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World J Gastroenterol 2006 January 7; 12(1): 70-74
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Gallbladder bile composition in patients with Crohn's disease

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Received: 2005-05-12 Accepted: 2005-06-02

Abstract

AIM: To further elucidate the pathogenesis and mechanisms of the high risk of gallstone formation in Crohn's disease.

METHODS: Gallbladder bile was obtained from patients with Crohn's disease who were admitted for elective surgery (17 with ileal/ileocolonic disease and 7 with Crohn's colitis). Fourteen gallstone patients served as controls. Duodenal bile was obtained from ten healthy subjects before and after the treatment with ursodeoxycholic acid. Bile was analyzed for biliary lipids, bile acids, bilirubin, crystals, and crystal detection time (CDT). Cholesterol saturation index was calculated.

RESULTS: The biliary concentration of bilirubin was about 50% higher in patients with Crohn's disease than in patients with cholesterol gallstones. Ten of the patients with Crohn's disease involving ileum and three of those with Crohn's colitis had cholesterol saturated bile. Four patients with ileal disease and one of those with colonic disease displayed cholesterol crystals in their bile. About 1/3 of the patients with Crohn's disease had a short CDT. Treatment of healthy subjects with ursodeoxycholic acid did not increase the concentration of bilirubin in duodenal bile. Several patients with Crohn's disease, with or without ileal resection/disease had gallbladder bile supersaturated with cholesterol and short CDT and contained cholesterol crystals. The biliary concentration of bilirubin was also increased in patients with Crohn's colitis probably not due to bile acid malabsorption.

CONCLUSION: Several factors may be of importance for the high risk of developing gallstones of both cholesterol and pigment types in patients with Crohn's disease.

Key words: Bile acid; Biliary lipid composition; Bilirubin; Crohn's disease; Gallstone disease

Lapidus A, Åkerlund JE, Einarsson C. Gallbladder bile composition in patients with Crohn's disease. *World J Gastroenterol* 2006; 12(1): 70-74

http://www.wjgnet.com/1007-9327/12/70.asp

INTRODUCTION

The prevalence of gallstone disease in patients with Crohn's disease is about two-fold higher than that in general population[1-8]. This is true not only for patients with ileal disease/resection but also for patients with Crohn's colitis. No large studies of gallstone composition are available in patients with Crohn's disease but in two small series, while both pigment stones and cholesterol-rich stones have been reported[9,10]. The pathogenesis of gallstones in patients with Crohn's disease still remains to be elucidated.

One hypothesis for the increased prevalence of gallstone disease in patients with Crohn's disease is that the bile acid malabsorption in patients with diseased or resected ileum may lead to cholesterol supersaturated bile. In fact, supersaturated bile has been reported in some[11-14] but not all studies of patients with ileal disease or resection[8-10,15-19].

Another hypothesis for gallstone formation in patients with Crohn's disease is that patients with ileal disease or resection develop pigment stones as a consequence of increased spillage of malabsorbed bile acids into the colon where they solubilize unconjugated bilirubin and promote its absorption and thereby increase the rate of bilirubin secretion into the bile. In support of this hypothesis, an increased concentration of bilirubin in gallbladder bile or duodenal bile of patients with chronic ileitis or previous ileectomy has been reported[10,14,17,18]. Brink *et al.* [20] demonstrated that bile acid malabsorption after ileectomy of rats induces enterohepatic circulation of bilirubin and doubles the secretion rate of bilirubin into the bile. The same research group has also shown that adding ursodeoxycholic acid to the diet of mice and rats can increase the cecal bile acid levels and bilirubin secretion rates into the bile probably by inducing enterohepatic cycling of bilirubin[21].

The aims of the present study were to determine the biliary lipid composition, occurrence of cholesterol crystals, crystallization time, and bilirubin concentration in gallbladder bile of patients with ileal Crohn's disease

Table 1 Characteristics of patients with Crohn's disease and controls with gallstone disease

Patient group	*n*	Sex (M/F)	Mean age (range) (yr)	Previous surgery	Present surgery	Medical treatment
Crohn's disease						
Ileitis or ileocolitis	17	8/9	38 (23-70)	Ileal or ileocolonic Ileal, resection (*n* = 12)	Colonic or ileocolonic resection (*n* = 15) Cholecystectomy (*n* = 2)	Steroids (*n* = 11) Azathioprine (*n* = 4) Nitromidazole (*n* = 6) 5-ASA (*n* = 2)
Colitis	7	5/2	42 (28-57)	Partial colonic resection (*n* = 3)	Partial colonic resection or colectomy (*n* = 7)	Steroids (*n* = 5) Azathioprine (*n* = 1) Nitromidazole (*n* = 3)
Gallstone disease	14	1/13	47 (27-57)		Cholecystectomy (*n* = 14)	

with or without the involvement of colon and especially patients with Crohn's colitis in comparison to patients with cholesterol gallstone disease undergoing cholecystectomy served as control group and to study the influence of treatment with ursodeoxycholic acid on the duodenal concentration of bilirubin in human subjects.

MATERIALS AND METHODS

Patients and healthy subjects

Twenty-four patients with Crohn's disease admitted for elective surgery were included in the study. Clinical details are given in (Table 1). One patient had slightly elevated serum alkaline phosphatase level and three had slightly elevated transaminase and/or gamma glutamyl transpeptidase (GGT)-level. All other patients had normal laboratory tests of liver function including serum bilirubin. Seventeen patients had ileal or ileocolonic disease and 7 patients had Crohn's disease confined solely to the colon. Two patients with gallstones were cholecystectomized, one of them simultaneously underwent colectomy. All other patients were admitted for ileal, ileocolonic or colonic resection due to the failure of pharmacological treatment.

Fourteen consecutive patients with cholesterol gallstones admitted for cholecystectomy served as controls (Table 1).

In another experiment, 10 healthy subjects (6 men and 4 women, mean age 44 years) were studied before and after the treatment with ursodeoxycholic acid.

Informed consent was obtained from all the participants. The ethical aspect of the study was approved by the Ethical Committee of Karolinska Hospital Huddinge.

Experimental procedures

After the abdomen was opened, bile from the gallbladder was obtained by needle aspiration. The bile was collected in sterile tubes surrounded by foil and sent to the laboratory for analysis.

The healthy subjects were treated with ursodeoxycholic acid (Ursofalk® in 250 mg capsules, obtained from Dr Falk Pharma, Freiburg, Germany) at a daily dose of 15 mg/kg for 3 wk. Before and after the treatment, the bile was collected with an oroduodenal tube in the morning after an overnight fast. Gallbladder contraction was stimulated by an intravenous injection of cholecystokinin and 5-10 mL of the concentrated bile was obtained through the tube. The bile was collected in a test tube surrounded by a foil and was sent to the laboratory for analysis. Serum samples were also collected for analysis of bilirubin.

Biliary bilirubin concentration

An aliquot of the bile was immediately diluted with saline and the bilirubin concentration was determined by a similar procedure as for serum bilirubin as described previously[18]. The biliary concentration was expressed as mmol/L in gallbladder bile and as micromoles of bilirubin per millimole bile acid in duodenal bile.

Biliary lipids and bile acid composition

A portion of the gallbladder bile was immediately extracted with 20 volumes of chloroform–methanol 2:1 (vol/vol) and analyzed for cholesterol and phospholipids. Cholesterol was determined by an enzymatic method[22] and phospholipids by the method of Rouser *et al*.[23] The total bile acid concentration in one aliquot of the bile sample was determined using 3-alpha-hydroxy steroid dehydrogenase assay[24]. The relative concentration of cholesterol bile acids and phospholipids was expressed as molar percentage of the total biliary lipids. The cholesterol saturation was calculated according to Carey[25]. Bile acid composition was determined using gas-liquid chromatography[26].

Analysis of cholesterol crystals and crystallization time (CDT)

Gallbladder bile samples were examined for typical rhomboid monohydrate cholesterol crystals by polarizing light microscopy on pre-heated slides. CDT was determined by the method of Holan *et al*.[27] with minor modifications[28]. After centrifugation of about 6 mL bile at 100 000 *g* for 2 h, 3 mL from the middle phase was transferred into a sterile glass vial and sealed with a cap equipped with permeable silicon membrane. The vial was stored in darkness in an incubator at 37 ℃. About 3 μL from the top, middle and bottom portions was aspirated each day, mixed and placed on a pre-heated slide and viewed thoroughly by polarizing light microscopy. CDT was defined as the number of days until the appearance of typical rhomboid monohydrate cholesterol crystals.

Statistical analysis

Data were given as mean ± SE. Comparisons of the data between patients and healthy subjects were calculated using Mann-Whitney's rank sum test and Wilcoxon's sum of rank test. $P < 0.05$ was considered statistically significant.

Table 2 Biliary lipid composition and cholesterol saturation (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 17)	Crohn's disease colitis (n = 7)	Crohn's disease all patients (n = 24)	Gallstone disease (n = 14)
Cholesterol (molar%)	7.4± 0.7	6.4 ± 0.9	7.1 ± 0.6	9.8 ± 1.2
Phospholipids (molar%)	22.3 ± 1.2	25.0 ± 1.8	23.1 ± 1.0	25.1 ± 1.8
Bile acids (molar%)	70.3 ± 1.6	68.6 ± 2.4	69.8 ± 1.3	65.1 ± 3.0
Cholesterol saturation (%)	103 ± 9	83 ± 9[a]	97 ± 7	138 ± 18

[a]$P < 0.05$ *vs* patients with gallstone disease.

Table 3 Biliary bile acid composition (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 17)	Crohn's disease colitis (n = 7)	Crohn's disease all patients (n = 24)	Gallstone disease (n = 14)
Cholic acid (%)	44.5 ± 3.3	43.2 ± 2.2	44.1 ± 2.4[a]	35.4 ± 2.7
Chenodeoxycholic acid (%)	43.0 ± 2.9[b]	45.3 ± 3.8[b]	43.7 ± 2.3[b]	31.2 ± 2.4
Deoxycholic acid (%)	8.4 ± 2.3[c]	10.7 ± 3.2[c]	9.1 ± 1.8[c]	30.7 ± 4.3
Lithocholic acid (%)	0.10 ± 0.09[c]	0.01 ± 0.01[c]	0.07 ± 0.07[c]	1.5 ± 0.2
Ursodeoxycholic acid (%)	3.6 ± 1.8	0.7 ± 0.5	2.7 ± 1.3	1.2 ± 0.3

$P < 0.05$, [2]$P < 0.01$, [3]$P < 0.001$ *vs* patients with gallstone disease.

Table 4 Bilirubin concentrations in bile (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 15)	Crohn's disease colitis (n = 6)	Crohn's diseaseall patients (n = 21)	Gallstone disease (n = 14)
Bilirubin (mmol/L)	4.6 ± 0.7[a]	5.9 ± 1.6	5.0 ± 0.7	2.6 ± 0.2

* Significantly different from corresponding value of patients with gallstone disease. [a]$P < 0.05$ *vs* patients with gallstone disease.

RESULTS

Gall bladder bile composition

Data on biliary lipid composition are given in (Table 2). The cholesterol saturation of bile was significantly lower in patients with Crohn's disease confined to the colon than in patients with the gallstone. In contrast, 10 out of 17 patients with Crohn's disease involving the ileum had cholesterol-saturated bile. Nevertheless, all patients with CD as a group tended to have lower cholesterol saturation compared to patients with gallstone ($P = 0.055$).

Bile acid composition is shown in (Table 3). Cholic acid, chenodeoxycholic acid and deoxycholic acid were the dominant bile acids both in patients with Crohn's disease and in patients with gallstone. The patients with Crohn's disease had significantly lower proportions of deoxycholic acid and lithocholic acid than the patients with gallstone. The proportions of cholic acid and chenodeoxycholic acid were concomitantly increased.

The biliary bilirubin concentration was about 50% higher in patients with Crohn's disease than in patients with gallstone (Table 4). No difference was obtained between patients with Crohn's disease confined to the colon and those with ileal involvement. The bilirubin concentration tended to be higher in patients with Crohn's disease confined to the colon in patients with ileal involvement but the difference did not reach statistical significance.

Cholesterol crystals and CDT

Cholesterol crystals were present in four gallbladder samples of the 17 patients with Crohn's disease involving the ileum. One of the patients with cholesterol crystals also had gallstones and was cholecystectomized. The gallbladder bile was saturated with cholesterol. Only one of the patients with Crohn's colitis displayed cholesterol crystals. Also this patient had gallstones in saturated bile and was cholecystectomized. Most of the patients (9 out of 14) with cholesterol gallstones displayed cholesterol crystals.

CDT was measured only in patients with Crohn's disease. Six out of sixteen patients with ileal involvement had a short CDT (mean 4 d, range 1-7 d). Two of six patients with Crohn's colitis also had a short CDT (4 and 5 d, respectively).

Treatment with ursodeoxycholic acid

The results are summarized in (Table 5). Ursodeoxycholic acid accounted for (0.5 ± 0.5)% of the total biliary bile acids before the treatment. Treatment with ursodeoxycholic acid increased the bile acid to (54.8 ± 3.8)%. Cholic acid, chenodeoxycholic acid, and deoxycholic acid were concomitantly decreased. Treatment with ursodeoxycholic acid decreased the cholesterol saturation from (101 ± 10)% to (52 ± 5)%. The biliary bilirubin concentration expressed as mmol/mol bile acid did not change after the treatment with ursodeoxycholic acid. The serum concentration of bilirubin also did not change after the treatment with ursodeoxycholic acid.

DISCUSSION

Several studies have shown that patients with ileal Crohn'

Table 5 Data on healthy subjects treated with ursodeoxycholic acid (UDCA)

Patients	Sex	Age (yr)	BMI	Cholesterol saturation (%)		Cholesterol (molar %)		Bile acids (molar %)		UDCA i (%)		Bile acids (mmol/mL)		Bilirubin (mmol/L)		Bilirubin/bile acids (mmol/mol)	
				A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	F	39	22.2	103	31	6.6	1.7	74.5	82.8	0.0	49.9	57.1	100.9	490	1 058	8.6	10.5
2	F	40	24.4	74	38	4.8	2.4	75.8	79.2	0.0	65.3	92.8	95.6	1 466	571	15.8	6.0
3	F	30	26.8	116	46	9.1	3.4	65.8	72.7	0.0	–	75.4	65.8	1 031	798	13.7	12.1
4	F	58	26.3	32	42	2.4	2.8	71.7	76.1	0.0	55.8	117.8	73.6	1 265	667	10.7	9.1
5	M	33	29.4	125	51	7.4	3.8	76.2	71.4	5.2	55.1	52.1	117.4	1 045	1 111	20.0	9.5
6	M	30	24.0	104	60	6.1	3.7	77.6	78.2	0.0	66.5	97.9	32.1	1 301	418	13.3	13.0
7	M	34	24.9	128	38	8.9	2.5	70.5	76.7	0.0	54.3	52.3	26.3	993	375	19.0	14.3
8	M	73	26.2	118	55	8.9	4.3	67.5	68.6	0.0	35.7	31.0	61.9	1 168	1 497	37.7	24.2
9	M	49	23.2	77	79	5.6	4.9	71.3	77.7	0.0	41.3	33.5	12.8	662	365	19.8	28.5
10	M	55	24.3	135	77	9.8	4.9	68.6	76.7	0.0	69.7	16.4	8.5	568	197	34.5	23.3
Mean		44	25.2	101	52[b]	7.0	3.4[b]	72.0	76.0[a]	0.5	54.8[b]	62.6	59.5	999	706	19.3	15.1
SE		4.5	0.7	10	5	0.7	0.3	1.26	1.3	0.5	3.8	10.3	12.1	104	130	3.1	2.4

A = before UDCA feeding; B = after UDCA feeding; [a]$P < 0.05$, [b]$P < 0.01$ *vs* before UDCA feeding.

s disease and/or previous ileal resection have elevated bilirubin levels in the bile[10,14,17,18]. Animal experiments showed that ileectomy-induced bile acid malabsorption increases bilirubin secretion into the bile[20], suggesting that the increased bilirubin levels in patients with ileal disease and/or resection may be due to induced enterohepatic cycling of bilirubin because of bile acid malabsorption. Orally given ursodeoxycholic acid can compete with ileal absorption of endogenous bile acids and cause bile acid malabsorption in rodents as well as in human subjects[29-31]. Meéndez-Saánchez *et al* [21] also showed that oral administration of ursodeoxycholic acid to rodents induces biliary secretion of bilirubin and increases cecal bile acid levels as well as bilirubin concentrations. In the present study however, oral administration of ursodeoxycholic to healthy subjects did not increase the bilirubin concentration in bile, which makes it unlikely that bile acid malabsorption increases bilirubin secretion into the bile in human beings. This is further confirmed by our finding in the present study that patients with Crohn's colitis but without the involvement of the distal ileum and apparent bile acid malabsorption also had elevated bilirubin levels in the bile. In fact, bilirubin concentrations tended to be higher in patients with Crohn's disease confined to the colon than in those with ileal involvement. In contrast to our results, Brink *et al* [10] and Pereira *et al* [14] have shown that bilirubin level is normal in the gallbladder bile of patients with Crohn's colitis.

If the increased bilirubin concentration in the bile of patients with Crohn's disease is not due to an enhanced enterohepatic circulation of bilirubin because of bile acid malabsorption, what could then be the explanation? Theoretically increased bilirubin content can be explained by an increased formation and excretion into the bile and/or a decreased metabolism of bilirubin in the intestine with subsequent absorption and enterohepatic circulation of bilirubin. An increased formation of bilirubin may originate from hemolysis. However, none of the patients in the present study had hemolysis or hyperbilirubinemia. Therefore, the most likely explanation for the increased biliary content of bilirubin in the patients is an increased intestinal absorption. Normally, bilirubin is deconjugated and degraded to urobilinogen and other products in the colon[32]. In Crohn's disease, an altered colonic bacterial flora may enhance the deconjugation with a subsequently increased absorption of unconjugated bilirubin from the intestine and an increased excretion of bilirubin into the bile[33].

Two patients with Crohn's disease, one with the disease involving the ileum and the other one with Crohn's colitis were cholecystectomized. The gallbladder bile in both of them was supersaturated with cholesterol and contained cholesterol crystals, indicating that the stones are cholesterol type. Another three patients with ileal involvement but with out Crohn's colitis displayed cholesterol crystals in the gallbladder bile. About 1/3 of the patients with Crohn's disease with but without the ileal disease had a short CDT. Half of these patients had unsaturated gallbladder bile. This finding is in agreement with a recent report by Keulemans *et al*[19] who showed that patients with Crohn's disease have an increased tendency to form cholesterol crystals. They have also found that the crystallization behavior is the same in patients with ileal disease as in those with the disease confined to the colon and is caused by increased cholesterol crystallization promoting activity.

Several of the patients with ileal disease but without gallstones displayed cholesterol saturated gallbladder bile (Table 5). However, the mean value of the cholesterol saturation in this group of patients was the same as that obtained in the healthy subjects, which is in agreement with our previous finding that patients with ileal resection due to Crohn's disease have a normal saturation of the bile[16,18].

In conclusion, patients with Crohn's disease involving the ileum and those with Crohn's colitis have elevated concentration of bilirubin in the gallbladder bile. Oral administration of ursodeoxycholic acid to healthy subjects does not increase the biliary concentration of bilirubin. These results speak against the previously described hypothesis that the increased concentration of bilirubin in the bile samples from patients with Crohn's ileitis

or previous ileal resection is due to malabsorption of bile acids that spill into the colon where they solubilize unconjugated bilirubin and increase its absorption and enterohepatic circulation. In some patients with ileal disease/resection, the gallbladder bile is supersaturated with cholesterol and contains cholesterol crystals. About 1/3 of the patients with Crohn's disease with but without the ileal involvement have a short CDT probably because of increased cholesterol crystallization promoting activity in the gallbladder bile. Thus, several factors including cholesterol supersaturated bile, short CDT and increased bilirubin concentration, may be of importance for the high risk of developing gallstones of both cholesterol and pigment types in Crohn's disease.

ACKNOWLEDGMENT

The skillful technical assistance of Ms Lisbet Benthin and Ms Ingela Arvidsson is acknowledged.

REFERENCES

1 **Cohen S**, Kpplan M, Gottlieb L, Patterson J. Liver disease and gallstones in regional enteritis. *Gastroenterology* 1971; **60**: 237-245
2 **Harrower AD**, Davidson NM, Yap PL, Nairn IM, Fyffe JA, Horn DB, Strong JA. Hypothalmic-pituitary adrenal responsiveness to dexamethasone-insulin tolerance test in acromegalic patients before and during treatment with bromocriptine. *Acta Endocrinol* (Copenh) 1978; **88**: 18-22
3 **Hill GL**, Mair WS, Goligher JC. Gallstones after ileostomy and ileal resection. *Gut* 1975; **16**: 932-936
4 **Whorwell PJ**, Hawkins R, Dewbury K, Wright R. Ultrasound survey of gallstones and other hepatobiliary disorders in patients with Crohn's disease. *Dig Dis Sci* 1984; **29**: 930-933
5 **Lorusso D**, Leo S, Mossa A, Misciagna G, Guerra V. Cholelithiasis in inflammatory bowel disease. A case-control study. *Dis Colon Rectum* 1990; **33**: 791-794
6 **Hutchinson R**, Tyrrell PN, Kumar D, Dunn JA, Li JK, Allan RN. Pathogenesis of gall stones in Crohn's disease: an alternative explanation. *Gut* 1994; **35**: 94-97
7 **Lapidus A**, Bångstad M, Aström M, Muhrbeck O. The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. *Am J Gastroenterol* 1999; **94**: 1261-1266
8 **Fraquelli M**, Losco A, Visentin S, Cesana BM, Pometta R, Colli A, Conte D. Gallstone disease and related risk factors in patients with Crohn disease: analysis of 330 consecutive cases. *Arch Intern Med* 2001; **161**: 2201-2204
9 **Magnuson TH**, Lillemoe KD, Pitt HA. How many Americans will be eligible for biliary lithotripsy? *Arch Surg* 1989; **124**: 1195-119; discussion 1195-119
10 **Brink MA**, Slors JF, Keulemans YC, Mok KS, De Waart DR, Carey MC, Groen AK, Tytgat GN. Enterohepatic cycling of bilirubin: a putative mechanism for pigment gallstone formation in ileal Crohn's disease. *Gastroenterology* 1999; **116**: 1420-1427
11 **Dowling RH**, Bell GD, White J. Lithogenic bile in patients with ileal dysfunction. *Gut* 1972; **13**: 415-420
12 **Marks JW**, Conley DR, Capretta TL, Bonorris GG, Chung A, Coyne MJ, Schoenfield LJ. Gallstone prevalence and biliary lipid composition in inflammatory bowel disease. *Am J Dig Dis* 1977; **22**: 1097-1100
13 **Rutgeerts P**, Ghoos Y, Vantrappen G. Effects of partial ileocolectomy and Crohn's disease on biliary lipid secretion. *Dig Dis Sci* 1987; **32**: 1231-1238
14 **Pereira SP**, Bain IM, Kumar D, Dowling RH. Bile composition in inflammatory bowel disease: ileal disease and colectomy, but not colitis, induce lithogenic bile. *Aliment Pharmacol Ther* 2003; **17**: 923-933
15 **Färkkilä MA**. Biliary cholesterol and lithogeneity of bile in patients after ileal resection. *Surgery* 1988; **104**: 18-25
16 **Lapidus A**, Einarsson K. Effects of ileal resection on biliary lipids and bile acid composition in patients with Crohn's disease. *Gut* 1991; **32**: 1488-1491
17 **Dawes L**, Stryker S, Rege R, Nahrwold D. Gallbladder bile composition in Crohn's disease. *Surg Forum* 1991; **42**: 188-189
18 **Lapidus A**, Einarsson C. Bile composition in patients with ileal resection due to Crohn's disease. *Inflamm Bowel Dis* 1998; **4**: 89-94
19 **Keulemans YC**, Mok KS, Slors JF, Brink MA, Gouma DJ, Tytgat GN, Groen AK. Concanavalin A-binding cholesterol crystallization inhibiting and promoting activity in bile from patients with Crohn's disease compared to patients with ulcerative colitis. *J Hepatol* 1999; **31**: 685-691
20 **Brink MA**, Méndez-Sánchez N, Carey MC. Bilirubin cycles enterohepatically after ileal resection in the rat. *Gastroenterology* 1996; **110**: 1945-1957
21 **Méndez-Sánchez N**, Brink MA, Paigen B, Carey MC. Ursodeoxycholic acid and cholesterol induce enterohepatic cycling of bilirubin in rodents. *Gastroenterology* 1998; **115**: 722-732
22 **Roda A**, Festi D, Sama C, Mazzella G, Alini R, Roda E, Barbara L. Enzymatic determination of cholesterol in bile. *Clin Chim Acta* 1975; **64**: 337-341
23 **Rouser G**, Fkeischer S, Yamamoto A. Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970; **5**: 494-496
24 **Fausa O**, Skålhegg BA. Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3alpha-hydroxysteroid dehydrogenase. *Scand J Gastroenterol* 1974; **9**: 249-254
25 **Carey MC**. Critical tables for calculating the cholesterol saturation of native bile. *J Lipid Res* 1978; **19**: 945-955
26 **Angelin B**, Einarsson K, Leijd B. Biliary lipid composition during treatment with different hypolipidaemic drugs. *Eur J Clin Invest* 1979; **9**: 185-190
27 **Holan KR**, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology* 1979; **77**: 611-617
28 **Sahlin S**, Ahlberg J, Angelin B, Reihnér E, Einarsson K. Nucleation time of gall bladder bile in gall stone patients: influence of bile acid treatment. *Gut* 1991; **32**: 1554-1557
29 **Keelan M**, Thomson AB. Feeding diets containing 2% cheno- or urso-deoxycholic acid or cholestyramine to rats for two weeks alters intestinal morphology and bile acid absorption. *Can J Physiol Pharmacol* 1991; **69**: 592-598
30 **Marteau P**, Chazouilléres O, Myara A, Jian R, Rambaud JC, Poupon R. Effect of chronic administration of ursodeoxycholic acid on the ileal absorption of endogenous bile acids in man. *Hepatology* 1990; **12**: 1206-1208
31 **Owen RW**, Dodo M, Thompson MH, Hill MJ. Faecal steroid loss in healthy subjects during short-term treatment with ursodeoxycholic acid. *J Steroid Biochem* 1987; **26**: 503-507
32 **LESTER R**, SCHMID R. Intestinal absorption of bile pigments. II. Bilirubin absorption in man. *N Engl J Med* 1963; **269**: 178-182
33 **Neut C**, Bulois P, Desreumaux P, Membré JM, Lederman E, Gambiez L, Cortot A, Quandalle P, van Kruiningen H, Colombel JF. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. *Am J Gastroenterol* 2002; **97**: 939-946

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Wang J