Digoxin Degradation in Acidic Dissolution Medium

TAKASHI SONOBE ˣ, **SHUNJI HASUMI, TAKASHI YOSHINO, YOICHI KOBAYASHI, HIROITSU KAWATA, and TSUNEJI NAGAI** *

Received January 8, 1979, from the *Institute of Research and Development, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8 Azusawa, Itabashi-ku, Tokyo 174, Japan.* Accepted for publication October 31, 1979. *Present address: Hoshi Institute of Pharmaceutical Sciences, Hoshi College of Pharmacy, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan.

Abstract □ The release of digoxin and its simultaneous conversion to digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin in a USP dissolution test medium were followed by high-pressure liquid chromatography. Two products, Tablets A and B, were manufactured by solvent deposition and simple blending methods, respectively. Tablet A released digoxin faster than Tablet B in distilled water and in artificial intestinal juice, and no decomposition was observed. In the USP dissolution test medium, the rate of hydrolysis to digoxigenin bisdigitoxoside was almost equal to that of hydrolysis to digoxigenin monodigitoxoside, and a comparatively large formation rate of digoxigenin was observed. Concentrations of digoxin and its decomposition products were described by differential equations that included dissolution rates of digoxin (rapidly dissolving digoxin and digoxin crystals) and an apparent hydrolysis rate. In the earlier stage of dissolution, hydrolysis was rate determining; in the later stage, dissolution became the rate-determining step for overall digoxin degradation. To suppress digoxin hydrolysis in the USP dissolution test medium, a developmental formulation study was performed. The incorporation of magnesium oxide and magnesium hydroxide–aluminum hydroxide in the tablet formulations inhibited digoxin hydrolysis by 15.3 and 14.5%, respectively, after dissolution for 30 min without serious delay of drug release.

Keyphrases □ Digoxin—dissolution and degradation in acidic solution, high-pressure liquid chromatography, kinetic study □ Dissolution—digoxin tablets, biphasic dissolution □ Stability—digoxin in acidic solution, high-pressure liquid chromatography, kinetic study, incorporation of antacid □ Antacids—suppression of digoxin degradation

The bioavailability of digoxin preparations was reported to vary significantly among brands and batches (1–5). Many studies revealed the correlation between the dissolution rate and the bioavailability of digoxin, but little attempt was made to separate digoxin from its degraded glycosides (6–8). Digoxin reportedly was hydrolyzed in acidic media (9), and the therapeutic efficacy may have decreased as a result (10). Although the absorption kinetics of digoxin, digoxigenin bisdigitoxoside, and digoxigenin monodigitoxoside were in the same range, the blood concentrations of the latter two substances were five to six times lower than that of digoxin (11). The cardioactivity of digoxigenin was one-tenth of that of digoxin, but the other compounds possessed approximately the same activity (12).

It was of interest to study the dissolution and simultaneous hydrolysis of digoxin *in vitro* since they may be potential causes of the variation of bioavailability and clinical efficacy in different digoxin formulations. To investigate digoxin hydrolysis, a quantitative assay that distinguishes between digoxin and its decomposition products is required. All of the current assays (13–15) fail to determine digoxin and its decomposition products individually without additional separations, except for a high-performance liquid chromatographic method (16).

The purposes of the present investigation were to use high-pressure liquid chromatography (HPLC) to study the hydrolysis of digoxin released from tablets during dissolution and to establish a pharmaceutical method of preventing digoxin hydrolysis in an acidic medium.

EXPERIMENTAL

Materials—Digoxin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin were used as received[1]. The following materials were USP grade: lactose, cornstarch, magnesium hydroxide–aluminum hydroxide, and magnesium oxide. Synthetic aluminum silicate was JP grade. The other reagents were reagent grade unless otherwise stated.

To prepare Tablet A, a 5% chloroform–ethanol solution (2:1 v/v) of digoxin was deposited on lactose–cornstarch (7:3 w/w) diluent and granulated with 10% cornstarch paste to make ~0.2% digoxin granules. The granules were compressed to tablets of 7 mm in diameter and 120 mg in weight by a single-punch tableting machine[2] (17).

To prepare Tablet B, digoxin crystals were blended with lactose–cornstarch (7:3 w/w) diluent to give a 10% digoxin powder. The powder was diluted repeatedly in the same manner to give a final digoxin content of ~0.2%. The resultant digoxin–diluent mixture was granulated and compressed to give tablets of the same size as Tablet A following the described procedure (17).

Tablets A and B each contained 0.25 mg of digoxin. A digoxin powder mixture also was prepared containing antacids to study the effect of antacids in suppressing digoxin hydrolysis in acidic media. This mixture was prepared by dissolving 0.25 g of digoxin crystals in 50 ml of ethanol–chloroform (1:2 v/v) solution and incorporating the resulting solution in 20 g of magnesium hydroxide–aluminum hydroxide, synthetic aluminum silicate, or magnesium oxide. After mixing, the solvents were evaporated by heating on a thermostated water bath at 40° for 4 hr. The powder mixture was blended with lactose–cornstarch (7:3 w/w) diluent and granulated with 10% cornstarch paste, and the mixture was dried and passed through a 32-mesh screen. The digoxin content of 120 mg of the powders was 0.25 mg.

Analyses of Digoxin and Glycosides—Spectrofluorometry was performed to determine the total amount of digoxin and glycosides. For the separate determination of digoxin and glycosides, an HPLC method was developed.

Spectrofluorometry—The digoxin concentration in solution was determined with a spectrofluorometer[3] following the assay described for the dissolution testing of digoxin tablets in USP XIX (13).

HPLC—The separation conditions developed by Castle (16) were modified for the present experiments. A high-pressure liquid chromatograph[4] was used. The eluent was monitored spectrophotometrically at 220 nm. The separation utilized a column[5] (4 mm × 15 cm) operating at 1.5 ml/min with 25% acetonitrile solution as the mobile phase.

The retention volumes for digoxin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin were 12.1, 7.2, 4.5, and 3.8 ml, respectively. The practical detection limit for digoxin was 50 ng. The resolution between the closest peaks of digoxigenin and digoxigenin monodigitoxoside was maintained at >1.3.

For the quantitation of digoxin and glycosides, aqueous standard solutions were prepared by dissolving accurately weighed quantities of digoxin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin and treating these solutions in the same manner as the sample solution. The amounts of the components were determined by comparing the peak heights with those of the standard solutions.

Dissolution Study—The dissolution tests for Tablets A and B were performed using a rotating-basket apparatus[6] described in USP XIX. Six tablets were used to determine the dissolution rate at 37° in 250 ml of distilled water, USP dissolution test medium (0.6% HCl), and artificial

[1] Boehringer, Mannheim GMBH, West Germany.
[2] Kimura KM-2, Okada Seiko Co., Tokyo, Japan.
[3] Hitachi MPF-4, Hitachi Ltd., Tokyo, Japan.
[4] The chromatographic system consisted of a 6000 A pump and a U6K universal injector (Waters Associates, Milford, Mass.) coupled with a SF-770 detector (Schoeffel Instrument Corp., Westwood, N.J.).
[5] LiChrosorb RP-18 (5 μm), Merck, Darmstadt, West Germany.
[6] Toyama Sangyo Co., Osaka, Japan.

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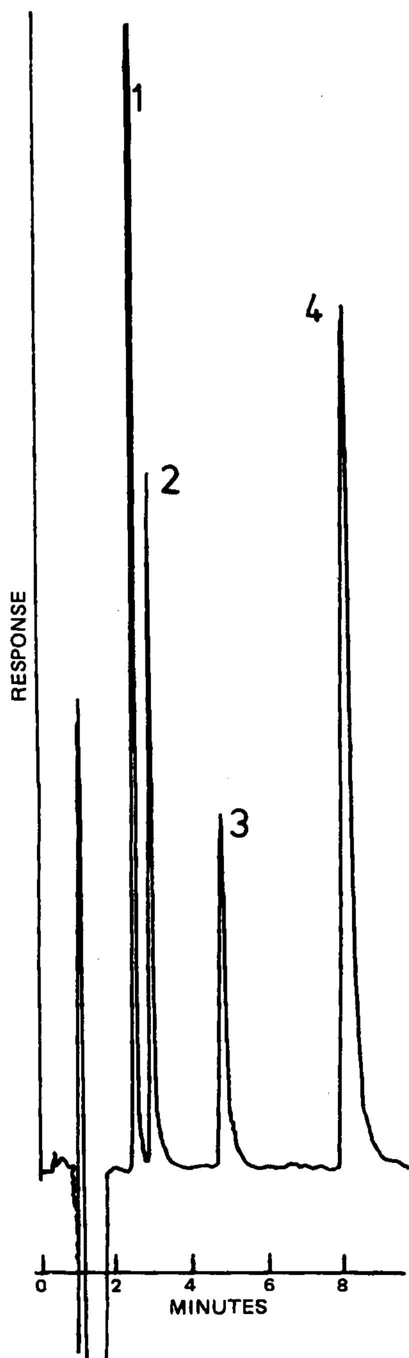

Figure 1—*Typical high-pressure liquid chromatogram of digoxin and its hydrolyzed products. Key: 1, digoxigenin (0.50 μg); 2, digoxigenin monodigitoxoside (0.50 μg); 3, digoxigenin bisdigitoxoside (0.50 μg); and 4, digoxin (2.5 μg).*

intestinal juice[7] at a basket rotation speed of 100 rpm. At the specified time, 5 ml of the test medium was withdrawn and filtered immediately through a membrane filter[8] (pore size of 0.22 μm).

With the USP dissolution test medium, 1.5 ml of Sørensen borate buffer (0.2 *M*, pH 10.0) was added to prevent further digoxin hydrolysis. Five milliliters of the dissolution medium was added at each sampling time to maintain a constant volume. Sample solutions were analyzed by spectrofluorometry and HPLC after appropriate dilution with distilled water.

[7] JP IX second disintegration test fluid: dissolve 35.8 g of dibasic sodium phosphate in 6 ml of 10% HCl and distilled water to make 1000 ml. The solution pH is ~7.5.

[8] Millipore, Japan Millipore Ltd., Tokyo, Japan.

RESULTS AND DISCUSSION

Dissolution—The individual determination of digoxin, digoxigenin bisdigitoxoside, digoxigenin monodigitoxoside, and digoxigenin was accomplished satisfactorily by HPLC as shown in Fig. 1. The total amount of digoxin detected by the conventional spectrofluorometric method was consistent with the HPLC results.

Digoxin instability in gastric juice was confirmed by analysis of digoxin and its degraded materials and was suggested as a possible cause of decreased oral efficacy (10). Degradation steps or pathways of digoxin in acidic media have been considered (18). The glycoside bond in the monoglycoside was suggested as being more resistant to acid hydrolysis than the bisglycoside and digoxin (9). Digoxin decomposition is closely related to reduced cardiac action. The rate constant of digoxigenin appearance might be clinically significant because of the lowered pharmacological action.

Figure 2 shows the dissolution of digoxin from Tablets A and B in distilled water and in artificial intestinal juice. Tablet A released digoxin faster than did Tablet B; their $t_{1/2}$ values were 1 and >60 min, respectively, which suggested that Tablet B would not meet the USP XIX requirement. This difference in the dissolution rates resulted mainly from the production procedure, because the same lot of digoxin crystals was incorporated in both digoxin tablets. For Tablet A, digoxin was deposited on diluents after complete dissolution in ethanol–chloroform, which might have resulted in easier dissolution of digoxin crystals. This deposit might be the amorphous layer, the existence of which was demonstrated by ball milling the digoxin crystals (19). No digoxin decomposition was observed in distilled water and in artificial intestinal juice; earlier work showed that digoxin mainly undergoes degradation at acidic pH (9, 10).

The amount of digoxin that dissolved from Tablet A in the USP XIX dissolution medium increased remarkably in a short time. Digoxin release reached a peak in ~6 min, and this release was followed by a gradual decrease that was related to the increase of decomposed digoxin. The digoxigenin bisdigitoxoside concentration reached a plateau in 10 min, while the digoxigenin monodigitoxoside concentration increased linearly with the time. These findings suggest that the degradation rate of digoxin to digoxigenin bisdigitoxoside is almost equal to the degradation rate of digoxin to digoxigenin monodigitoxoside and that a comparatively high formation rate of digoxigenin exists. In a previous study (20) with a conventional solvent extraction method, 75% of the dissolved digoxin was degraded 60 min after the initiation of the dissolution test in the USP XIX dissolution test medium; that finding supports results obtained in this study.

After the disintegration of digoxin tablets, the digoxin dissolution is assumed to consist of two stages; the dissolution of digoxin into the medium from crystals and the simultaneous hydrolysis of digoxin by acid. It also is assumed that a freely soluble amorphous layer exists in crystals (21) and that the hydrolysis of digoxin at the surface of the digoxin solid is negligibly small compared to that in solution.

With these assumptions, the following differential equations are obtained for the description of the dissolution and degradation:

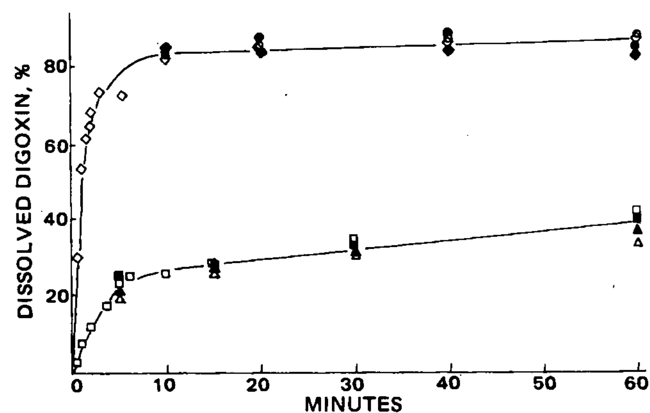

Figure 2—*Dissolution of digoxin tablets in distilled water or in artificial intestinal juice at 37°. Key: ♦, fluorometry, Tablet A in distilled water; ◇, HPLC, Tablet A in distilled water; ■, fluorometry, Tablet B in distilled water; □, HPLC, Tablet B in distilled water; ●, fluorometry, Tablet A in artificial intestinal juice; ○, HPLC, Tablet A in artificial intestinal juice; ▲, fluorometry, Tablet B in artificial intestinal juice; and △, HPLC, Tablet B in artificial intestinal juice.*

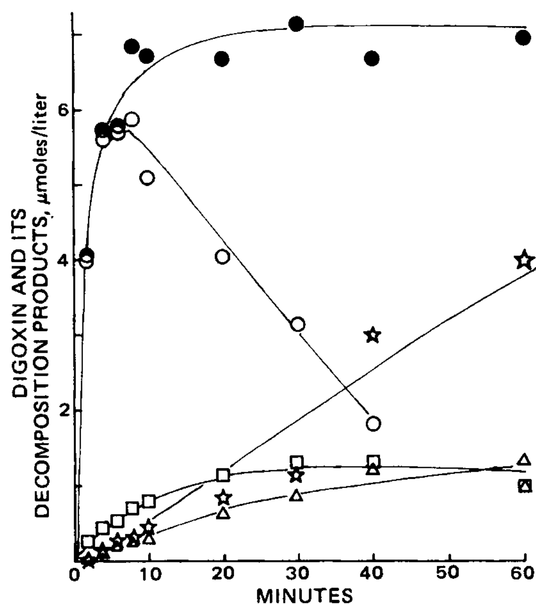

Figure 3—*Dissolution and degradation of digoxin Tablet A in the USP XIX dissolution test medium at 37°. Key:* ●, *total amount dissolved;* ○, *digoxin;* ☆, *digoxigenin;* △, *digoxigenin monodigitoxoside; and* □, *digoxigenin bisdigitoxoside.*

$$dA/dt = -K_{diss}A \qquad \text{(Eq. 1)}$$

$$dB/dt = K_{diss}A - K_{degr}B \qquad \text{(Eq. 2)}$$

$$dC/dt = K_{degr}B \qquad \text{(Eq. 3)}$$

$$A + B + C = A_0 \qquad \text{(Eq. 4)}$$

where $A = A_I + A_{II}$, $B = B_I + B_{II}$, $C = C_I + C_{II}$, and $A_0 = A_{0I} + A_{0II}$, in which A, B, C, and A_0 are the amounts of solid digoxin, digoxin in solution, digoxin degradation products (total amount of digoxigenin monodigitoxoside, digoxigenin bisdigitoxoside, and digoxigenin), and initial digoxin, respectively. The subscripts I and II denote the amorphous digoxin and digoxin crystals, respectively.

When $t = 0$, $A = A_0$ and $B = C = 0$. When t = infinity, $A = B = 0$ and $C = A_0$. Under these conditions, Eqs. 1–4 were integrated to give the following solutions:

$$A = A_0 \exp(-K_{diss}t) \qquad \text{(Eq. 5)}$$

$$B = A_0 \left(\frac{K_{diss}}{K_{degr} - K_{diss}}\right) [\exp(-K_{diss}t) - \exp(-K_{degr}t)] \qquad \text{(Eq. 6)}$$

$$C = A_0 \left[1 - \frac{K_{degr}\exp(-K_{diss}t) - K_{diss}\exp(-K_{degr}t)}{K_{degr} - K_{diss}}\right] \qquad \text{(Eq. 7)}$$

For both solids I and II, the same solutions are given. Therefore, the residual digoxin dissolved in the solution could be described by the following equation:

$$B = A_{0I}\left(\frac{K_{dissI}}{K_{degr} - K_{dissI}}\right)[\exp(-K_{dissI}t) - \exp(-K_{degr}t)] + A_{0II}\left(\frac{K_{dissII}}{K_{degr} - K_{dissII}}\right)[\exp(-K_{dissII}t) - \exp(-K_{degr}t)] \qquad \text{(Eq. 8)}$$

For Tablets A and B, the dissolution data were fitted to Eq. 8 by the least-squares method[9]. Figures 3 and 4 show the data points for the release of digoxin from Tablets A and B in the USP XIX dissolution test medium. Table I summarizes the rate constants calculated by the iterative least-squares method. The K_{degr} value is in good agreement with that determined in a hydrolysis study of digoxin in solution (20).

Tablet A should have essentially the same dissolution rate constants, K_{dissI} and K_{dissII}, as those of Tablet B. However, in this study, these kinetic constants were expressed as apparent constants which included

Table I—Values of K_{diss} and K_{degr} of Digoxin Obtained from Dissolution Tests of Tablet A and Tablet B

Parameter	Tablet A	Tablet B
K_{dissI}, min^{-1}	1.23	0.269
K_{dissII}, min^{-1}	0.00995	0.00429
K_{degr}, min^{-1}	0.0367 (0.0216)[a]	

[a] From Ref. 20.

effective surface area. Therefore, the differences of the diffusion rates indicate the existence of a more soluble layer and smaller digoxin crystals in Tablet A. The degradation rate constant was smaller than K_{dissI} but was larger than K_{dissII} in both Tablets A and B. This result indicates that degradation was rate determining in the earlier dissolution stage while dissolution became the rate-determining step in the later stage.

Suppression of Digoxin Hydrolysis—Digoxin is adsorbed by some antacids in aqueous solution (22). Moreover, when a large amount of an antacid is incorporated into a drug, the bioavailability of the drug may be reduced considerably (23). However, when specific antacids that have a large adsorption capability and dissolve comparatively quickly in acidic solution are selected, the stability of digoxin preparations can be enhanced without serious delay of drug release.

In this study, digoxin powders containing magnesium hydroxide–aluminum hydroxide, synthetic aluminum silicate, or magnesium oxide were placed in the dissolution apparatus in two positions, outside and inside the basket, to study the influence of granule dispersion on hydrolysis. The release of digoxin and hydrolyzed glycosides from digoxin powders in the USP XIX dissolution test medium was observed at different basket rotation speeds (Table II).

When the sample was placed inside the basket at a rotation speed of 50 rpm, magnesium oxide and magnesium hydroxide–aluminum hydroxide prevented the degradation of digoxin in the dissolution medium most, although the latter compound suppressed the release of digoxin. Aluminum silicate released digoxin more easily than did the other antacids but showed little antidegradation effect. When powders were placed outside the basket at a rotation speed of 100 rpm, synthetic aluminum silicate showed an inhibitory effect that probably was due to a reduced digoxin release rate because it released only 44.2% of the total amount of digoxin; magnesium hydroxide–aluminum hydroxide released 98.3% of the digoxin in 30 min. When samples were placed inside the basket at a rotation speed of 100 rpm, magnesium oxide and magnesium hydroxide–aluminum hydroxide released 100% of the digoxin in 30 min and still exerted a degradation inhibitory effect.

The dissolution rate was influenced considerably by the stirring conditions and the sample position in the dissolution apparatus. However, all samples showed reduced decomposition compared to that of the ref-

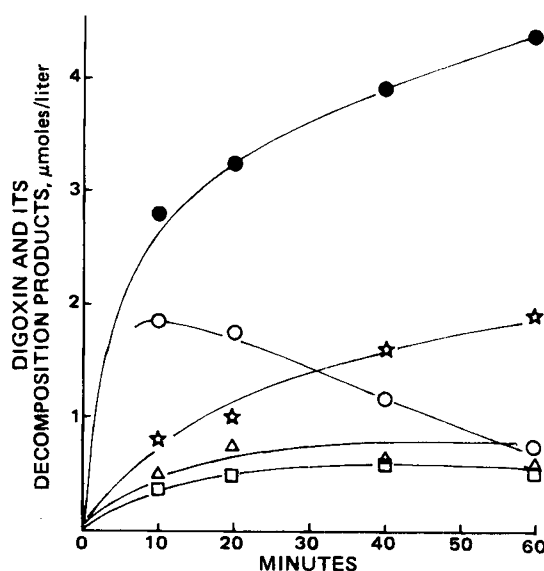

Figure 4—*Dissolution and degradation of digoxin Tablet B in the USP XIX dissolution test medium at 37°. Key:* ●, *total amount dissolved;* ○, *digoxin;* ☆, *digoxigenin;* △, *digoxigenin monodigitoxoside; and* □, *digoxigenin bisdigitoxoside.*

[9] Calculations were made by a digital computer, IBM S/370 model 158, with the NONLIN FORTRAN program developed by C. M. Metzler, G. L. Elfring, and A. J. McEwen, The Upjohn Co., Kalamazoo, Mich.

Table II—Effect of Antacids on Digoxin Hydrolysis in the USP XIX Dissolution Test Medium

Basket Rotation Speed, Sample Location	Antacid[a]	Total Amount Dissolved, %			Residual Digoxin in Solution, %		
		10 min	20 min	30 min	10 min	20 min	30 min
100 rpm, outside the basket	A	59.5	87.8	98.3	84.3	76.1	63.4
	B	30.0	39.8	44.2	78.3	65.7	53.6
	Reference	96.4	99.3	100	77.3	59.2	45.7
50 rpm, inside the basket	A	29.3	42.8	59.4	84.5	66.1	56.7
	B	37.1	55.4	73.7	70.4	59.2	48.4
	C	30.5	46.3	66.3	77.5	60.5	57.5
	Reference	56.4	60.4	69.4	76.4	55.2	42.2
100 rpm, inside the basket	A	94.8	99.7	100	75.4	53.7	39.3
	C	94.4	89.5	100	75.0	55.8	45.4
	Reference	94.7	94.0	100	72.5	47.4	30.5

[a] A = magnesium hydroxide-aluminum hydroxide, B = synthetic aluminum silicate, and C = magnesium oxide.

erence sample, which contained no antacid. Throughout the dissolution test, the dissolution medium pH was constant (1.3–1.4), and no significant influence of antiacidic agents on the medium pH was observed. This finding suggests that suppressed degradation of digoxin in the acidic medium by the incorporation of specific antacids is due to the antiacidic effect of those substances at the local dissolution site but is not due to an overall change in pH of the dissolution medium.

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Adsorption of Lecithin by Cholesterol

ANNIE HOELGAARD *x and SVEN FRØKJAER ‡

Received September 17, 1979, from the *Department of Pharmaceutics, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark, and the ‡NOVO Research Institute, Novo Allé, DK-2880 Bagsvaerd, Denmark. Accepted for publication November 2, 1979.

Abstract □ Egg lecithin was adsorbed significantly by cholesterol monohydrate crystals. Adsorption data obtained at initial concentrations of <1.1 m*M* lecithin fitted the Langmuir equation. The calculated adsorption capacity suggested formation of a lecithin bilayer or a mixed bilayer of lecithin and cholesterol. The amount of lecithin adsorbed was highly dependent on the cholate concentration in the incubation medium. Minimal adsorption was observed at ~5 m*M* cholate. The presence of quaternary ammonium salts and dioctyl sodium sulfosuccinate caused desorption. The finding of an adsorptive layer supported the existence of an interfacial barrier that controls cholesterol dissolution.

Keyphrases □ Adsorption—formation of adsorptive lecithin layer on cholesterol, effect of detergents □ Lecithin—adsorption by cholesterol, effect of total bile acid concentration, lecithin concentration and type of bile acid □ Cholesterol—effect of lecithin adsorption on cholesterol dissolution, interfacial barrier formation

The main constituent of most gallstones is cholesterol monohydrate, which is insoluble in water. The bile containing bile acids and phospholipid can dissolve some cholesterol in mixed micelles. Recent investigations (1–4) established that the *in vitro* dissolution of both human cholesterol gallstones and cholesterol monohydrate crystals in bile salt–lecithin media and human bile is controlled largely by an interfacial barrier. The cholesterol mono-

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