Pathogenesis of gallstones in Crohn's disease
Brink, M.A.

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Chapter 5

ENTEROHEPATIC CYCLING OF BILIRUBIN:
A PUTATIVE MECHANISM FOR PIGMENT GALLSTONE FORMATION IN ILEAL CROHN'S DISEASE
ENTEROHEPATIC CYCLING OF BILIRUBIN: A PUTATIVE MECHANISM FOR PIGMENT GALLSTONE FORMATION IN ILEAL CROHN'S DISEASE

Menno A. Brink¹, J. Frederik M. Slors², Yolande C.A. Keulemans², Kam S. Mok¹, Rudi de Waart¹, Martin C. Carey³, Albert K. Groen¹, G.N.J. Tytgat¹

Department of Gastroenterology¹ and Surgery², Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam; Department of Medicine, Harvard Medical School and Harvard Digestive Diseases Center, Gastroenterology Division, Brigham and Women's Hospital, Boston MA 02115, USA.³

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Background & Aims: Patients with ileal disease, bypass or resection are at increased risk for developing gallstones. In ileectomized rats, bilirubin secretion rates into bile are elevated, caused most likely by increased colonic bile salt levels, which solubilize unconjugated bilirubin, prevent calcium complexing, and promote its absorption and enterohepatic cycling. The hypothesis that ileal disease or resection engenders the same pathophysiology in humans was tested.

Methods: Sterile gallbladder biles were obtained intraoperatively from 29 patients with Crohn's disease and 19 patients with ulcerative colitis. Bilirubin, total calcium, biliary lipids, β-glucuronidase activities, and cholesterol saturation indices in bile were measured and markers of hemolysis and ineffective erythropoiesis in blood were assessed.

Results: Bilirubin conjugates, unconjugated bilirubin and total calcium levels were increased three- to tenfold in bile of patients with ileal disease and/or resection compared to patients with Crohn's colitis or ulcerative colitis. Biliary bilirubin concentrations correlated positively with the anatomical length and duration of ileal disease. Endogenous biliary β-glucuronidase activities were comparable in all groups and both the hemogram and serum vitamin B₁₂ levels were normal.

Conclusions: This study establishes that increased bilirubin levels in bile of patients with Crohn's disease are caused by lack of functional ileum and support the hypothesis that enterohepatic cycling of bilirubin occurs.
Introduction

Crohn's disease, particularly with prior ileal resection, and ileal bypass for morbid obesity or hypercholesterolemia increase the risk for developing gallstones two- to threefold. In contrast, among patients with ulcerative colitis the prevalence of gallstone disease is similar to that of the general population. Although there is controversy as to whether patients with ileal disease, resection or bypass are at increased risk for cholesterol or pigment gallstone disease, gallbladder biles of patients with Crohn's disease contain increased levels of conjugated and unconjugated bilirubin (UCB), but normal cholesterol saturation indexes. These studies strongly suggest an increased prevalence of pigment as opposed to cholesterol gallstones after ileal resection or bypass, a concept which is supported by several animal studies. It is unknown whether the observed increases in biliary pigments were caused exclusively by ileal disease or by inflammatory bowel disease per se. Furthermore, in the earlier reports, small numbers of patients were studied, fasting duodenal biles were used and neither hemolysis, ineffective erythropoiesis from vitamin B12 deficiency nor elevated bacterial \( \beta \)-glucuronidase activities were excluded as causes of the elevated pigment levels in bile.

In recent work from our laboratories, we observed a twofold increase in bilirubin secretion rates into bile of ileectomized but not jejunectomized rats and we attributed this to increased enterohepatic cycling of bilirubin from the colon as a result of bile salt malabsorption. This concept was supported further by similar increases in biliary bilirubin secretion rates in rodents ingesting ursodeoxycholic acid as well as cholesterol. The hydrophilic bile acid competes with endogenous bile salts for the apical sodium-dependent bile salt transporter (ASBT) in the ileum and cholesterol apparently downregulates ASBT at the transcriptional level, both leading to bile salt malabsorption. We have proposed that increased colonic bile salt levels solubilize UCB, prevent calcium complexing and promote its absorption from the large intestine, such as is believed to occur for oxalic acid in enteric hyperoxaluria. If increased enterohepatic cycling of bilirubin from the colon is a pathophysiological mechanism in humans as well, then bilirubin levels in gallbladder bile should correlate with the extent of ileal dysfunction or resection. The specific aims of this
study were to investigate in a large cohort of Crohn’s disease patients whether alterations in bilirubin concentrations in gallbladder bile are related to dysfunction or resection of the ileum.

**Patients and Methods**

**Patients**

After written informed consent and with approval of the medical ethics committee at the Academic Medical Center, Amsterdam, we studied 48 patients with radiologically or endoscopically suspected inflammatory bowel disease when they were admitted for an elective bowel resection, that was indicated as part of their medical care. The diagnosis of Crohn’s disease or ulcerative colitis was confirmed by histological examination of the resected bowel segments. Serum indices of inflammation, vitamin B₁₂ status and hemolysis were determined preoperatively.

**Bile collection**

After the gallbladder was examined by palpation to assess calculi, gallbladder bile was obtained by needle aspiration as described by Strasberg et al. In three patients with Crohn’s disease who suffered from symptomatic gallstone disease, gallbladder bile was sampled immediately following surgical removal of the gallbladder. To protect bilirubin from photodegradation, tubes were covered with aluminum foil and transported to the laboratory for immediate processing. A portion of bile was stored on ice in the dark and analyzed within one hour for bilirubins by high-performance liquid chromatography. Bile was then centrifuged (10 min, 1500xg) and stored at -20°C for later determinations of biliary lipid concentrations and β-glucuronidase activity. All biles were sterile except for that of a single Crohn’s disease patient, which was excluded from subsequent analysis.

**Laboratory Methods**

Gallstones were typed on the basis of their cholesterol content, using isopropanol extraction and chemical analysis. Stones with more than 70% cholesterol were classified as cholesterol gallstones, and those with less than 20% cholesterol were designated pigment gallstones. Aliquots of fresh biles were examined using polarized light microscopy, cultured
aerobically and anaerobically for microorganisms, and pH was measured. Bilirubin conjugates and UCB were quantified by high-performance liquid chromatography, according to the procedure of Spivak and Yuey.\textsuperscript{27} Total calcium was determined by atomic absorption spectrometry. Biliary bile salts, phospholipids and cholesterol were measured by standard enzymatic procedures.\textsuperscript{26,28,29} Total lipid concentration (TLC) was calculated from the sum of cholesterol, bile salt and phospholipid concentrations and expressed in g/dL. Cholesterol saturation indices were calculated according to Carey’s critical tables.\textsuperscript{30} Endogenous β-glucuronidase activity was determined at pH 5.2 with 4-methyl-umbelliferone-β-glucuronide (Sigma, St. Louis, MO) as substrate according to Ho.\textsuperscript{31} Although the pH optimum of the human β-glucuronidase is 5.2, the activity is approximately 25% at pH 7.0,\textsuperscript{31} hence, the enzyme activity was adequate to deconjugate bilirubin diglucuronide (BDG) and monoglucuronide (BMG) at pH of native bile.

**Statistical Analysis**

All values are expressed as means ± S.D. For multiple comparisons of data from different groups, we used a one-way nonparametric test (analysis of variance) and the Student’s t-test for comparisons between two groups. Distributions in the different groups were compared using the chi-square test. \( P \) values less than 0.05 were judged to be significant.

**Results**

**Patient characteristics**

As shown in Table 1, 48 patients with inflammatory bowel disease were divided histologically and clinically into 29 with Crohn’s disease and 19 with ulcerative colitis. The Crohn’s disease patients were subdivided into i) patients with prior ileal resection (ileectomy), ii) Crohn’s ileitis (ileitis) and iii) Crohn’s colitis (colitis) in whom disease involved the colon exclusively. Patients with prior ileectomy had greater than 40 cm ileum resected, 8.8 ± 2.1 years prior to the current surgery and sampling of gallbladder bile. Eight of the 19 patients with ulcerative colitis, but none of patients with Crohn’s disease, had undergone total or partial colonic resections.
The four groups were comparable in terms of mean age, gender distribution and duration of disease (Table 1). Immunosuppressive therapy, as exemplified by the use of corticosteroids, mesalazine and azathioprine, was similar in all four groups. The plasma leucocyte counts were highly comparable among the groups, but the erythrocyte sedimentation rate was significantly higher ($P = 0.03$) in Crohn’s colitis compared to ulcerative colitis patients. None of the patients had ever been, or currently were treated with partial or total parenteral nutrition. Patients who in the past were shown to be malabsorbing vitamin B$_{12}$ as inferred from serum vitamin B$_{12}$ levels were treated routinely with i.m. Vitamin B$_{12}$.

Table 1. Characteristics of Patients With Crohn’s Disease and Ulcerative Colitis

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileectomy</td>
<td>Ileitis</td>
</tr>
<tr>
<td>$n$</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43 ± 10</td>
<td>34 ± 19</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>4/1</td>
<td>6/12</td>
</tr>
<tr>
<td>Duration of disease (yr)</td>
<td>9.2 ± 3.9</td>
<td>7.8 ± 8.1</td>
</tr>
<tr>
<td>Immunosuppressive treatment</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Mesalamine</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/hr)</td>
<td>26 ± 14</td>
<td>33 ± 30</td>
</tr>
<tr>
<td>White blood cell count (10$^9$/L)</td>
<td>8.5 ± 1.6</td>
<td>9.4 ± 4.4</td>
</tr>
</tbody>
</table>

NOTE. All data are expressed as means ± SD.

$^a$Statistically significant difference between patients with Crohn’s disease and ulcerative colitis.

Serum Hemogram Analyses and Vitamin B$_{12}$-Levels

Table 2 lists the preoperative blood tests on the patients with respect to hemolysis in the different groups. No evidence for hemolysis was found and all indices were within normal limits. Plasma UCB levels, although tending to be slightly higher in ileectomy patients, were not significantly different from Crohn’s ileitis/colitis and ulcerative colitis patients. Preoperative vitamin B$_{12}$ levels in ileectomized and ileal Crohn’s disease patients were within the normal range (200-800 ng/L) with mean vitamin B$_{12}$ concentrations being 503 ± 254 and 387 ± 96 ng/L, respectively, whereas the serum hemogram did not reveal megaloblastosis in any of the patients.
gallbladder bile in crohn's disease

Table 2. Preoperative Blood Tests of Patients With Crohn's Disease and Ulcerative Colitis

<table>
<thead>
<tr>
<th></th>
<th>Crohn's disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ileectomy (n = 5)</td>
<td>Ileitis (n = 18)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.6 ± 1.0</td>
<td>13.4 ± 1.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.0 ± 3.0</td>
<td>39.0 ± 4.0</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>84.0 ± 39.0</td>
<td>105.0 ± 32.0</td>
</tr>
<tr>
<td>Haptoglobin (g/L)</td>
<td>3.2 ± 1.1</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>UCB (µmol/L)</td>
<td>7.0 ± 3.2</td>
<td>6.1 ± 2.6</td>
</tr>
</tbody>
</table>

NOTE. All data are expressed as means ± SD.

Gallstones, biliary sludge and cholesterol crystals in gallbladder bile

Gallstones were not found in any patient with ulcerative colitis. One patient with a history of ileectomy presented with multiple “black” pigment gallstones (16% cholesterol by weight) and two patients with colonic and ileal Crohn’s disease had solitary cholesterol gallstones (92% and 73% cholesterol by weight, respectively), the latter with a dense black pigmented shell. Gallbladder sludge containing microscopic calcium bilirubinate precipitates was observed in another patient with ileal Crohn’s disease. Cholesterol monohydrate crystals were detected microscopically in gallbladder biles of two patients with prior ileectomy, three patients with Crohn’s ileitis, one patient with Crohn’s colitis and one patient with ulcerative colitis.

Biliary lipids and endogenous β-glucuronidase activity

Table 3 lists the results of gallbladder bile analysis in each patient group. Gallbladder bile was just saturated with cholesterol in Crohn’s disease patients with prior ileectomy and in ulcerative colitis patients, and marginally supersaturated in patients with Crohn’s ileitis or colitis. Nonetheless, there were no significant differences in relative or absolute biliary lipid compositions among the four groups. Endogenous β-glucuronidase activities of gallbladder bile were also similar in the three groups, but were significantly elevated in the Crohn’s colitis group (Table 3).
Table 3. Gallbladder Bile Analysis in Patients With Crohn's Disease and Ulcerative Colitis

<table>
<thead>
<tr>
<th></th>
<th>Crohn's disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ileectomy (n = 5)</td>
<td>ileitis (n = 18)</td>
</tr>
<tr>
<td>Phospholipid (mmol/L)</td>
<td>$52.0 \pm 10.0$</td>
<td>$51.0 \pm 17.0$</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>$18.0 \pm 5.0$</td>
<td>$19.0 \pm 7.0$</td>
</tr>
<tr>
<td>Bile salts (mmol/L)</td>
<td>$167.0 \pm 40.0$</td>
<td>$156.0 \pm 49.0$</td>
</tr>
<tr>
<td>Total lipid concentration (g/dL)</td>
<td>$12.9 \pm 2.7$</td>
<td>$12.4 \pm 3.6$</td>
</tr>
<tr>
<td>CSI°</td>
<td>$1.0 \pm 0.3$</td>
<td>$1.1 \pm 0.3$</td>
</tr>
<tr>
<td>Endogenous $\beta$-glucuronidase (U/L)</td>
<td>$9.6 \pm 1.9$</td>
<td>$8.0 \pm 6.3$</td>
</tr>
</tbody>
</table>

NOTE. All data are expressed as means ± SD.
°Biliary lipids were measured by enzymatic methods. 26,28,29
°Total lipid concentration was the sum of bile salt, phospholipid, and cholesterol concentrations expressed in grams per deciliter.
°Cholesterol saturation indices (CSI) were calculated according to Carey's critical tables.30
°Endogenous $\beta$-glucuronidase was measured according to Ho.31
°Statistical significant difference between patients with Crohn's colitis and ulcerative colitis.

**Biliary bilirubin and total calcium concentrations**

Table 4 summarizes concentrations of individual bilirubin conjugates and UCB as well as total calcium concentrations in the gallbladder biles. Total bilirubin concentrations and their individual subfractions did not differ between Crohn's colitis and ulcerative colitis patients. However, concentrations of total conjugated bilirubins and UCB were elevated significantly ($P < 0.05$) in gallbladder biles of Crohn's patients with ileitis, as well as those with prior ileectomy. Bilirubin levels were strikingly increased in patients with prior ileal resection or extensive Crohn's ileitis (>50 cm ileum; Table 4) being significantly higher ($P < 0.05$) compared to patients with lesser (<50 cm ileum) involvement of the ileum. Only in ileectomized patients and Crohn's ileitis patients with >50 cm ileum involved were there larger proportions of bilirubin monoconjugates and UCB in gallbladder bile, as evidenced by the significant ($P < 0.05$) increases of BMG/BDG and UCB/total bilirubin molar ratios (Table 4). Total bilirubin/bile salt molar ratios is a normalized index for the concentrating power of the gallbladder since bile salts are not absorbed (24). The ratio was increased significantly ($P < 0.05$) in ileectomized patients, and in the patients with Crohn's ileitis, outruling a lipid concentration artefact. No significant differences in gallbladder bile pH were found among the four groups.

Total calcium concentrations were increased markedly in bile of ileectomized and Crohn's ileitis (>50 cm ileum) patients, compared to patients with ulcerative colitis, and this was reflected in the total calcium/bile salt molar ratio, which was increased significantly ($P <$
In general, total calcium was increased in groups with high conjugated bilirubins or UCB levels or both (Table 4).

### Table 4. Bilirubin Conjugates, UCB, and Total Calcium Concentrations in Gallbladder Bile Obtained Peroperatively in Patients With Crohn’s Disease and Ulcerative Colitis

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileectomy (n = 5)</td>
<td>All (n = 15)</td>
</tr>
<tr>
<td>Bilirubin conjugates (mmol/L)</td>
<td>13.6 ± 5.7</td>
<td>10.3 ± 5.5</td>
</tr>
<tr>
<td>BDG</td>
<td>6.4 ± 2.9</td>
<td>6.2 ± 3.8</td>
</tr>
<tr>
<td>BMGGI</td>
<td>1.4 ± 0.5</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>BMGI</td>
<td>0.4 ± 0.5</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>BMGI</td>
<td>4.4 ± 1.9</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>BMGI</td>
<td>0.5 ± 0.3</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>BMX</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>UCB (μmol/L)</td>
<td>610.0 ± 300.0</td>
<td>157.0 ± 82.0</td>
</tr>
<tr>
<td>Total bilirubin (nmol/L)</td>
<td>14.2 ± 5.8</td>
<td>10.5 ± 5.6</td>
</tr>
<tr>
<td>UCB/total bilirubin X 100</td>
<td>4.3 ± 2.3</td>
<td>15.6 ± 0.6</td>
</tr>
<tr>
<td>BMG/BDG mole ratio</td>
<td>0.69 ± 0.16</td>
<td>0.36 ± 0.14</td>
</tr>
<tr>
<td>Total bilirubin/bile salt mole ratio</td>
<td>0.10 ± 0.06</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>Total calcium (mmol/L)</td>
<td>15.9 ± 4.3</td>
<td>10.6 ± 3.3</td>
</tr>
<tr>
<td>Total calcium/bile salt mole ratio</td>
<td>0.10 ± 0.03</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>pH</td>
<td>6.9 ± 0.1</td>
<td>7.1 ± 0.3</td>
</tr>
</tbody>
</table>

NOTE. All data are expressed as means ± SD.

BMGGI, bilirubin monoglucuronide-glucoside; BDG1, bilirubin diglucoside; BMGI, bilirubin monoglucoside; BMX, bilirubin monoxyloside.

*Anatomic length of ileum involved.

Bilirubin molecular species were measured by high-performance liquid chromatography. 37

Total calcium was measured by anatomic absorption spectrometry.

Statistically significant difference (P < 0.05) between patients with Crohn’s ileitis or ileectomy and ulcerative colitis.

Statistically significant difference (P < 0.05) among patients with ileectomy and Crohn’s ileitis (<50 cm), colitis, or ulcerative colitis.

Figure 1 shows mean ± SD values for total bilirubin (Panel A), UCB (Panel B) and total calcium levels (Panel C) in patients with Crohn’s disease in relation to duration of ileal disease (< or > 4 yrs). Biliary bilirubin levels (Panel A) were significantly (P = 0.05) higher in patients with longer duration of ileal disease, but UCB concentrations although increased (Panel B) missed statistical significance (P = 0.21). Further, total calcium levels (Panel C) in gallbladder bile were nonsignificantly (P = 0.12) decreased in patients with longer duration of ileal disease.
Discussion

In this study, we observed that in inflammatory bowel diseases, concentrations of bilirubin conjugates were increased two- to threefold in gallbladder bile of patients with involvement of the ileum, but not in patients with colonic involvement, e.g. Crohn's colitis or ulcerative colitis. The BMG/BDG ratios in gallbladder bile of ileectomy patients were reminiscent of the altered pigment conjugation patterns in patients with chronic hemolysis, but hematologic indices in plasma did not reveal any evidence of overt hemolysis (Table 2), although we acknowledge that moderate levels could be compensated for without changes in these indices. In patients with ileal Crohn's disease and particularly ileectomy, vitamin B\textsubscript{12} malabsorption may occur, causing megaloblastic anemia, hyperbilirubinemia, increased conjugated bilirubin levels in bile and pigment gallstone disease. However, prior to surgery neither vitamin B\textsubscript{12} deficiency nor megaloblastosis was found in our patients with ileectomy or ileal Crohn's disease, although several patients had received i.m. vitamin B\textsubscript{12} supplements in the past. Increased bilirubin levels in gallbladder bile in the absence of hemolysis and ineffective erythropoiesis may occur because of increased heme destruction from nonhemoglobin sources or by enhanced return of intestinal bilirubin to the liver via enterohepatic cycling of UCB. There is no a priori reason why increased heme enzyme catabolism e.g. by rapid liver regeneration secondary to toxic, infectious or metabolic injury to the liver, could have occurred in our patients with Crohn's disease. Contrariwise, in recent laboratory animal work, we presented evidence that in acutely ileectomized rats increased enterohepatic cycling of bilirubin occurred in association with bile salt malabsorption. As a
mechanism we have proposed\textsuperscript{21,22} that as a consequence of bile salt malabsorption, increased colonic bile salt levels solubilize UCB, prevent calcium complexing and promote passive UCB absorption from the large intestine, analogous to the situation with dietary oxalic acid in enteral hyperoxaluria.\textsuperscript{23} Because the first-pass extraction of UCB by the liver\textsuperscript{41} is believed to be constant (\textasciitilde30%), some bilirubin will spill-over into the systemic circulation. The concept of enterohepatic cycling of bilirubin mediated by bile salt malabsorption is supported by our recent observations in inbred mice and rats.\textsuperscript{22} Oral ingestion of ursodeoxycholic acid by the rodents increased cecal bile salt levels as well as UCB and urobinogen concentrations and decreased fecal bilirubin outputs, consistent with colonic absorption. As a result, we found increased bilirubin secretion rates into bile as well as elevated serum UCB concentrations.\textsuperscript{22} It is attractive to propose that bile salt malabsorption is also the underlying factor for the increases in biliary bile pigments in our patients with a non-functional ileum.\textsuperscript{42} Our study does not provide direct evidence that bilirubin uptake from the colon occurs, but this mechanism could explain the high biliary concentrations of bilirubin conjugates in patients with ileal resection or disease, and the slight elevations in plasma UCB in the absence of other causes such as vitamin B\textsubscript{12} deficiency, hemolytic anemia or increased turnover of tissue hemes. It is highly unlikely that small intestinal bacterial overgrowth, which occurs in about one-quarter of patients with Crohn's disease,\textsuperscript{43,44} would have contributed, since this condition also leads to deconjugation of bile salt conjugates and thereby loss of the vehicle that promotes UCB's absorption from the small intestine.\textsuperscript{21-24} Nonetheless, as a consequence of UCB levels being high in gallbladder bile in ileectomized patients, there may be some shunting of UCB from the gallbladder to plasma\textsuperscript{45} as well as enterohepatic cycling from all levels of the small intestine,\textsuperscript{40} thereby amplifying the pathophysiological cycling.

We believe that the best interpretation for the elevated UCB concentrations in gallbladder biles of our Crohn's patients (Table 4) is non-enzymatic or enzymatic hydrolysis of the increased bilirubin conjugate concentrations in the gallbladder. However, in ileectomy subjects, the markedly increased UCB/total bilirubin molar ratios compared with the other Crohn's disease patients, suggests that additional factors contribute to hydrolysis of conjugated bilirubins to UCB. First, increased BMG/BDG ratios in these patients, as seen with increased bilirubin loads delivered to the liver in hemolytic syndromes\textsuperscript{32} and impaired diglucuronidation of bilirubin by the liver in Gilbert's syndrome\textsuperscript{46} can elevate UCB levels in bile, probably
because BMG with only one ester linkage is easier hydrolyzed to UCB than BDG. Second, in Crohn's disease patients especially following ileectomy, the bile salt pool may be depleted diurnally. This would give rise to deficient micellar solubilization of dietary lipid resulting in a weaker fatty acid stimulus to cholecystokinin release from the proximal small intestine and thereby prolonged retention of bile in the gallbladder.

The elevated calcium level in bile of patients with a dysfunctional ileum may at least be due in part to increased levels of BMG and BDG, causing diffusion of calcium ions into bile by Gibbs-Donnan forces. It is well known that the concentrations of free ionized calcium and unbound UCB monoanions control formation of the sparingly soluble calcium bilirubinate salts in bile. Since total calcium and total UCB were increased about threefold in our patients, the facility with which calcium bilirubinates would form should be increased markedly, especially in Crohn's disease patients with extensive ileitis and ileectomy.

We observed a low gallstone prevalence in our Crohn's patients, possibly due to their relatively short duration of disease. Furthermore, ultrasonography of the gallbladder for gallstones was not performed preoperatively, and therefore very small gallstones could have been missed detection during intraoperative examination of the gallbladder. We found both cholesterol as well as pigment gallstones and sludge in our patients with Crohn's disease, although stone prevalence in each category was small. A significant number of our Crohn's disease patients had mild degrees of cholesterol supersaturated bile, and displayed cholesterol crystals microscopically. Studies in Crohn's disease patients by Färkkilä in Finland have shown that biles were supersaturated with cholesterol when fecal bile salt losses were minor, whereas biliary cholesterol secretion and bile lithogenicity decreased with large fecal bile salt losses.

In summary, we observed a striking increase in bile pigments (both conjugates and UCB) and calcium in biles of Crohn's disease patients with a non-functional or compromised ileum. Based on recent animal studies and by exclusion of other causes, such as hemolysis, increased tissue heme turnover and vitamin B_{12} deficiency, we postulate that enterohepatic cycling of UCB is the dominant mechanism as depicted in Figure 2. In this scenario, conjugated bilirubins in the cecum, in the presence of malabsorbed bile salts, are hydrolyzed to UCB without subsequent precipitation or rapid urobilinogen formation. Following bile salt-facilitated passive absorption, UCB recycles to the liver to be extracted, reconjugated and
resorbed into bile. Because of the higher bilirubin conjugate levels in bile and gallbladder hypomotility from defective cholecystokinin release, UCB forms, which if not precipitated as a calcium salt can be reabsorbed into blood from the gallbladder and small intestine. We speculate that the enterohepatic cycling of bilirubin once initiated may become amplified by this means, thereby constituting a vicious cycle, placing the patient at high risk for pigment gallstones.

Figure 2. In health, bilirubin conjugates are hydrolyzed by bacterial β-glucuronidases in the large intestine, and UCB either precipitates as insoluble calcium salts, or is further converted by bacterial catabolism into urobinogen. We postulate that in patients with ileal exclusion or disease, bile salt malabsorption increases colonic bile salt levels, which solubilize UCB in dimeric and micellar complexes and prevent precipitation and calcium complexing. Solubilization facilitates passive colonic UCB absorption and UCB returns to the liver bound to albumin in portal venous blood. Following hepatic sinusoidal uptake, UCB is efficiently reconjugated but now principally as monoglucuronides because of the increased flux, and re-secreted into bile, establishing a pathological enterohepatic cycling of bilirubin. At the level of the gallbladder, conjugated bilirubins are hydrolyzed in part by endogenous biliary β-glucuronidase and non-enzymatically, and UCB levels exceed normal values, principally because of elevated conjugated bilirubin levels, increased BMG/BGD ratios and prolonged gallbladder residence time. The gallbladder hypomotility is promoted by deficient cholecystokinin release from the proximal small intestine because of poor bile salt micellar solubilization of dietary fatty acid secondary to non-compensated bile salt malabsorption. If UCB is not complexed with calcium to precipitates as pigment stones, it may be absorbed passively at both gallbladder and small intestinal sites, thereby amplifying the enterohepatic cycling.
References


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