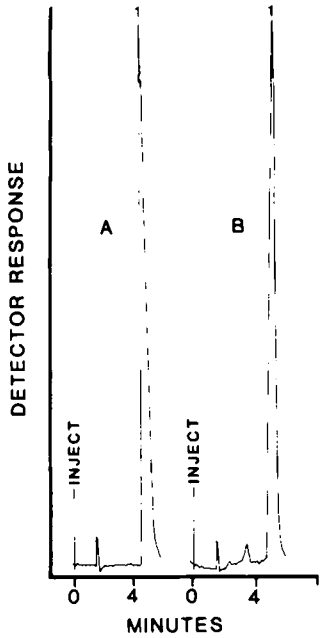

Figure 2—*Typical chromatograms at 245 nm. Peak 1 is from phenobarbital sodium (II). Key: (A) standard solution; (B) injection containing 130 mg/mL of II.*

RESULTS AND DISCUSSION

The assay results indicate (Table I) that the proposed HPLC method can be adopted for the quantitation of phenobarbital and phenobarbital sodium in pharmaceutical dosage forms. The method is accurate and precise. The percent relative standard deviations based on six injections were 0.9 and 0.7 at 245 and 230 nm (with internal standard), respectively. The internal standard was not used for assays at 245 nm due to poor absorption of mezlocillin at this wavelength. However, the results by both assay methods were similar (Table I). The concentrations *versus* peak heights were directly related in the 7- to 21-μg range at 245 nm. The ratio of peak heights (drug to internal standard) were directly related to drug concentrations at the 1.5- to 4.5-μg range at 230 nm. The method is ~1.7 times more sensitive at 230 than at 245 nm, as determined by using a standard solution (300 μg/mL in water) and a sensitivity of 0.1. Moreover, the method is at least two times more sensitive than the USP-NF method (3), as determined by UV spectrometry with the two mobile phases. The elixirs and injections can be simply diluted and assayed for phenobarbital and phenobarbital sodium. A simple preliminary extraction procedure is required to assay phenobarbital in tablets. There was no interference from excipients present in the tablets and the red colorant of the elixir (Table I).

The decomposed samples of phenobarbital sodium showed a new peak (peak 1, Fig. 1C) in the chromatogram, and the potency of the drug remaining intact was only 15.4% after ~30 min of boiling (see above). After ~4 min of boiling, there were two small peaks (side by side) in the chromatogram at about the same location as peak 1, Fig. 1C. The scheme of decomposition has been reported previously in the literature (4). It is interesting to point out that there were two additional peaks when the same lot of phenobarbital was assayed at 245 nm *versus* only one peak when assayed at 230 nm. It is perhaps due to the facts that (*a*) the concentration of excipients was 3.33 times higher (also considering AUFS of 0.2 at 245 nm and 0.1 at 230 nm) at 245 *versus* 230 nm and (*b*) there was poor or no absorption from one of the excipients at 230 nm. Furthermore, the same lot of phenobarbital sodium injection showed a small additional peak (Fig. 2B) when assayed at 245 nm *versus* almost none (Fig. 1B) when assayed at 230 nm.

REFERENCES

(1) W. F. Schmidt and L. J. Pennington, *J. Pharm. Sci.*, **71**, 954 (1982).

(2) R. Gloor and E. L. Johnson, *J. Chromatogr. Sci.*, **15**, 413 (1977).

(3) Third Supplement to USP XX and to NF XV, The U.S. Pharmacopeial Convention, Rockville, Md., 1982, pp. 216–217.

(4) K. A. Connors, G. L. Amidon, and L. Kennon, "Chemical Stability of Pharmaceuticals—A Handbook for Pharmacists," Wiley, New York, N.Y., 1979, p. 280.

Mesophase Formation During Cholesterol Gallstone Dissolution in Human Bile: Effect of Bile Acid Composition

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Abstract □ Duodenal bile obtained from patients with gallstones who were acutely infused with chenodeoxycholic acid, ursodeoxycholic acid, or cholic acid were examined for the propensity toward the formation of a liquid crystalline mesomorphic phase when cholesterol gallstones were incubated in these bile acids. Bile taken from patients infused with ursodeoxycholic acid was found to be enriched in ursodeoxycholic acid; mesophase formation was detected in these samples but not in bile from patients infused with chenodeoxycholic acid or cholic acid.

Keyphrases □ Cholesterol—gallstone dissolution in human bile, effect of bile acid composition □ Gallstone dissolution—human bile, effect of bile acid composition □ Bile acids—mesophase formation during cholesterol gallstone dissolution

Clinical investigations have shown that ursodeoxycholic acid is as effective or more effective than chenodeoxycholic acid in gallstone dissolution. From the physicochemical standpoint, these results are interesting, because ursodeoxycholic acid (1) and its conjugates are generally not as effective as chenodeoxycholic acid and its conjugates in solubilizing cholesterol in the bile acid or the bile acid–lecithin solution (2). We have recently suggested (2, 3) that during *in vitro* dissolution of cholesterol monohydrate in the form of powder, compressed disks, single crystals, or in gallstones, a transition from the micellar phase to the liquid crystalline phase occurs when the dissolution media contain lecithin and predominantly ursodeoxycholic acid conjugates, but not chenodeoxycholic acid or cholic acid conjugates.

It was shown that mesophase formation and subsequent dispersion may contribute to the total dissolution/disintegration of cholesterol in the solid phase. To determine whether mesophase formation would occur during the *in vitro* disso-

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Table I—Biliary Lipid Composition During Infusion with Chenodeoxycholic, Ursodeoxycholic, and Cholic Acid [a]

Bile Acid Infused	Bile Acid, mol %	Phospholipids, mol %	Cholesterol, mol %	Phospholipids-Cholesterol Molar Ratio
Chenodeoxycholic acid	87.2	8.3	4.5	1.84
Ursodeoxycholic acid	86.2	11.4	2.4	4.75
Cholic acid	89.2	6.7	4.1	1.63

[a] Averages of two samples are presented.

lution of cholesterol gallstones in human bile, we studied the mesophase formation tendency with gallstones in human bile samples which were selectively enriched with conjugates of ursodeoxycholic acid, chenodeoxycholic acid, or cholic acid by prior infusion of these bile acids into the jejunum of patients with gallstones.

EXPERIMENTAL SECTION

Materials—A triple-lumen orojejunal tube was placed into the patient with gallstones fluoroscopically[1], and synthetically prepared conjugates of chenodeoxycholic, ursodeoxycholic, or cholic acids were infused into the jejunum at 2 mmol/h for 8 h (3). Bile was aspirated from the duodenal port, beginning at 8 h, at which time replacement of the bile acid pool by the infused bile acid was 69–90% complete. Bile was collected in 60-min pools for the subsequent 28 h and frozen. Each bile acid was infused in random order in three consecutive 72-h periods. Before the experiments, the bile samples were freeze-dried, weighed, and reconstituted to predetermined concentrations by equilibrating in water at 37°C for 3 d. After inactivating the enzymes by heating at 60°C for 30 min, the reconstituted bile was filtered through a 0.45-μm filter[2]. The biliary lipid compositions were determined by enzymatic methods[3] (4, 5).

Gallstones were obtained at routine cholecystectomy from patients[1] who had not taken a gallstone-dissolving bile acid. Gallstones were washed free of bile and mucus and stored for short periods in normal saline solutions under nitrogen at 4°C in the dark.

Mesophase Detection—Two gallstones were placed in 2.5 mL of the filtered bile in a test tube. The test tube was flushed with nitrogen, capped, and then shaken with a wrist-action shaker in a water bath at 37°C. At predetermined intervals, samples of the dissolution media were taken and examined immediately under a polarizing microscope. The observation of birefringent spherules was interpreted to mean that the formation of a liquid crystalline phase had occurred.

RESULTS AND DISCUSSION

The effect of infused ursodeoxycholic acid on hepatic secretion of biliary lipids is shown in Table I. The better suppression of hepatic cholesterol output during ursodeoxycholic acid infusion than under the other experimental conditions has also been reported in healthy volunteers either acutely infused with (6, 7) or chronic enterally administered this bile acid and in patients with radiolucent gallstones (8). It is interesting to note that acute administration of ursodeoxycholic acid increased the amount of phospholipids secreted per mole of cholesterol (6). Table II indicates that during infusion with ursodeoxycholic acid, this bile acid increased to 73% of the bile, whereas chenodeoxycholic acid decreased to 11.5% of the total biliary bile acids. An even higher (>80%) enrichment of ursodeoxycholic acid in bile has recently been reported by others (6) in patients during the postreplacement phase of ursodeoxycholic acid studies.

Table III presents the results of the mesophase formation studies performed with the polarizing microscope. A transition from the micellar phase to the liquid crystalline phase was detected in the ursodeoxycholic acid-enriched bile samples but not in the chenodeoxycholic acids or cholic acid-enriched bile (Table III).

Table II—Bile Acid Composition in Reconstituted Duodenal Bile

Bile Acid Infused	Molar % Chenodeoxycholic Acid	Ursodeoxycholic Acid	Cholic Acid	Deoxycholic Acid	Lithocholic Acid
Chenodeoxycholic acid	51.1	37.4	10.5	0.6	0.4
Ursodeoxycholic acid	11.5	73.1	12.0	3.0	0.4
Cholic acid	1.3	—	98.2	0.5	—

Table III—Mesophase Formation in Reconstituted Duodenal Bile

Bile Acid Infused	Bile Acid, mM	Phospholipid, mM	Cholesterol, mM	Time Mesophase Observed
Chenodeoxycholic acid				
Sample 1	87.2	8.1	4.3	—[a]
Sample 2	142.5	13.9	7.6	—
Ursodeoxycholic acid				
Sample 3	81.3	13.2	2.1	<10 d
Sample 4	123.6	12.7	3.9	<2 d
Cholic acid				
Sample 5	52.6	4.2	2.1	—
Sample 6	90.4	6.4	4.6	—

[a] Not observed.

Although these bile samples were obtained from patients with acute infusion of bile acids and although ursodeoxycholic acid concentrations in these bile samples appear to be higher than those taken from patients taking ursodeoxycholic acid orally (8), these results provide the first insights regarding the possibility of mesophase formation during ursodeoxycholic acid administration *in vivo* in patients undergoing ursodeoxycholic acid therapy for gallstone dissolution. Mesophase formation is believed (3) to be the consequence of thermodynamics favoring the formation of a lecithin-cholesterol-rich mesophase from cholesterol monohydrate in the presence of aqueous bile acid–lecithin solutions when the bile acid is an ursodeoxycholic acid conjugate. The subsequent dispersion of the mesophase may then contribute to the total dissolution/disintegration of cholesterol in the solid phase (2, 3), and this process may effect mass transfer significantly beyond the micellar phase saturation limit. Thus, this finding that ursodeoxycholic acid conjugates, but not chenodeoxycholate conjugates, induced the formation of mesophases during the gallstone dissolution process in the conjugated ursodeoxycholic acid-enriched bile may relate to the equal or superior clinical efficacy of ursodeoxycholic acid compared with chenodeoxycholic acid.

REFERENCES

(1) H. Igimi and M. C. Carey, *J. Lipid Res.*, **22**, 254 (1981).

(2) O. I. Corrigan, C. C. Su, W. I. Higuchi, and A. F. Hofmann, *J. Pharm. Sci.*, **69**, 869 (1980).

(3) C. C. Su, J. Y. Park, W. I. Higuchi, M. H. Alkan, O. I. Corrigan, A. F. Hofmann, and R. G. Danzinger, *J. Pharm. Sci.*, **70**, 713 (1981).

(4) I. T. Gilmore, K. Stokes, A. F. Hofmann, D. Gurantz, and D. Lorenzo, *Gastroenterology*, **79**, 1023 (1980).

(5) H. Dam, I. Kruse, I. Prange, H. E. Kallehauge, H. J. Fenger, and M. Krogh Jensen, *Z. Ernaehrungswiss.*, **10**, 160 (1971).

(6) C. Sama, N. F. Larusso, V. Lopez del Pino, and J. L. Thistle, *Gastroenterology*, **82**, 515 (1982).

(7) T. Schersten and L. Lindblad, in "Biological Effects of Bile Acids," G. Paumgartner, A. Stiehl, and W. Gerok, Eds., MTP Press, Lancaster, England, 1978, pp. 53–60.

(8) K. von Bergmann, M. Gutsfeld, K. Schulze-Hagen, and G. von Unruh, in "Biological Effects of Bile Acids," G. Paumgartner, A. Stiehl, and W. Gerok, Eds., MTP Press, Lancaster, England, 1978, pp. 61–66.

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[1] Dr. A. F. Hofmann, University of California, San Diego, Calif.; written informed consent and institutional human subjects committee approval were obtained.

[2] Millipore Corp., Bedford, Mass.

[3] Cholesterol Reagent Set; Bio-Dynamics/bmc, Indianapolis, Ind; Sterognost-3 Set; Accurate Chemical & Scientific Corp., Hicksville, N.Y.; PL Kit-K; Nippon Shouji Kaisha Ltd., Osaka, Japan.