Digestive Diseases and Sciences, Vol. 32, No. 1 (January 1987), pp. 33–44

Absence of Significant Role of Bile Acids in Diarrhea of a Heterogeneous Group of Postcholecystectomy Patients

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Twenty-five postcholecystectomy (PC) patients who underwent a diagnostic work-up for persistent diarrhea and six control subjects were studied. Fourteen of the 25 patients were also characterized by conditions other than PC which could play a role in the pathogenesis of the diarrhea. However, none of the patients had evidence of ileal disease or resection. The average follow-up of the patients after the study was approximately 4.4 years. Excretion, composition, and aqueous-phase concentrations of fecal bile acids were analyzed using gas–liquid chromatography. Eleven of the 25 PC patients showed an increased fecal bile acid excretion. In three of the 11 patients, the magnitude of the bile acid loss, which ranged from 2.26 to 3.34 mmol/24 hr, indicated the presence of severe bile acid malabsorption. The fecal bile acid composition showed a significant shift from secondary to primary bile acids. In spite of the presence of marked bile acid malabsorption, the aqueous-phase concentrations of the dihydroxy bile acids, chenodeoxycholic and deoxycholic acids, did not, with one exception, reach the secretory level of 1.5 mM. The relatively low aqueous concentrations were the result of low bile acid solubility, due to an acidic fecal pH. Only two of nine patients, one with severe, and the other with equivocal bile acid malabsorption, who were treated with cholestyramine, showed an improvement of the diarrhea. The findings of subsecretory bile acid concentrations in the fecal aqueous phase and of inconsistent therapeutic responses to cholestyramine indicate that, in spite of the presence of bile acid malabsorption, the diarrhea was, with few exceptions, not bile acid-induced. The results of the study also suggest that the diarrhea in many PC patients is multifactorial in origin.

Although postcholecystectomy (PC) diarrhea is considered to be a clinical entity (1), little information can be found in the literature regarding the frequency with which it occurs, the clinical conditions with which it is associated, its mechanism, or its cause.

Bile acid malabsorption has been suspected of playing a role in the pathogenesis of this disorder (1–2). A report by Hutcheon et al, in which three cases of PC diarrhea are described, indicates that fecal bile acid loss may be increased in this syndrome (2). The development of various degrees of ileal malabsorption of bile acids, and of a consequently increased passage of bile acids into the colon, is also suggested by the finding of a PC increase in the $^{14}CO_2$ excretion in breath after oral administration of [1-^{14}C]glycine-labeled glycocholic

Manuscript received April 18, 1985; revised manuscript received February 3, 1986; accepted March 17, 1986.

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This paper was presented, in part, at the American Gastroenterological Association Meeting, New Orleans, Louisiana, May 1984. Dr. Fromm was a recipient of Research Career Development Award AM 00290 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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0163-2116/87/0100-0033$05.00/0

TABLE 1. PERTINENT CLINICAL AND LABORATORY DATA ON SUBJECTS STUDIED

*Subject**	*Age*	*Sex*	*Body wt (kg)*	*Conditions other than PC*	*Onset of diarrhea in relation to C and other conditions*	*Follow-up information, duration of diarrhea*	*Response to cholestyramine*	*Response to other treatments*	*Fecal wt (g/24 hr)*	*Fecal fat (g/24 hr)*	*Fecal ^{51}Cr (% dose/24 hr)†*
CO1	35	M	106	NA	NA	NA	NA	NA	113	3.3	10.6
CO2	31	M	89	NA	NA	NA	NA	NA	125	2.7	NM
CO3	44	F	97	NA	NA	NA	NA	NA	55	3.3	22.9
CO4	25	F	60	Lower abdominal pain. Extensive gastrointestinal and gynecological work-up negative	NA	NA	NA	NA	85	2.5	0.3
CO5	27	F	60	Hiatal hernia, mild gastroesophageal reflux	NA	NA	NA	NA	88	1.5	9.4
CO6	55	F	80	Mild symptoms of gastroesophageal reflux	NA	NA	NA	NA	262	7.8	35.0
PC1	61	F	45	Choledochojejunostomy at time of C	Immediately PC	Ceased spontaneously 16 mo PC	No cholestyramine taken	No history of drug treatment for diarrhea	51	3.8	11.3
PC2	60	F	89	Partial hysterectomy, hypothyroidism (treated), lactose intolerance	10 yr prior to C, worsened PC	Continued diarrhea 5 yr after study	No cholestyramine taken	No response to lactose-free diet	87	1.0	22.3
PC3	65	F	62	Hypothyroidism (treated), hysterectomy	3 yr PC	Ceased spontaneously 1 yr after onset	Temporary	No response to Imodium	154	1.0	16.7
PC4	27	M	80	Appendectomy	1 yr prior to C	Continued diarrhea 6 yr after study	No cholestyramine taken	Response to Lomotil	40	2.4	0
PC5	53	F	61	Relapsing acute pancreatitis (ERCP normal)	8 yr PC when pancreatitis started	Continued intermittent diarrhea 2 yrs after study	No cholestyramine taken	Unconvincing response to pancreatic enzymes	121	4.1	1.0
PC6	63	F	70	Hysterectomy, partial gastrectomy and vagotomy	15 yr PC, 1½ yr post gastrectomy	Ceased spontaneously within 1 yr	No cholestyramine taken	No history of drug treatment	169	2.7	0.1
PC7	29	F	50	Distal colitis without small bowel involvement	5 mo PC	Ceased after treatment with Prednisone within 1 yr after study	No cholestyramine taken	Response to prednisone	144	0.6	63.2
PC8	77	F	43	None	Prior to C, but worse PC	Ceased after 1 yr of treatment with cholestyramine	Improvement	No history of other treatment	135	4.8	39.0

TABLE 1. CONTINUED

*Subject**	*Age*	*Sex*	*Body wt (kg)*	*Conditions other than PC*	*Onset of diarrhea in relation to C and other conditions*	*Follow-up information, duration of diarrhea*	*Response to cholestyramine*	*Response to other treatments*	*Fecal wt (g/24 hr)*	*Fecal fat (g/24 hr)*	*Fecal ^{51}Cr (% dose/24 hr)†*
PC9	42	F	139	Massive obesity	Immediately PC	Had jejunoileal bypass, diarrhea worsened	No cholestyramine taken	No history of other treatment	112	3.7	54.2
PC10	30	F	83	None	Immediately PC	Ceased spontaneously within 1 yr after study	No response	No history of other treatment	188	7.6	41.0
PCD1	39	F	53	Subtotal villous atrophy in jejunum	1 yr PC	No follow-up information available			585	23.8	4.4
PCD2	79	F	58	Lysis of intraabdominal adhesions and resection of segment of sigmoid colon	Several yr PC after sigmoid resection	Continued intermittent diarrhea 8 yr after study	Temporary	Response to Tinctura opii	274	2.3	71.1
PCD3	70	F	49	Billroth II gastrectomy	Several yr PC, 26 yr post gastrectomy	Ceased after nutmeg treatment	No response	Response to nutmeg	1547	7.1	96.9
PCD4	63	M	88	Billroth II gastrectomy, hiatal hernia repair	4 yr PC, 14 yr post gastrectomy	Less pronounced intermittent diarrhea 6 yr after study	No cholestyramine taken	Questionable response to Bentyl	416	9.2	NA
PCD5	69	M	66	Billroth II gastrectomy	7 yr PC, 28 yr post gastrectomy	Unchanged intermittent diarrhea 2 yr after study	No cholestyramine taken	Response to Imodium	1015	3.6	72.0
PCD6	69	F	41	Billroth II gastrectomy	21 yr PC, 9 yr post gastrectomy	Diarrhea improved 2 yr after study	No cholestyramine taken	Response to Lomotil	422	1.8	31.2
PCD7	28	F	80	Appendectomy, right tubal ligation, right ovarian cystectomy, lysis of intraabdominal adhesions	8 mo PC	Unchanged intermittent diarrhea 4 yr after study	No cholestyramine taken	Response to Imodium	344	6.5	73.5
PCD8	66	F	70	None	36 yr prior to C, worsened PC	Unchanged diarrhea 3 yr after study	No cholestyramine taken	Response to Imodium	299	11.4	48.7
PCD9	40	F	70	Relapsing acute pancreatitis (ERCP normal)	15 yr PC	Slightly improved intermittent diarrhea 2 yr after study	No cholestyramine taken	No history of drug treatment for diarrhea	1251	16.6	87.0

TABLE 1. CONTINUED

*Subject**	*Age*	*Sex*	*Body wt (kg)*	*Conditions other than PC*	*Onset of diarrhea in relation to C and other conditions*	*Follow-up information, duration of diarrhea*	*Response to cholestyramine*	*Response to other treatments*	*Fecal wt (g/24 hr)*	*Fecal fat (g/24 hr)*	*Fecal ^{51}Cr (% dose/24 hr)†*
PCD10	52	F	77	None	Immediately PC	Unchanged diarrhea 3 yr after study	No response	No satisfactory response to various antidiarrheal drugs	393	10.1	53.0
PCD11	65	F	59	Hysterectomy (Mullerian carcinoma), chemotherapy	7 yr PC, probably related to metastatic cancer	Patient died 2 yr after study	No cholestyramine taken	No history of drug treatment for diarrhea	487	8.1	80.1
PCD12	72	F	70	None	Immediately PC	Continued diarrhea 4 yr after study	No information obtainable regarding antidiarrheal treatment		1079	5.0	97.6
PCD13	32	F	85	Lactose intolerance	Preceded C, worsened PC	Continued diarrhea 5 yr after study	No response	Partial response to Imodium	883	10.4	98.9
PCD14	60	F	91	Stomach stapling for morbid obesity 5 yr PC	10 yr PC, immediately after stomach stapling	Continued diarrhea 1 year after study	Temporary	No response to multiple antidiarrheal drugs	627	42.7	85.2
PCD15	50	M	53	Billroth II gastrectomy	Few weeks PC	Continued intermittent diarrhea 8 yr after study	Yes	No history of other treatment	421	13.1	85.7

*CO = control; C = cholecystectomy; PC = postcholecystectomy, normal stool weight at time of study; PCD = postcholecystectomy, increased stool weight at time of study; NA = not applicable; NM = not measured.
†After 2 μCi of $^{51}CrCl_3$ *per os*.

acid (positive bile acid breath test) (3). However, systematic studies of fecal bile acid excretion in PC patients are lacking.

In the present study, fecal bile acid excretion is analyzed in a relatively large and heterogeneous group of PC patients with diarrhea. The reason for studying a relatively heterogeneous group of PC patients lies in the clinically important observation that the majority of the PC patients who were referred to the authors' laboratory for a diagnostic study of bile acid absorption were also characterized by conditions other than PC. These conditions could, either alone or in concert with the cholecystectomy, influence bile acid excretion. The latter possibility is suggested by the history of several patients according to which either a preexistent diarrhea worsened after a cholecystectomy or diarrhea developed when there was a combination of a cholecystectomy and other gastroenterological conditions. Since the fecal aqueous concentration and composition of bile acids constitute critical determinants of bile acid-induced diarrhea (4–6), the principal aim of this study was to evaluate these potentially important pathogenetic aspects of the PC syndrome.

MATERIALS AND METHODS

Study Subjects. The study was approved by the Committee on the Use of Human Subjects for Research of Montefiore Hospital, University Health Center, Pittsburgh, Pennsylvania, and written informed consent was obtained from all subjects before the study. Twenty-five PC patients and six control subjects were studied. The pertinent clinical and laboratory characteristics of the

TABLE 2. FECAL pH, BILE ACID EXCRETION, AND AQUEOUS-PHASE DIHYDROXY BILE ACID CONCENTRATIONS

Subject*	Total fecal bile acid excretion (mmol/24 hr)	Fecal pH	Fecal aqueous phase dihydroxy bile acid concentrations (mM)†	
			Actual	Maximum possible
C01	0.70	6.7	0.30	4.25
C02	0.72	6.1	0.17	2.94
C03	0.68	6.4	0.25	6.80
C04	0.88	7.2	0.45	4.01
C05	0.52	5.8	0.26	2.90
C06	0.61	8.2	0.10	1.13
PC1	0.15	6.6	0.10	1.94
PC2	0.47	6.2	0.52	3.69
PC3	0.34	7.6	0.48	0.97
PC4	0.28	7.1	0.38	3.50
PC5	0.61	6.9	0.18	1.25
PC6	0.43	6.8	0.30	1.21
PC7	0.37	7.8	1.31	1.75
PC8	0.77	6.3	0.57	4.13
PC9	0.96	7.1	1.46	5.63
PC10	3.53	6.6	1.08	10.56
PCD1	0.64	7.8	0.05	0.40
PCD2	0.72	7.0	0.76	1.82
PCD3	0.42	6.7	0.10	0.16
PCD4	0.48	7.5	0.06	0.08
PCD5	1.48	7.3	0.26	0.98
PCD6	1.25	8.8	2.73	2.92
PCD7	1.37	7.9	0.56	2.59
PCD8	1.22	6.3	0.30	2.20
PCD9	1.24	7.9	0.25	0.74
PCD10	1.31	6.6	0.54	2.61
PCD11	1.80	7.7	1.02	2.06
PCD12	1.79	8.2	0.87	1.04
PCD13	2.26	6.7	0.65	1.75
PCD14	3.34	6.1	0.10	3.61
PCD15	2.75	6.8	0.68	4.33

*For explanation of abbreviations, see Table 1.
†For definition of actual and maximum possible aqueous concentrations see text of Materials and Methods.

study subjects are listed in Table 1. The patients are grouped according to 24-hr stool weight and fecal bile acid excretion. Fifteen of the 25 PC patients were documented to have an increased 24-hr stool weight at the time of the study. The other 10 PC patients gave a history of chronic diarrhea, but had a normal stool weight. These patients were included for the following reasons: (1) They gave a credible history of diarrhea, which could have been intermittent in nature, with infrequent intervals of normal fecal weight. (2) These patients can also serve as a PC control group.

All patients were studied in the context of a diagnostic work-up for diarrhea. They were referred by their physicians to the authors' laboratory in order to be tested for the presence of bile acid malabsorption. The study was conducted in 18 of the 25 patients during a hospital stay and in the remaining seven on an outpatient basis. None of the patients had evidence of ileal disease or resection. The presence of ileal disease had been excluded by roentgenographic examination of the distal small bowel. However, as pointed out above and shown in Table 1, the majority of the patients was also characterized by the presence of conditions which, in addition to the cholecystectomy, could play a role in the pathogenesis of the diarrhea.

The control group consisted of one healthy subject, two subjects who were, with the exception of obesity, healthy, and three "disease controls" who were characterized by conditions which have no known association with bile acid malabsorption (Table 1). Previous studies have shown that diarrhea and accelerated intestinal transit may, also in the absence of ileal disease or resection, lead to borderline increases in the fecal excretion of bile acids without elevations in their fecal aqueous concentrations (7).

Protocol. The study was carried out in a manner similar to that previously described (6–8). In brief, the subjects were on a regular diet which supplied an average of 22 kcal/kg body weight and approximately 80 g of fat per day. On the day of the study, after a 12-hr fast, the subjects ingested a liquid breakfast (300 ml Ensure® which supplied 35 cal of protein, 79 cal of fat, and 136 cal of carbohydrates) containing 2 μCi of $^{51}CrCl_3$ as a nonabsorbable marker of intestinal transit (6–8). After the

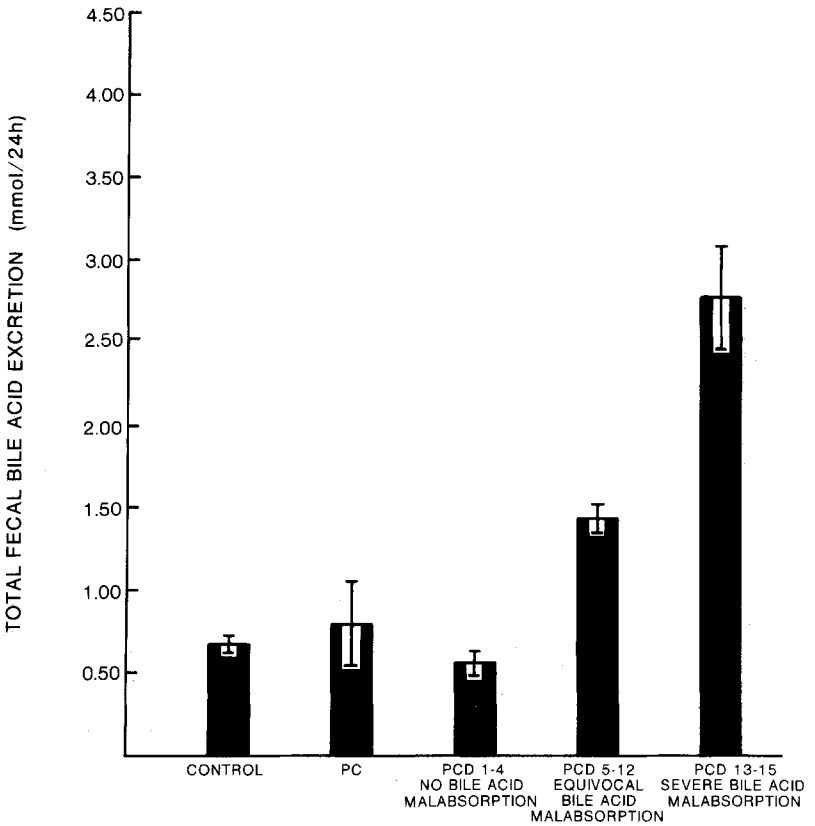

Fig 1. Total 24-hr fecal bile acid excretion (M ± SE) in the study groups. For abbreviations, see Table 1 and for individual data see Table 2.

liquid breakfast, the subjects resumed a regular diet for the subsequent meals. Stool was collected in preweighed paint cans on ice for 24 hr.

Processing and Analysis of Fecal Samples. Following the measurements of the fecal weight, the pH was recorded using a combination electrode (Brinkman Instruments, Westbury, New York, Model E-388) (7, 8). To solid stool, a known volume of deionized distilled water was added, resulting in an approximately 1:1 dilution. Stool samples of patients with diarrhea usually did not require dilution by addition of water. The stool samples were then homogenized, as previously described (6–8). Following homogenization, the fecal pH was recorded again and, in agreement with previous studies, no change was noted in comparison to the initial value (6, 8). The samples were either analyzed freshly or after storage at −20° C. In the latter case, the pH measurement was repeated after thawing of the sample and again found to be unchanged. Experiments were also carried out in order to determine the stability of the fecal pH between the time of a bowel movement and up to 24 hr of storage of the stool sample on ice. The pH was found to be stable, irrespective of whether the initial values were in the alkaline or acid range.

The fecal ^{51}Cr radioactivity after the administration of the nonabsorbable marker, $^{51}CrCl_3$, was determined by analysis of 2-g aliquots of the stool homogenates in a gamma-well counter (6–8).

Fecal bile acids were analyzed qualitatively and quantitatively by gas-liquid chromatography, as previously described (6, 8). For quantitative analysis, 0.5–1.0 mg of nordeoxycholic acid was added as internal standard to duplicate or triplicate 1-g aliquots of stool homogenate. These aliquots were subjected to alkaline hydrolysis in a 3-ml solution of 2 N NaOH and 50% methanol at 116° C for 16 hr. The samples were then transferred, dried, and resuspended in 30 ml of distilled water (resulting in a 0.1 N NaOH solution). One gram of Amberlite-XAD-7 resin (Polysciences, Inc., Warrington, Pennsylvania) was added, and the suspension was rotated for 60 min in order to adsorb bile acids to the resin. The aqueous portion was removed and the resin was washed once more with 10 ml of water. The bile acids were eluted from the resin with methanol. Samples were then dried and passed through a Florisil column to remove lipids and acidic pigments (6, 9). The resultant purified bile acids were further extracted with dichloromethane, esterified using ethereal diazomethane, and acetylated with perchloric acid, glacial acetic acid, and acetic anhydride (0.1:14:10, v/v). The bile acid methyl ester acetates were then extracted with diethyl ether, dried, and dissolved in *N,N*-dimethyl formamide, and analyzed by gas–liquid chromatography, employing a 6-ft U-column with a cyanosilicone stationary phase (AN-600 Supelco, Inc., Bellefonte, Pennsylvania).

The recovery of bile acids in this extraction system has previously been shown in this laboratory to be 90 ± 1.0%.

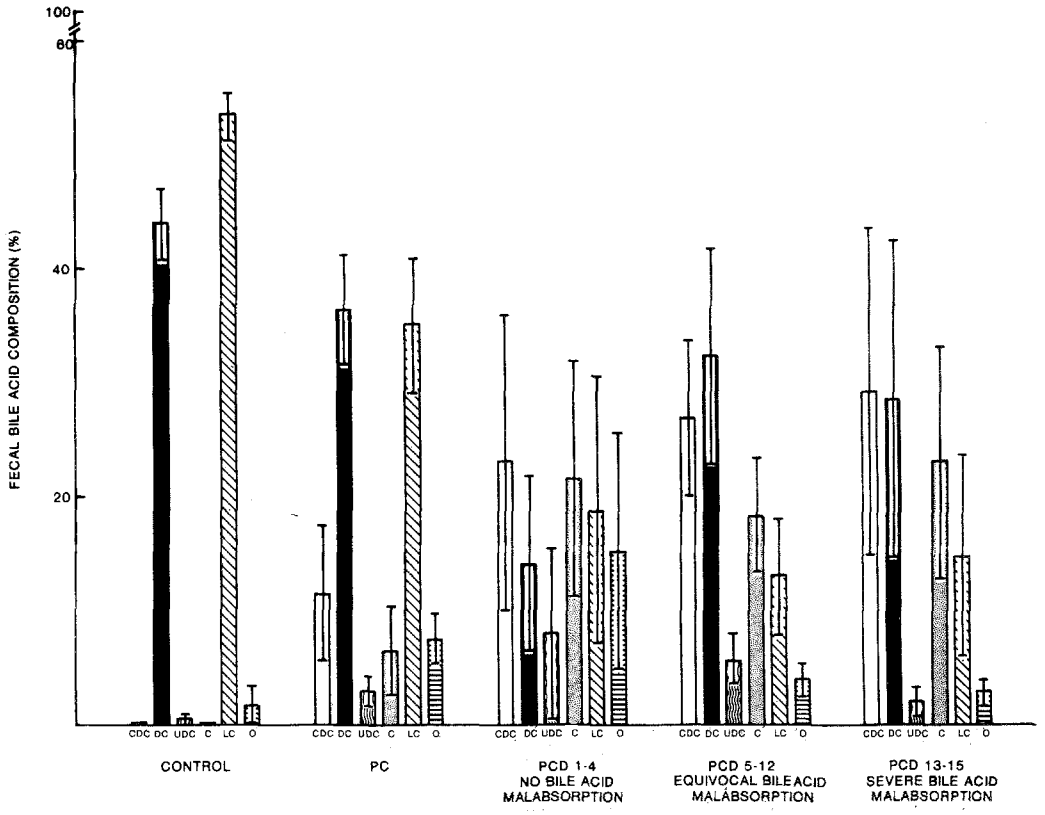

Fig 2. Fecal bile acid composition (M ± SE) in the study groups. For abbreviations see Tables 1 and 3.

The reproducibility of the entire extraction and analytical process, including gas–liquid chromatography, has also previously been demonstrated to be very satisfactory. The average difference from the mean was 6 ± 2.6% (6). It has been shown in previous studies of both fecal fat and bile acid excretion that the respective analyses on 24-hr collections give results similar to those of much longer collection periods in patients with diarrhea (7). Therefore, values for fecal bile acids in 24-hr collections can be considered to be valid in patients with diarrhea if the ^{51}Cr recovery is 50% or higher (50% of the ^{51}Cr label recovered in stool 24 hr after administration of $^{51}CrCl_3$).

Aqueous phase bile acids were determined by employing the above extraction procedure on 1–2 ml of ultracentrifuged supernatant (6). The supernatant was obtained by centrifuging stool homogenate at 100,000*g* for 2.5 hr at room temperature.

Assessment of Measurements and Statistics. Fecal weight was considered to be increased if the 24-hr value exceeded 200 g. The maximum possible aqueous-phase dihydroxy bile acid concentrations were estimated assuming that stool consists of 85% water. If distilled water was added for homogenization, correction was made for dilution. The term "maximum possible aqueous dihydroxy bile acid concentration" implies that all fecal dihydroxy bile acids are in aqueous solution (6, 8).

The upper limit of normal fecal bile acid excretion was defined to be represented by the mean ± 2SD control value, namely 0.926 mmol/24 hr. Equivocal bile acid malabsorption represented fecal bile acid excretion values which ranged between the upper limit of normal level and the mean ± 2 SD excretion value, 2.243 mmol/24 hr, previously observed in a so-called diarrhea control group (6). The diarrhea control group in the previous study consisted of patients with diarrhea in whom there was no evidence for the presence of an ileopathy. Severe bile acid malabsorption was considered to be present if fecal bile acid excretion exceeded 2.243 mmol/24 hr.

The statistical significance of the differences among the mean values of the measurements was calculated using the Student's *t* test. The correlation between the percent available dihydroxy bile acids in fecal aqueous solution and the pH was computed by least-square regression analysis (10).

RESULTS

Clinical Features at Time of Study. As detailed in Table 1, the relation between the onset of diarrhea and the time of cholecystectomy and other conditions varied considerably among the patients. In 20 patients, the diarrhea began either immediately (N = 5), within a few weeks (N = 1), or 5 months to 21 years (average approximately 6.9 years, N = 4) after the cholecystectomy. While five patients experienced diarrhea already before the cholecystec-

TABLE 3. STATISTICAL SIGNIFICANCE OF DIFFERENCES AMONG STUDY GROUPS

Groups compared*	Fecal wt (g/24 hr)	Fecal pH	Fecal fat (g/24 hr)	Total fecal bile acids (mmol/24 hr)	Dihydroxy bile acids (CDC and DC) in fecal aqueous phase (mM)†	
					Actual concentration	Maximal possible concentation
PC vs CO	NS	NS	NS	NS	$P < 0.05$	NS
PC vs PCD without bile acid malabsorption	NS	NS	NS	NS	NS	$P < 0.02$
PC vs PCD with equivocal bile acid malabsorption	$P < 0.01$	NS	$P < 0.02$	NS	NS	NS
PC vs PCD with severe bile acid malabsorption	$P < 0.01$	NS	NS	$P < 0.01$	NS	NS
PCD without bile acid malabsorption vs CO	NS	NS	NS	NS	NS	$P < 0.01$
PCD without bile acid malabsorption vs PCD with equivocal bile acid malabsorption	NS	NS	NS	$P < 0.01$	NS	$P < 0.05$
PCD without bile acid malabsorption vs PCD with severe bile acid malabsorption	NS	NS	NS	$P < 0.01$	NS	$P < 0.05$
PCD with equivocal bile acid malabsorption vs CO	$P < 0.01$	NS	$P < 0.05$	$P < 0.01$	NS	NS
PCD with equivocal bile acid malabsorption vs PCD with severe bile acid malabsorption	NS	$P < 0.02$	NS	$P < 0.01$	NS	NS
PCD with severe bile acid malabsorption vs CO	$P < 0.01$	NS	NS	$P < 0.01$	NS	NS

*NS = not significant; for abbreviations, see Table 1.
†For definition of actual and maximum possible concentrations of dihydroxy bile acids, see Materials and Methods.
‡CDC = chenodeoxycholic acid, DC = deoxycholic acid, UDC = ursodeoxycholic acid, C = cholic acid, LC = lithocholic acid, O = other, includes 7-ketolithocholic acid and unidentified bile acids.

tomy, four of them noticed it to worsen after the operation. The majority of the patients was also characterized by conditions other than PC, such as Billroth II gastrectomy ($N = 5$), Billroth I gastrectomy and vagotomy ($N = 1$), distal colitis ($N = 1$), subtotal villous atrophy ($N = 1$), metastatic Mullerian carcinoma ($N = 1$), stomach stapling for morbid obesity ($N = 1$), relapsing acute pancreatitis ($N = 2$), and lactose intolerance ($N = 2$) (Table 1). However, five patients, in whom the diarrhea started immediately PC (PC1, PC9, PC10, PCD10, and PCD12), were free of conditions other than cholecystectomy which could influence gastrointestinal function. Two other PC patients (PC8 and PCD8), in whom the diarrhea began before, but worsened after the cholecystectomy, were also free of other significant conditions.

Clinical Follow-Up. Follow-up information regarding the duration and treatment of the diarrhea after the study was, with one exception, obtained in all patients (Table 1). The average length of follow-up was 4.4 years. In two (PC1 and PC10) of the five patients in whom the diarrhea had begun immediately PC, it stopped spontaneously after 1.3 and 1 year, respectively. In a third patient (PC8), the diarrhea, which had started before, but worsened after the cholecystectomy, ceased after one year of cholestyramine treatment. In three other patients (PC7, PC3, and PC6) in whom the diarrhea had begun 0.4, 3, and 15 years PC, respectively, it stopped after about one year. A total of nine patients were treated with cholestyramine (Table 1). Only two of the nine, one with evidence of severe bile acid malabsorption (PCD15) and the second with equivocally increased fecal bile acid loss (PC8), responded satisfactorily to this treatment. In three other patients (PC3, PCD2, and PCD14), the diarrhea improved only temporarily on cholestyramine.

Total Fecal Bile Acid Excretion. Although all 25 PC patients gave a history of diarrhea and underwent a diagnostic work-up for this symptom, 10 of them (PC1-10) were found to have a normal 24-hr stool weight at the time of the study (Table 1). This finding may be the result of incomplete stool collection by the patient or the manifestation of a diarrhea-free interval of the illness. The former possibility appears, at least in several of the patients, to

TABLE 3. CONTINUED.

Fecal bile acid composition (%)‡					
CDC	*DC*	*UDC*	*C*	*LC*	*O*
$P < 0.01$	NS	NS	$P < 0.01$	$P < 0.02$	NS
NS	$P < 0.05$	NS	NS	NS	NS
NS	NS	NS	NS	$P < 0.02$	NS
NS	NS	NS	NS	NS	NS
$P < 0.01$	$P < 0.01$	NS	$P < 0.01$	$P < 0.02$	NS
NS	NS	NS	NS	NS	NS
NS	NS	NS	NS	NS	NS
$P < 0.01$	NS	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS
NS	NS	NS	NS	NS	
$P < 0.01$	NS	NS	$P < 0.01$	$P < 0.01$	NS

be the less likely one, since more than 50% of the nonabsorbable marker of intestinal transit, ^{51}Cr, was recovered during the 24-hr collection period (Table 1). With the exception of one patient (PC 10), all of them showed either normal (PC18) or only equivocally increased (PC9) fecal bile acid excretion (Table 2, Figure 1). The exception was a patient who, despite a normal stool weight, had a marked increase in fecal bile acids (Table 2). The remaining 15 PC patients were characterized by an increased fecal weight. In these patients, the bile acid excretion was either normal (PCD1–4), equivocally increased (PCD5–12, $P < 0.01$ vs controls), or greatly increased (PCD13–15, $P < 0.01$ vs controls) (Figure 1, Tables 2 and 3).

Fecal Bile Acid Composition. The statistical significance of the differences which existed in the fecal bile acid composition among the groups is detailed in Table 3. The most consistent differences were found among the chenodeoxycholic and cholic acid, as well as the lithocholic acid levels. The former were significantly higher and the latter significantly lower in the PC groups than in the controls. The differences among the groups were less frequent in regard to deoxycholic and ursodeoxycholic acids (Figure 2, Table 3).

Aqueous-Phase Dihydroxy Bile Acids and Fecal pH. There were, with one exception, no statistically significant differences in the actual fecal aqueous-phase dihydroxy bile acid concentration among the different study groups. The exception was the group of PC patients with a normal 24-hr stool weight at the time of the study which showed a significantly higher actual aqueous concentration than the control group (Figure 3). Only one subject (PCD6), a patient with evidence of equivocal bile acid malabsorption, showed a markedly increased aqueous-phase concentration of dihydroxy bile acids (Table 2). In two other patients (PC7 and PC9), who had a normal 24-hr stool weight at the time of the study, the aqueous dihydroxy bile acids were also increased, but remained, with concentrations of 1.31 and 1.46 mM, respectively, below the secretory level of 1.5 mM.

The percent available dihydroxy bile acids in fecal aqueous solution [(actual aqueous dihydroxy bile acid concentrations × 100) ÷ maximum possible aqueous dihydroxy bile acid concentrations)] showed a close correlation with fecal pH ($r = 0.639$, $P < 0.001$). Since chenodeoxycholic and deoxycholic acids are virtually insoluble below a pH of 6.8 and 7.0, respectively, an alkaline fecal pH, in addition to sufficient maximum possible aqueous concentrations of these bile acids, is usually required to raise the dihydroxy bile acids in the aqueous phase to secretory levels (4, 6, 20–22). With the aforementioned exception, the PC cases fulfilled either none or only one of the two critical requirements (Table 2, Figure 3).

DISCUSSION

According to previous studies by other investigators, the bile acid metabolism in PC subjects is characterized by an increased dehydroxylation of cholic to deoxycholic acid (3, 11, 12). This alteration is usually associated with a contraction of the total bile pool, with the deoxycholic acid pool being either normal or enlarged (3, 11, 12). The reason for this change, which indicates an increased exposure of the bile acid pool to the enzymatic actions of intestinal bacteria, is uncertain. One possible explanation could be the presence of an augmented passage of bile acids into the colon. This possibility is not necessarily inconsistent with the finding by several groups of investigators, according to which

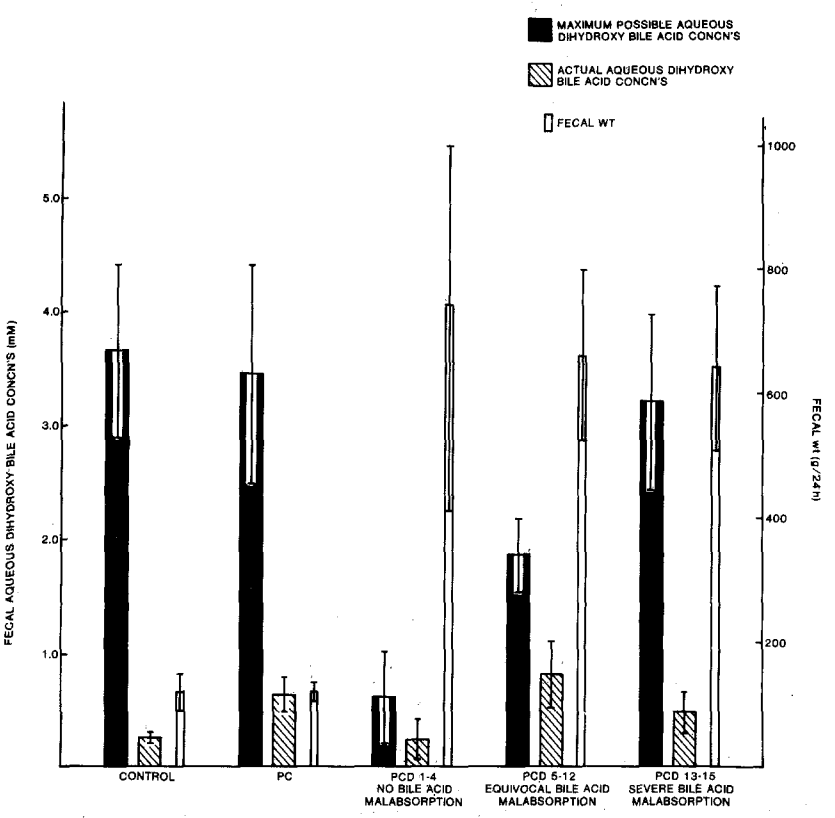

Fig 3. Fecal aqueous-phase dihydroxy bile acid (chenodeoxycholic and deoxycholic acid) concentrations and fecal weight (M ± SE) in the study groups. The maximum possible concentrations of dihydroxy bile acids in the aqueous phase represent the theoretical values assuming complete solubilization of bile acids. The actual concentrations represent the measured values. For abbreviations (PC and PCD) see footnote of Table 1. For calculation of maximum possible aqueous concentrations of dihydroxy bile acids and the definition of bile acid malabsorption see Materials and Methods.

the bile acid synthesis rate remains normal PC (3, 11, 12). Fecal loss and, consequently, compensatory hepatic synthesis of bile acids may remain normal as long as increases in the passage of bile acids from the ileum do not exceed the reabsorption capacity of the colon. Since considerable amounts of bile acids can be absorbed in the colon (13, 14), minor degrees of ileal malabsorption may be manifested by the colonic bacterial dehydroxylation of correspondingly increased amounts of bile acids, without noticeable increments in their fecal loss and hepatic synthesis. The latter was probably the case in the aforementioned studies by other authors which, apparently, involved PC patients who were free of diarrhea (3, 11, 12).

In the majority of the PC patients in the present study with diarrhea, fecal bile acid excretion was either normal or within the equivocal range, as it is prevalent in conditions associated with moderate ileal dysfunction and/or accelerated intestinal transit (6, 7). Only three PC patients were found to have unequivocally increased fecal bile acid losses, indicating the presence of severe bile acid malabsorption as is typically seen in conditions of marked ileal dysfunction (5, 6, 15–17). These cases resembled those with bile acid malabsorption due to ileopathies also in regard to fecal bile acid composition, which was characterized by a significant shift from secondary to primary bile acids (5, 6, 18, 19). However, neither of these three patients with severe bile acid malabsorption and only one with evidence of equivocal bile acid malabsorption showed concentrations of bile acids in the fecal aqueous phase which are considered to be high enough to induce diarrhea (4). Fecal aqueous concentrations of the two secretory bile acids, chenodeoxycholic and deoxycholic acids, of about 1.5 mM or greater, are prerequisites for bile acid-induced diarrhea (4). The main reason for aqueous bile acids remaining at subsecretory levels, in spite

of high total (maximum possible) concentrations, lies in the presence of suboptimal solubility due to an acidic fecal pH (6, 8). Chenodeoxycholic and deoxycholic acids are only slightly soluble below a fecal pH of 6.8 and 7.0, respectively (6, 20–22). However, their solubility rises sharply if the pH increases above these levels (6, 20–22).

The present study, therefore, indicates that, with few exceptions, secretory effects of bile acids do not constitute a major factor in the mechanism of the diarrhea in PC patients. Although an increased sensitivity of the intestinal epithelium to the secretory stimulus by bile acids, similar to that observed by Oddsson et al (23) in patients with irritable bowel syndrome, has to be considered, several observations speak against this possibility. First, in most patients who were placed on cholestyramine, the diarrhea failed to respond to this treatment. Although the improvement of diarrhea during cholestyramine treatment does not establish bile acids as the pathogenetic factor, the absence of a therapeutic response to this drug makes it unlikely that bile acids are significantly involved in the causation of the diarrhea. On the one hand, cholestyramine can exert a nonspecific antidiarrheal effect unrelated to its bile acid-binding action. On the other, bile acid-induced diarrhea invariably improves upon the administration of cholestyramine (5). The second observation, which speaks against the presence of an increased sensitivity of the intestinal epithelium to secretory bile acids, relates to that of relatively high fecal aqueous-phase dihydroxy bile acid concentrations in three PC patients (PC7, PC9, and PC10) who had a normal 24-hr stool weight at the time of the study (Table 2).

The present data are consistent with the occurrence of several types and mechanisms of diarrhea in PC patients, none of which were significantly related to a secretory effect of bile acids. In one, an important pathogenetic role of the cholecystectomy is suggested by the close temporal relationship which existed between the operation and the onset of the diarrhea. In 10 of the PC patients, the diarrhea either started shortly PC or, if it existed prior to the cholecystectomy, it worsened after this operation. The second type of diarrhea, prevalent in the patients studied, was characterized by significant conditions other than cholecystectomy. For example, different gastric operations could either by themselves or in conjunction with the cholecystectomy and/or unidentified factors be responsible for the diarrhea. It appears possible that a cholecystectomy decreases the threshold of the intestine for a diarrheal response to various secretory, toxic, and/or infectious agents. However, although the cause of the diarrhea in several of the PC patients is consistent with this possibility, systematic epidemiological, clinical, or experimental studies of this question are lacking.

In summary, the finding of subsecretory bile acid concentrations in the fecal aqueous phase and/or unsatisfactory therapeutic responses to cholestyramine indicates that, in spite of the presence of bile acid malabsorption, PC diarrhea is, with few exceptions, not bile acid-induced. The results of the present study also suggest that the diarrhea in many PC patients is multifactorial in origin. Future studies are necessary to determine whether a cholecystectomy leads to a lower threshold for diarrheal responses of the intestine to various stimuli.

ACKNOWLEDGMENT

The authors wish to thank Prafulla Amin for skillful technical assistance.

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