Digestive Diseases and Sciences, Vol. 41, No. 12 (December 1996), pp. 2409–2416

Biliary Electrolytes and Enzymes in Patients with and Without Gallstones

KANG-JEY HO, MD, PhD

pH, osmolarity, various electrolytes, nine enzymes, and bile acid were determined in hepatic and gallbladder biles from 108 and 100 patients, respectively, relating to various types of gallstones. The pH, osmolarity, and electrolytes were essentially identical in all groups of patients except for slightly higher Ca and Mg in the hepatic bile in patients with muddy pigment stones. The gallbladder bile contained much higher inorganic cations yet remained isosmotic as a result of their sequestration into bile acid micelles. Excluding extremely high values, the activities of nine enzymes in the bile showed only minor differences among four groups of patients except for a high β-glucuronidase activity in the hepatic bile in patients with muddy pigment stones. The biliary baseline activities of various enzymes and the relation to their serum levels were determined by their sources and subcellular localization in the hepatocytes. We concluded that biliary electrolytes and enzymes were basically similar in patients with and without gallstones except for higher levels of Ca, Mg, and β-glucuronidase in hepatic bile in patients with muddy pigment stones.

KEY WORDS: hepatic bile; gallbladder bile; electrolyte; aspartate transaminase; alanine transaminase; γ-glutamyl transaminase; lactate dehydrogenase; leucine aminopeptidase; alkaline phosphatase; creatine phosphokinase; β-glucuronidase; cholelithiasis.

Water and electrolytes diffuse from the hepatocytes to the bile canaliculi when the bile acids are secreted. This component of the bile is referred to as the bile acid-dependent fraction (1–3). Some water and electrolytes are also secreted into the canaliculi in association with the active transport of nonbile acid organic anions. This portion of the bile is known as the bile acid-independent fraction (3–5). The canalicular bile must be further modified by the lining epithelial cells of the bile ductules and ducts before reaching the common bile duct (6). The hepatic bile is then concentrated and modified during storage in the gallbladder (7). The first goal of the present study is to measure and compare the osmolality and the contents of various electrolytes in the hepatic bile and the gallbladder bile for understanding of such modification in normal and disease (cholelithiasis) states.

The protein concentration in human bile varies greatly: 2 to 530 mg per 100 ml for the hepatic bile and up to 550 mg/100 ml for the gallbladder bile (8). The bile proteins are very much heterogeneous with serum albumin as the major component. Enzymes derived from hepatocytes are also an important source of biliary protein (9). The second goal of this study is to determine the activities of nine representative enzymes derived from various subcellular locations of the hepatocytes and from extrahepatic source in the hepatic and gallbladder biles in patients with no gallstones and in those with various types of gallstones. The mechanisms for the presence of such various enzymes in the bile and their possible role in the gallstone formations are discussed.

Manuscript received August 11, 1995; revised manuscript received January 19, 1996; accepted August 13, 1996.

From the Department of Laboratory Medicine, National Taiwan University, College of Medicine, Taipei, Taiwan, and University of Alabama at Birmingham and Veterans Affairs Medical Center, Birmingham, Alabama 35233.

Address for reprint requests: Dr. Kang-Jey Ho, Department of Pathology, University of Alabama at Birmingham Medical Center, UAB Station, Birmingham, Alabama 35233.

0163-2116/96/1200-2409$09.50/0

MATERIALS AND METHODS

Bile Sampling. Gallbladder biles were obtained from 100 patients admitted to National Taiwan University Hospital, Taipei, during 1992–1993 period. Twenty of these patients had no gallstones, 35 had high-residue black pigment stones, 15 low-residue brown pigment stones, and 30 mixed stones. The methods for chemical analysis of the stones and their classification according to the chemical constituents are reported separately (10). Those patients with no gallstones underwent laparotomy for reasons other than biliary tract disease and were free of obvious liver disease as judged by the liver function tests and direct observation of the liver at surgery. The bile was aspirated directly from the gallbladder using an 18-gauge needle attached to a syringe during abdominal surgery. The puncture wound was closed by subserosal sutures. For the patients with stones, the bile was obtained by the same technique as soon as the gallbladder was removed from the body. Informed consent was obtained from all patients. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institution's human study committee.

Bile samples were also collected from the common bile duct from 108 patients by two methods. In 28 patients with indwelling common duct T-tube the bile was collected directly from the T-tube to the collecting container after complete re-establishment of the enterohepatic circulation. In the remaining 80 patients the bile was collected through a nasobiliary catheter. Among these patients 20 did not have gallstones, 32 had high-residue black pigment stones, another 32 had either low-residue brown pigment stones or muddy pigment stones, and 24 had mixed stones.

Analysis of the Bile. As soon as the bile specimens were received in the lab the pH and osmolality were determined by a pH meter and an osmometer, respectively (the pH did not change over a period of 3 hr when the containers were tightly capped), and then appropriate dilutions with demineralized distilled water were made. For the hepatic bile 1-, 2-, and 4-fold dilutions were made. The usual dilutions for the gallbladder bile were 5-, 10-, and 20-fold. The samples were then analyzed by a Hitachi 747 chemical autoanalyzer for electrolytes and various enzymes. Sodium, potassium, and chloride were measured by means of ortho-cresolphthalein complexone of Connerty and Briggs (11), inorganic phosphorus by the reaction with acidic ammonium molybdate (12), and magnesium by the reaction with calmagite to form a reddish violet chromophore (13). The following enzymes were measured by the same Hitachi 747 chemical autoanalyzer: aspartate transaminase (AST or glutamic-oxaloacetic transaminase; EC 2.6.1.1) (14), alanine transaminase (ALT or glutamic-pyruvic transaminase; EC 2.6.1.2) (14), γ-glutamyl transaminase (GGT; EC 2.3.2.1) (15), lactate dehydrogenase (LDH; EC 1.1.1.27) (15), leucine amino peptidase (LAP; EC 3.4.1.1 or naphthylamidase) (14, 15), alkaline phosphatase (ALP; EC 3.1.3.1) (16), creatinine phosphokinase (CPK or CK; EC 2.7.3.2) (14, 15), and amylase (EC 3.2.1.1) (17). In addition, the maximal velocity of β-glucuronidase of both human and bacterial origins devoid of intrinsic interference by bilirubin and bile acid were determined by the method developed previously by us (18, 19). Bile acid was measured by the enzyme method of Talalay with 3-α-hydroxysteroid NAD oxidoreductase (20) after 5- to 10-fold dilution of the hepatic bile or 20- to 100-fold dilution of the gallbladder bile with methanol.

Statistical Analysis. Duncan's multiple-range test (21) was used for comparison of parameters in more than two groups of subjects.

RESULTS

pH, Osmolality, and Electrolytes. The pH of hepatic bile was around 7.4 in all four groups of patients regardless of presence or absence of gallstones and the kinds of stones (Table 1). The pH decreased to about 7.0 in the gallbladder bile in all four groups (Table 2). The osmolality of the gallbladder bile was identical to that of the common duct bile. In fact the osmolality was essentially identical in all groups of patients (Tables 1 and 2). Among the electrolytes measured chloride was also identical in both hepatic bile and gallbladder bile in all four groups. The other electrolytes became more concentrated in the gall-

TABLE 1. BILE ACID, pH, OSMOLALITY, AND ELECTROLYTES OF THE HEPATIC BILE IN FOUR GROUPS OF PATIENTS

Common duct bile	*Without stones (n = 20)*	*With formed black pigment stones (n = 32)*	*With muddy pigment stones (n = 32)*	*With mixed stones (n = 24)*
Bile acid*	16.0 ± 10.2† A‡	14.4 ± 13.8 A	20.7 ± 16.0 A	13.8 ± 11.6 A
pH	7.40 ± 0.41 A	7.38 ± 0.36 A	7.52 ± 0.42 A	7.48 ± 0.39 A
Osmolality	285 ± 8 A	290 ± 12 A	284 ± 9 A	289 ± 14 A
Cl	107 ± 11 A	118 ± 16 A	110 ± 12 A	110 ± 10 A
Na	169 ± 18 A	157 ± 17 A	163 ± 16 A	157 ± 13 A
K	5.4 ± 1.6 A	5.2 ± 0.9 A	4.9 ± 1.1 A	5.1 ± 0.9 A
Ca	1.8 ± 0.4 B	1.6 ± 0.4 B	2.1 ± 0.5 A	1.6 ± 0.3 B
Mg	0.36 ± 0.30 B	0.34 ± 0.36 B	0.58 ± 0.36 A	0.27 ± 0.41 B
P	4.0 ± 2.7 A	2.7 ± 1.6 A	3.5 ± 1.5 A	3.3 ± 1.8 A

* Units for bile acid, μmol/L; osmolality, mOsm/L of water; and all electrolytes, mmol/L.
† Mean ± SD.
‡ Grouping by Duncan multiple-range test at 1% significant level.

TABLE 2. BILE ACID, pH, OSMOLALITY, AND ELECTROLYTE CONTENTS OF THE GALLBLADDER BILE IN FOUR GROUPS OF PATIENTS

Gallbladder bile	*Without stones (n = 20)*	*With high-residue black pigment stones (n = 35)*	*With low-residue brown pigment stones (n = 15)*	*With mixed stones (n = 22)*
Bile acid*	202 ± 70† A‡	119 ± 90 B	91 ± 66 B	109 ± 80 B
pH	6.96 ± 0.35 A	6.90 ± 0.32 A	7.05 ± 0.40 A	7.10 ± 0.39 A
Osmolality	282 ± 9 A	288 ± 15 A	280 ± 14 A	286 ± 12 A
Cl	110 ± 29 A	108 ± 36 A	113 ± 62 A	118 ± 39 A
Na	216 ± 42 A	220 ± 51 A	199 ± 56 A	218 ± 50 A
K	10 ± 4 A	11 ± 6 A	10 ± 4 A	11 ± 3 A
Ca	6.8 ± 4.4 A	7.8 ± 6.2 A	6.2 ± 5.0 A	7.2 ± 4.3 A
Mg	3.2 ± 2.2 A	4.1 ± 2.4 A	2.9 ± 2.6 A	4.0 ± 2.7 A
P	10 ± 5 A	11 ± 6 A	12 ± 9 A	9 ± 4 A

* Units for bile acid, μmol/L; osmolality, mOsm/L; and all electrolytes, mmol/L.
† Mean ± SD.
‡ Grouping by Duncan multiple-range test at 1% significant level.

bladder bile than in the hepatic bile. On average there were 1.3-, 2.1-, 3.2-, 4.1-, and 10.2-fold increases for sodium, potassium, phosphorus, calcium, and magnesium, respectively (Table 3). The concentrations of various electrolytes in the gallbladder bile showed no different among the four groups of patients with no stone or with various types of gallstones (Table 2). The hepatic bile in patients with muddy pigment stones had a slightly but significantly higher concentration of calcium and magnesium than the other three groups (Table 1).

Enzymes. Unlike electrolytes, the protein concentration including all the enzymes in the bile varied greatly depending upon the concentration state of the bile. Therefore, in addition to the absolute activity per unit volume of the bile (U/L), the enzyme activity was also "normalized" by the bile acid concentration, expressed as activity per micro-mole of bile acid (U/μmol). Furthermore, extreme values of enzyme activity, often 10-, 100-, and even 1000-fold of the normal, could be occasionally observed. Such obviously extreme values were excluded when the mean was computed for the group. The number of bile samples excluded and the highest values for each enzyme are shown in Table 4. After exclusion of those extreme values the enzyme activities in all four groups with or without normalization for bile acid concentration were quite similar to each other. Therefore, instead of meticulously presenting all the data, only those values of the control group are shown in Table 5. The few exceptions included a lower AST and a higher amylase activity found in the hepatic bile from patients with muddy pigment stones and from those with mixed stones, respectively. The activity of β-glucuronidase in the gallbladder bile was higher in the control than in the other three groups. However, such difference disappeared after normalization with bile acid concentration (Figure 1). β-Glucuronidase activity in the hepatic bile was higher in the muddy pigment stone group than in others. After normalization it remained highest in the muddy pigment stone group. The activity in formed pigment stone and mixed stone groups was statistically significantly higher than in the control group (Figure 1). The ratios of the enzyme activity in the gallbladder bile to that in the hepatic bile are shown in Table 6.

TABLE 3. THE RATIO OF BILE ACID, OSMOLALITY, AND ELECTROLYTES IN THE GALLBLADDER BILE TO THAT IN THE HEPATIC BILE

	[Gallbladder bile] / [Hepatic bile]
Bile acid	8.3 (4.4–12.6)*
Osmolality	0.99 (0.98–0.99)
Cl	1.01 (0.92–1.07)
Na	1.32 (1.22–1.40)
K	2.09 (1.92–2.20)
Ca	4.11 (2.95–4.90)
Mg	10.2 (5.0–14.8)
P	3.25 (2.55–4.22)

* Mean (range).

DISCUSSION

Both bile acid and a number of non-bile acid organic anions are secreted into bile canaliculus primarily by various ATP-dependent active transport systems but also by a membrane potential-dependent mechanism (5, 22–26). Their secretion is accompanied by passive diffuse of water and inorganic ions (1–5, 24). Under the influence of secretin the lining

TABLE 4. NUMBER OF PATIENTS WITH EXCESSIVE HIGH VALUES OF ENZYME ACTIVITY EXCLUDED FROM COMPUTATION OF THE MEAN OF EACH GROUP AND THE HIGHEST VALUE OF EACH ENZYME

Enzyme in the bile	Hepatic bile			Gallbladder bile		
	No. of patients with high values	Highest value		No. of patients with high values	Highest value	
		U/μmol BA	U/L bile		U/μmol BA	U/L bile
AST	2	60	1,048	2	132	1,205
ALT	3	46	104	1	30	2,430
GGT	6	470	1,824	13	570	2,000
LDH	6	1,526	1,740	14	750	6,855
LAP	1	392	3,275	2	145	460
ALP	13	1,300	10,849	11	146	17,450
CPK	4	182	207	10	187	15,370
Amylase	18	23,760	60,827	8	25	80
β-Glu	0	—	—	0	—	—

epithelial cells of the biliary tract also secrete bicarbonate and water (7). The bile collected from the common duct T-tube or nasobiliary catheter in this study was the hepatic bile with above modification. The pH, osmolality, and various electrolytes were essentially identical in the hepatic biles in patients with various types of stones or without stones except for a slightly higher calcium and magnesium in the muddy pigment stone group (Table 1). Calcium plays an important role in the formation of gallstones, particularly pigment gallstones (27–29). Calcium in the bile exists as either free Ca^{2+} ions or as bound/complexed calcium. The major calcium binding ligands include premicellar and micellar bile salts, bilirubin, and mucin (27–29). It has been shown that due to its high pH canine ductular bile, but not gallbladder bile, is supersaturated with calcium carbonate (30). Although we did not determine Ca^{2+} ions in the bile, the high total calcium in the hepatic bile of patients with muddy pigment stones suggests that the bile must be extremely supersaturated with calcium carbonate and might contribute to the formation of such stones often located intrahepatically (10). The calcium concentration of the gallbladder bile in average as four times higher than that in the hepatic bile (Table 3) and was essentially identical among the four groups of patients (Table 2). Such an increase in calcium concentration in the gallbladder bile is due to (a) concentration of bile acids that allows more Ca^{2+} to be bound to bile acid micelles (31), (b) absorption and neutralization of HCO_3^- by H^+ that lowers the pH from 7.4 to 7.0 and prevents precipitation of $CaCO_3$ (3, 32, 33), and (c) a Gibbs–Donnan effect induced by impermeable, negatively charged bile acid molecules that determines the free Ca^{2+} in the bile (31, 34). This is consistent with the fact that muddy pigment stones rarely occur in the gallbladder (10).

As compared with the hepatic bile, osmolality and chloride were unchanged and all inorganic cations were increased to various degrees in the gallbladder bile (Table 3). In average, bile acids were concentrated about 8-fold in the gallbladder, all electrolytes

TABLE 5. ACTIVITY OF VARIOUS ENZYMES IN HEPATIC AND GALLBLADDER BILES IN THE CONTROL GROUP WITHOUT STONES

Enzyme	Hepatic bile		Gallbladder bile	
	U/L	U/mmol BA	U/L	U/mmol BA
AST	38 ± 36*	3.4 ± 2.56	1012 ± 806	17 ± 18
ALT	3.8 ± 4.9	0.35 ± 0.61	98 ± 79	2.1 ± 2.3
GGT	382 ± 298	34 ± 30	1511 ± 1206	15 ± 7
LDH	53 ± 48	3.8 ± 2.6	1400 ± 1322	18 ± 22
LAP	173 ± 167	15 ± 13	782 ± 690	9.2 ± 4.9
ALP	317 ± 330	32 ± 29	822 ± 750	6.4 ± 3.1
CPK	8.6 ± 6.9	1.1 ± 1.5	404 ± 320	3.8 ± 4.2
Amylase	4.0 ± 8.9	0.34 ± 0.76	0.8 ± 1.2	0.08 ± 0.10
	(1827 ± 2547)†	(144 ± 233)	(11 ± 56)	(0.08 ± 0.10)
β-Glu	2.8 ± 2.5	180 ± 110	92 ± 63	422 ± 319

* Mean ± SD.
† Mean ± SD without exclusion of extreme values.

except magnesium, were concentrated to a lesser degree than the bile acids. The total electrolyte concentration in the gallbladder bile was higher than that in the hepatic bile, yet the gallbladder bile remained isosmotic to the hepatic bile, and thus also to the plasma. Some of the electrolytes must be sequestered in the bile and became osmotically inactive. The bile salts, cholesterol, and phospholipids form virtually osmotically inactive micelles in the bile (35). Since bile salts are anions, the inorganic cations, particularly calcium and magnesium, can be bound within the micelles and become osmotically inactive (36, 37). This might also explain the excess of inorganic cations over inorganic anions in the gallbladder bile.

As a consequence of biliary obstruction, particularly by gallstones, and subsequent hepatocellular damage, some of these enzymes could increase even to an extremely high level in the bile as show in Table 4. After exclusion of such extreme values, the enzyme activities showed only minor variation among the four groups of patients which might represent their basal or normal ranges (Table 5). Despite that the serum enzymes are derived from multiple sources, a comparison of their levels with that in the hepatic bile yields some important information. The low CPK in the bile as compared with the serum level indicates that CPK and most likely other serum enzymes too are normally not excreted directly from circulation to the bile. The fact that CPK could not be detected in 20% (22 of 108) of hepatic biles suggests that any presence of CPK in the bile represents an abnormal leakage from sinusoid through the paracellular space to the bile canaliculus. Amylase is another example. Forty percent (43 of 108) of the hepatic biles exhibited no detectable activity of amylase. When present in the bile the amylase activity was much higher than in the serum. Eighteen samples had extremely high values. The amylase in the hepatic bile is therefore mostly derived from the pancreas secondary to the obstruction of the orifice of the common channel and not from the circulation. In most instances, the obstruction was secondary to stone impaction. Apparently amylase rarely backflowed and entered the gallbladder because the activity in the gallbladder remained low despite extremely high activity in the common duct (Table 5). ALP, GGT, and LAP were present in much higher concentrations in the hepatic bile than in the serum. ALP and GGT are bound to both the sinusoidal and the canalicular membrane, whereas LAP bound only to the canalicular membrane (38–43). Their high concentrations in the bile canalicular membrane are linked to their high activities in the bile. AST exists in many organs which all contribute to the serum level, whereas ALT is found primarily in the cytosal and mitochondria in the liver and kidney (44–47). The fact that the AST level in the hepatic bile tended to be higher than in the

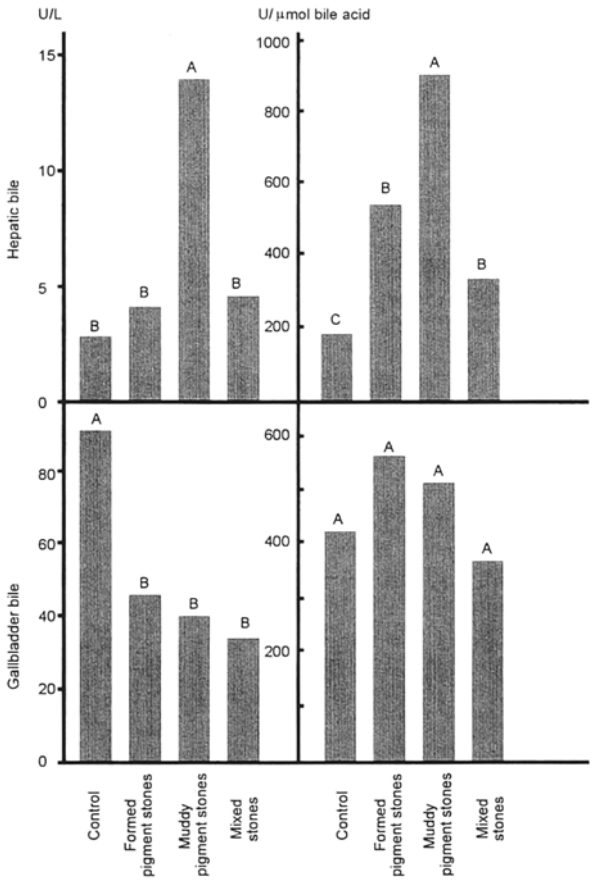

Fig 1. Endogenous β-glucuronidase activity in hepatic and gallbladder biles with or without normalization with bile acid concentration in the control and the patients with various types of gallstones. A, B, C: grouping by Duncan multiple-range test at 1% significance level.

TABLE 6. THE RATIO OF ENZYME ACTIVITY IN THE GALLBLADDER BILE TO THAT IN THE HEPATIC BILE AFTER NORMALIZATION WITH BILE ACID CONCENTRATION

	Gallbladder bile activity / Hepatic bile activity
AST	6.0 (3.3–11.9)*
ALT	5.9 (4.9–6.6)
GGT	0.37 (0.32–0.45)
LDH	3.3 (1.9–4.9)
LAP	0.60 (0.38–1.01)
ALP	0.26 (0.20–0.28)
CPK	5.1 (3.5–7.2)
Amylase	0.10 (0.01–0.23)
β-Glu	1.28 (0.57–2.34)

* Mean (range).

serum, whereas the ALT level tended to be lower suggests that relatively more AST than ALT is excreted into the bile canaliculus. LDH, another high molecular weight enzyme (MW 135,000), is distributed throughout but contained within the cytoplasm (44). Its serum level, contributed by many organs, is much higher than the level in the hepatic bile. Release of LDH to the plasma and bile is usually considered to reflect an increase in permeability of the plasma membrane to large molecules.

β-Glucuronidase, a lysosomal enzyme, is secreted from the hepatocyte into bile canaliculus by exocytosis of lysosome-derived vesicles (48–50). The lining epithelia of the biliary tract including gallbladder also contain lysosomes and are another possible source of biliary β-glucuronidase (51, 52). However, while the bile acids were, on average, concentrated 8-fold in the gallbladder, the β-glucuronidase activity in the gallbladder bile was only 1.28-fold that in the hepatic bile (Table 6). The high content of bile acids and bilirubin in the gallbladder bile should not affect the enzyme activity determination since they were removed before enzyme kinetic study (19). Furthermore, D-glucaro-1,4-lactone, a specific β-glucuronidase inhibitor, is virtually absent from the bile due to the high pH of the bile (53). Therefore, the main source of β-glucuronidase in the bile was the hepatocytes while the gallbladder contributed only to a small fraction of the total enzyme activity. The hepatic bile from the patients with intrahepatic and/or extrahepatic muddy pigment stones had the highest endogenous β-glucuronidase content in addition to bacterial enzyme (Figure 1). The excessive enzyme could come from injured hepatocytes and/or biliary mucosal cells and inflammatory cells. A detailed histological study with ultrastructural immunolabeling for β-glucuronidase should be able to distinguish the types of cells involved. A similar process but to a lesser degree might also be present in the formed pigment stone and the mixed stone groups because they had a higher β-glucuronidase activity in the hepatic bile than the control (Figure 1). Bacterial β-glucuronidase was detected in 0, 3, 53, and 12% of the hepatic biles from the control, formed pigment, muddy pigment, and mixed stone groups, respectively. The high detectability of bacterial enzyme in the muddy pigment stone group indicates that biliary tract infection with β-glucuronidase producing bacteria initiates the disease process with the human β-glucuronidase as the secondary responder.

When the enzyme activity was corrected for the bile acid content AST, ALT, and CPK were 5- to 6-fold concentrated in the gallbladder, and LDH 3-fold, but GGT, LAP, and ALP only 0.2- to 0.6-fold (Table 6). Since we determined the activity of the enzyme instead of its mass, the enzyme activity can be affected by many factors. The lost activity of GGT, LAP, and ALP might be the result of inactivation of these enzymes by denaturation of the enzyme, the presence of specific or nonspecific inhibitors, or interference with the assay procedure.

In summary, since the biliary electrolytes and enzymes were basically identical in patients with and without gallstones, it was unlikely that they played significant roles in the gallstone formation. The only exceptions were higher levels of calcium and magnesium and higher activity of β-glucuronidase in the hepatic bile in patients with muddy pigment stones. The high activity of β-glucuronidase might facilitate the hydrolysis of conjugated bilirubin and high content of calcium would favor the formation of calcium bilirubinate and its subsequent polymerization and precipitation. In fact, over 50% of such biles also contained a significant amount of bacterial β-glucuronidase. Even the hepatic bile from patients with formed black pigment stones and mixed stones had a β-glucuronidase activity higher than the control. Therefore, β-glucuronidase was the biliary enzyme which might contribute to stone formation.

ACKNOWLEDGMENTS

This work was supported in part by Research Grant 820412B002-342 from the National Science Council, Executive Yuen, Republic of China, and the Medical Research Fund from the Department of Veterans Affairs, USA.

REFERENCES

1. Wheeler HO: Water and electrolytes in bile. *In* Handbook of Physiology, Alimentary Canal Section, CF Code (ed). Baltimore, Williams & Wilkins, 1968, Vol 5, pp 2409–2431
2. O'Maille ERL, Richards TG, Short AH: Factors determining the maximal rate of organic anion secretion by the liver and further evidence on the hepatic site of action of the hormone secretin. J Physiol London 186:424–438, 1966
3. Anwer MS: Transheptic solute transport and bile formation. Adv Vet Sci Comp Med 37:1–29, 1993
4. Erlinger S, Dhumeaux D: Mechanisms and control of secretion of bile water and electrolytes. Gastroenterology 66:281–304, 1974
5. Ballatori N, Truong AT: Glutathione as a primary osmotic driving force in hepatic bile formation. Am J Physiol 263:G617–G624, 1992
6. Klaassen CD, Watkins JB III: Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacol Rev 36:1–67, 1984
7. Moser AJ, Abedin MZ, Abedin ZR, Rosyln JJ: Ca^{2+} calmod-

ulin regulates basal gallbladder absorption. Surgery 114:300–307, 1993
8. Reuben A: Biliary proteins. Hepatology 4:46S–50S, 1984
9. Godfrey PP, Warner MT, Coleman R: Enzymes and proteins in bile, variations in output in rat cannula bile during and after depletion of bile-salt pool. Biochem J 196:11–16, 1981
10. Ho KJ, Lin XZ, Yu SC, Chen JS, Wu CZ: Cholelithiasis in Taiwan: Gallstone characteristics, surgical incidence, bile lipid composition, and role of b-glucuronidase. Dig Dis Sci 40:1963–1973, 1995
11. Connerty HY, Briggs AS: Determination of serum calcium by means of orthocresolphthalein complexone. Am J Clin Pathol 45:290–296, 1966
12. Bakker AJ, Bosma H, Christen PJ: Influence of monoclonal immunoglobulins in three different methods for inorganic phosphorus. Ann Clin Biochem 27:227–231, 1990
13. Sky-Peck HH: A method for determination of magnesium in serum and urine. Clin Chem 10:391–398, 1964
14. Ono T, Kitaguchi K, Takehara M, Shiiba M, Hayami K: Serum-constituents analyses: Effects of duration and temperature of storage of clotted blood. Clin Chem 27:35–38, 1981
15. Rosalki SB: Enzyme tests in diseases of the liver and hepatobiliary tract. *In* The Principles and Practice of Diagnostic Enzymology, JH Wilkinson (ed), London, Edward Arnold, 1975, p 303
16. Thefeld W, Hoffmeister H, Busch EW, Koller PU, Vollmar J: Referenzwerte für die Bestimmangen der Transaminasen GOT und GPT sowie der Alkalischen Phosphatase im Serum mit optimierten Standardmethoden. Dtsch Med Wschr 99:343–351, 1974
17. Hohenwallner W, Stein W, Hafkenscheid JC, Kruse-Jarres JD, Kaiser C, Hubbuch H, Klein G: Reference ranges for alpha-amylase in serum and urine with 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-alpha-D-mattohepataoside as substrate. J Clin Chem Clin Biochem 27:97–101, 1989
18. Ho YC, Ho KJ: Differential quantitation of urinary b-glucuronidase of human and bacterial origin. J Urol 134:1227–1234, 1985
19. Ho YC, Ho KJ: Human b-glucuronidase: Measurement of its activity in the gallbladder bile devoid of intrinsic interference. Dig Dis Sci 33:435–442, 1988
20. Talalay P: Enzymic analysis of steroid hormones. Methods Biochem Anal 8:119–143, 1960
21. Duncan DB: Multiple range and multiple F tests. Biometrics 11:1–42, 1955
22. Inoue M, Kinne R, Tran T, Arias IM: Taurocholate transport by rat liver canalicular membrane vesicles: Evidence for the presence of an Na^+-independent transport system. J Clin Invest 73:659–663, 1984
23. Adachi Y, Kobayashi H, Kurumi Y, Shouji M, Kitano M, Yamamoto T: ATP-dependent taurocholate transport by rat liver canalicular membrane vesicles. Hepatology 14:655–659, 1991
24. Arias IM, Che M, Gatmaitan Z, Leveille C, Hishida T, Pierre MS: The biology of the bile canaliculus. Hepatology 17:318–329, 1993
25. Nishida T, Gatmaitan Z, Roy-Chowdhry J, Arias IM: Two distinct mechanisms for bilirubin glucuronide transport by rat bile canalicular membrane vesicles: Demonstration of defective ATP-dependent transport in rats (TR^-) with inherited conjugated hyperbilirubinemia. J Clin Invest 90:2130–2135, 1992
26. Ishikawa T, Müller M, Klünemann C, Schaub T, Keppler D: ATP-dependent primary active transport of cysteinyl leukotrienes across liver canalicular membrane: Role of the ATP-dependent transport system for glutathione S-conjugates. J Biol Chem 265:19279–19286, 1990
27. Moore EW: Biliary calcium and gallstone formation. Hepatology 12:206S–214S, 1990
28. Dawes LG, Nahrwold DL, Rege RV: Increased total and free ionized calcium in a canine model of pigment gallstones. Surgery 104:86–90, 1988
29. Shiffman ML, Sugerman HJ, Kellum JM, Moore EW: Calcium in human gallbladder bile. J Lab Clin Med 120:875–884, 1992
30. Rege RV, Moore EW: Pathogenesis of calcium-containing gallstones: Canine ductular bile, but no gallbladder bile, is supersaturated with calcium carbonate. J Clin Invest 77:21–26, 1986
31. Rege RV, Hahrwold DL, Moore EW: Absorption of biliary calcium from the canine gallbladder: Protection against the formation of calcium-containing gallstones. J Lab Clin Med 110:381–386, 1987
32. Rege RV, Moore EW: Evidence for H^+ secretion by the in vivo canine gallbladder. Gastroenterology 92:281–289, 1987
33. Reuss L: Ion transport across gallbladder epithelium. Physiol Rev 69:503–545, 1989
34. Dawes LG, Moore EW, Rege RV: Gibbs-Donnan equilibrium is the major determinant of free CA^{++} in canine bile. Gastroenterology 94:A534, 1988
35. Carey MC, Cahalane MJ: Enterohepatic circulation. *In* The Liver: Biology and Pathobiology, 2nd ed, IM Arias, WB Jakoby, H Popper, D Schachter, DA Shafritz (eds), New York, Raven Press, 1988, pp 573–616
36. Moor EW, Celic L, Ostrow JD: Interactions between ionized calcium and sodium taurocholate: Bile salts are important buffers for prevention of calcium-containing gallstones. Gastroenterology 83:1079–1089, 1982
37. Gleeson D, Murphy GM, Dowling RH: Calcium binding by bile acids: in vitro studies using a calcium ion electrode. J Lipid Res 31:781–791, 1990
38. Spies M, Hunziker W, Lodish HF, Semenza G: Molecular cell biology of brush border hydrolases: sucrase-isomaltase and r-glutamyltransferase. *In* Mammalian Ectoenzymes, J Kenny (ed), Amsterdam, Elsevier, 1987, pp 87–110
39. Lanca AJ, Israel Y: Histochemical demonstration of sinusoidal r-glutamyltransferase activity by substrate protection fixation: Comparative studies in rat and guinea pig liver. Hepatology 14:857–863, 1991
40. Alpeso DH, Eliakim R, DeSchryver-Kecskemeti K: Secretion of hepatic and intestinal alkaline phosphatases: Similarities and differences. Clin Chim Acta 186:211–224, 1989
41. Tanaka K, Omori K, Tashiro Y: Quantitative immunoferritin localization of leucine aminopeptidase on canine hepatocyte cell surface. J Histochem Cytochem 34:775–784, 1986
42. Low MG: Biochemistry of the glycosyl-phosphatidylinositol membrane protein anchors. Biochem J 244:1–13, 1987
43. Low MG, Prasad ARS: A phospholipase D specific for the phosphatidylinositol anchor of cell surface proteins is abundant in plasma. Proc Natl Acad Sci USA 85:980–984, 1988
44. Ishiguro M, Takio K, Suzuki M, Oyama R, Matsuzawa T, Titani K: Complete amino acid sequence of human liver cystolic alanine aminotransferase (GPT) determined by a combination of conventional and mass spectral methods. Biochemistry 30:10451–10457, 1991

45. Pavé-Preux M, Ferry N, Bouguet J, Hanoune J, Barouki R: Nucleotide sequence and glucocorticoid regulation of the mRNAs for the isoenzymes of rat aspartate aminotransferase. J Biol Chem 263:17459–17466, 1988
46. Barouki R, Pavé-Preux M, Bousquet-Lemercier B, Pol S, Bouquet J, Hanoune J: Regulation of cytosolic aspartate aminotransferase mRNA in the Fao rat hepatoma cell line by dexamethasone, insulin and cyclic AMP. Eur J Biochem 186:79–85, 1989
47. Pincus MR, Zimmerman HJ, Henry JB: Chapter 13, Clinical Enzymology. *In* Clinical Diagnosis and Management by Laboratory Methods, 18th ed, JB Henry, DA Nelson, RH Tomas, JA Washington, GA Threatte (eds), Philadelphia, W. B. Saunders, 1991, pp 250–284
48. Sewell RB, Dillon C, Grinpukel S, Yeomans ND, Smallwood RA: Pericanalicular location of hepatocyte liposomes and effects of fasting: A morphometric analysis. Hepatology 6:305–311, 1986
49. LaRusso NF, Fowler S: Coordinate secretion of acid hydrolases in rat bile: Hepatocyte exocytosis of lysosomal protein. J Clin Invest 64:948–954, 1979
50. LeSage GD, Robertson WE, Baumgart MA: Bile acid-dependent vesicular transport of lysosomal enzymes into bile in the rat. Gastroenterology 105:889–900, 1993
51. Simert G, Hammar E, Nansson JA, Hägerstrand I, Wang J: Enzyme histochemical studies in rabbit bile ducts with and withou bile flow. Acta Pathol Microbiol Scand Sect A 84:33–39, 1976
52. Kouroumalis E, Hopwood D, Ross PE, Miles G, Bouchier IAD: Gallbladder epithelial acid hydrolases in human cholecystitis. J Pathol 139:179–191, 1983
53. Ho YD, Ho KJ: Biliary D-glucaric acid: Its quantitation and preventive role in gallstone formation. Scand J Gastroenterol 25:631–640, 1990