipid Res.* **36:** 2251–2260.
24. Nishina, P. M., J. Verstuyft, and B. Paigen. 1990. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J. Lipid Res.* **31:** 859–869.
25. Eriksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. *Proc. Soc. Exp. Biol. Med.* **94:** 578–582.
26. Dowling, R. H., E. Mack, and D. M. Small. 1970. Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis, and pool size in the Rhesus monkey. *J. Clin. Invest.* **49:** 232–242.
27. Dowling, R. H., E. Mack, and D. M. Small. 1971. Biliary lipid secretion and bile composition after acute and chronic interruption of the enterohepatic circulation in the Rhesus monkey. IV. Primate biliary physiology. *J. Clin. Invest.* **50:** 1917–1926.
28. Wang, D. Q-H., and M. C. Carey. 1996. Characterization of crystallization pathways during cholesterol precipitation from human gallbladder biles: identical pathways to corresponding model biles with three predominating sequences. *J. Lipid Res.* **37:** 2539–2549.
29. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
30. Gurantz, D., M. F. Laker, and A. F. Hofmann. 1981. Enzymatic measurement of choline-containing phospholipids in bile. *J. Lipid Res.* **22:** 373–376.
31. Rossi, S. S., J. L. Converse, and A. F. Hofmann. 1987. High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugated bile acids. *J. Lipid Res.* **28:** 589–595.
32. Goh, E. H., S. M. Colles, and K. D. Otte. 1989. HPLC analysis of desmosterol, 7-dehydrocholesterol, and cholesterol. *Lipids.* **24:** 652–655.
33. Carey, M. C. 1978. Critical tables for calculating the cholesterol saturation of native bile. *J. Lipid Res.* **19:** 945–955.
34. Heuman, D. M. 1989. Quantitative estimation of the hydrophilic-hydrophobic balance of mixed bile salt solutions. *J. Lipid Res.* **30:** 719–730.
35. Boyer, J. L. 1980. New concepts of mechanisms of hepatocyte bile formation. *Physiol. Rev.* **60:** 303–326.
36. Nervi, F., M. Bronfman, W. Allalon, E. Depiereux, and R. Del Pozo. 1984. Regulation of biliary cholesterol secretion in the rat. Role of hepatic cholesterol esterification. *J. Clin. Invest.* **74:** 2226–2237.
37. Oude Elferink, R. P. J., R. Ottenhoff, M. van Wijland, J. J. M. Smit, A. H. Schinkel, and A. K. Groen. 1995. Regulation of biliary lipid secretion by mdr2 P-glycoprotein in the mouse. *J. Clin. Invest.* **95:** 31–38.
38. Shaffer, E. A., J. W. Braasch, and D. M. Small. 1972. Bile composition at and after surgery in normal persons and patients with gallstones. Influence of cholecystectomy. *N. Engl. J. Med.* **287:** 1317–1322.
39. Mazer, N. A., and M. C. Carey. 1984. Mathematical model of biliary lipid secretion: a quantitative analysis of physiological and biochemical data from man and other species. *J. Lipid Res.* **25:** 932–953.
40. Carey, M. C., and W. C. Duane. 1994. Enterohepatic Circulation. *In* The Liver: Biology and Pathobiology. I. M. Arias, J. L. Boyer, N. Fausto, W. B. Jakoby, D. A. Schachter, and D. A. Shafritz, editors. Raven Press, Ltd., New York. 719–767.
41. Madsen, D., M. Beaver, L. Chang, E. Bruckner-Kardoss, and B. Wostmann. 1976. Analysis of bile acids in conventional and germ-free rats. *J. Lipid Res.* **17:** 107–111.
42. Carey, M. C., and J. T. LaMont. 1992. Cholesterol gallstone formation. 1. Physical-chemistry of bile and biliary lipid secretion. *Prog. Liver Dis.* **10:** 139–163.
43. Pedreira, F., and H. M. Tepperman. 1964. Bile flow rate and cholesterol content in mice fed a gallstone-inducing diet. *Am. J. Physiol.* **206:** 635–640.
44. Tepperman, J., F. T. Caldwell, and H. M. Tepperman. 1964. Induction of gallstones in mice by feeding a cholesterol-cholic acid containing diet. *Am. J. Physiol.* **206:** 628–634.
45. Ishikawa, Y., K. Uchida, and T. Akiyoshi. 1984. Increased biliary cholesterol secretion in alloxan diabetic mice. *Jpn. J. Surg.* **14:** 174–183.
46. Akiyoshi, T., K. Uchida, H. Takase, Y. Nomura, and N. Takeuchi. 1986. Cholesterol gallstones in alloxan-diabetic mice. *J. Lipid Res.* **27:** 915–924.
47. Lee, S. P., J. T. LaMont, and M. C. Carey. 1981. Role of gallbladder mucin hypersecretion in the evolution of cholesterol gallstones: studies in the prairie dog. *J. Clin. Invest.* **67:** 1712–1723.
48. Brenneman, D. E., W. E. Connor, E. L. Forker, and L. DenBesten. 1972. The formation of abnormal bile and cholesterol gallstones from dietary cholesterol in the prairie dog. *J. Clin. Invest.* **51:** 1495–1503.
49. DenBesten, L., S. Safaie-Shirazi, W. E. Connor, and S. Bell. 1974. Early changes in bile composition and gallstone formation induced by a high cholesterol diet in prairie dogs. *Gastroenterology.* **66:** 1036–1045.
50. Bergman, F., A. H. Juul, and W. van der Linden. 1970. Development and regression of morphological and biochemical changes in hamsters and mice fed a cholesterol-cholic acid containing diet. *Acta Pathol. Microbiol. Scand.* **78:**179–191.
51. Shioda, R. 1965. Experimental studies on gallstone formation. *Arch. Jpn. Chir.* **34:** 571–586.
52. Crawford, J. M., G-M. Möckel, A. R. Crawford, S. J. Hagen, V. C. Hatch, S. Barnes, J. J. Godleski, and M. C. Carey. 1995. Imaging biliary lipid secretion in the rat: ultrastructural evidence for vesiculation of the hepatocyte canalicular membrane. *J. Lipid Res.* **36:** 2147–2163.
53. Hofmann, A. F., S. M. Grundy, J. M. Lachin, S-P. Lan, R. A. Baum, R. F. Hanson, T. Hersh, N. C. Hightower, Jr., J. W. Marks, H. Mekhjian, R. A. Shaefer, R. D. Soloway, J. L. Thistle, F. B. Thomas, M. P. Tyor, and the National Cooperative Gallstone Study Group. 1982. Pretreatment biliary lipid composition in white patients with radiolucent gallstones in the National Cooperative Gallstone Study. *Gastroenterology.* **83:**738–752.
54. Erlinger, S. 1994. Bile flow. *In* The Liver: Biology and Pathobiology. I. M. Arias, J. L. Boyer, N. Fausto, W. B. Jakoby, D. A. Schachter, and D. A. Shafritz, editors. Raven Press, Ltd., New York. 769–785.
55. Nathanson, M. H., and J. L. Boyer. 1991. Mechanisms and regulation of bile secretion. *Hepatology.* **14:** 551–566.
56. Bouchard, G., H. C. Chao, F. Lammert, D. Q-H. Wang, M. C. Carey, and B. Paigen. 1998. *Cmoat* is a candidate gene for *Lith2*, a cholesterol gallstone gene found on chromosome 19 in inbred mice. *Hepatology.* **28:** 502A (Abstract).
57. Müller., M., H. Roelofsen, and P. L. M. Jansen. 1996. Secretion of organic anions by hepatocytes: involvement of homologues of the multidrug resistance protein. *Semin. Liver Dis.* **16:** 211–220.
58. Strichartz, S. D., M. Z. Abedin, M. S. Abdou, and J. J. Roslyn. 1989. The effects of amiloride on biliary calcium and cholesterol gallstone formation. *Ann. Surg.* **209:**152–156.
59. Rutgeerts, P., Y. Ghoos, and G. Vantrappen. 1983. The enterohepatic circulation of bile acids during continuous liquid formula perfusion of the duodenum. *J. Lipid Res.* **24:** 614–619.
60. Wang, D. Q-H., B. Paigen, and M. C. Carey. 1998. Genetic variations in cholesterol absorption efficiency are associated with cho-

lesterol gallstone formation in inbred mice. *Hepatology.* **28:** 163A (Abstract).
61. Robins, S. J., J. M. Fasulo, M. A. Collins, and G. M. Patton. 1985. Evidence for separate pathways of transport of newly synthesized and preformed cholesterol into bile. *J. Biol. Chem.* **260:** 6511–6513.
62. Botham, K. M., and E. Bravo. 1995. The role of lipoprotein cholesterol in biliary cholesterol secretion: studies with *in vivo* experimental models. *Prog. Lipid Res.* **34:** 71–97.
63. Robins, S. J., and J. M. Fasulo. 1997. High density lipoproteins, but not other lipoproteins, provide a vehicle for sterol transport to bile. *J. Lipid Res.* **99:** 380–384.
64. Fuchs, M., G. Steimann, O. Müller, C. Schalla, P. Bartsch, and E. F. Stange. 1998. Hepatic overexpression of class B scavenger receptor (Sr-b1) during cholesterol gallstone formation in inbred mice. *Hepatology.* **28:** 502A (Abstract).
65. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, and M. Krieger. 1997. Overexpression of the HDL receptor Sr-b1 alters plasma HDL and bile cholesterol levels. *Nature.* **387:** 414–417.
66. Fuchs, M., F. Lammert, D. Q-H. Wang, B. Paigen, M. C. Carey, and D. E. Cohen. 1998. Sterol carrier protein 2 participates in hypersecretion of biliary cholesterol during gallstone formation in genetically gallstone–susceptible mice. *Biochem. J.* **336:** 33–37.
67. Puglielli, L., A. Rigotti, L. Amigo, L. Nuñez, A. V. Greco, M. J. Santos, and F. Nervi. 1996. Modulation of intrahepatic cholesterol trafficking: evidence by in vivo antisense treatment for the involvement of sterol carrier protein-2 in newly synthesized cholesterol transport into rat bile. *Biochem. J.* **317:** 681–687.
68. Welch, C. L., Y-R. Xia, I. Shechter, R. Farese, M. Mehrabian, S. Mehdizadeh, C. H. Warden, and A. J. Lusis. 1996. Genetic regulation of cholesterol homeostasis: chromosomal organization of candidate genes. *J. Lipid Res.* **37:** 1406–1421.
69. Xia, Y-R., D. R. Bachinsky, J. A. Smith, R. T. McCluskey, C. H. Warden, and A. J. Lusis. 1993. Mapping of the glycoprotein 330 (*Gp330*) gene to mouse chromosome 2. *Genomics.* **17:** 780–781.
70. Lammert, F., D. R. Beier, D. Q-H. Wang, M. C. Carey, B. Paigen, and D. E. Cohen. 1997. Genetic mapping of hepatocanalicular transporters establishes sister-P-glycoprotein (*Spgp*) as a candidate for the major gallstone gene (*Lith1*). *Hepatology.* **26:** 358A (Abstract).
71. Childs, S., R. L. Yeh, E. Georges, and V. Ling. 1995. Identification of a sister gene to P-glycoprotein. *Cancer Res.* **55:** 2029–2034.
72. Gerloff, T., B. Stieger, B. Hagenbuch, J. Madon, L. Landmann, J. Roth, A. F. Hofmann, and P. J. Meier. 1998. The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. *J. Biol. Chem.* **273:** 10046–10050.