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.

REFERENCES

(1) J. Lindenbaum, M. H. Mellow, M. O. Blackstone, and V. P. Butler, *N. Engl. J. Med.*, **285**, 1344 (1971).

(2) V. Manninen, J. Melin, and G. Hartel, *Lancet*, **2**, 934 (1971).

(3) T. R. D. Shaw, M. R. Howard, and J. Harmer, *ibid.*, **2**, 303 (1972).

(4) C. Ampolsuk, J. V. Mauro, A. A. Nyuis, N. Shah, and C. I. Jarowski, *J. Pharm. Sci.*, **63**, 117 (1974).

(5) P. R. Klink, R. I. Poust, J. L. Colaizzi, and R. H. McDonald, Jr., *ibid.*, **63**, 1231 (1974).

(6) B. F. Johnson and S. Lader, *Br. J. Clin. Pharmacol.*, **1**, 329 (1974).

(7) P. Ylitalo, G. Wilen, and S. Lundall, *J. Pharm. Sci.*, **64**, 1264 (1975).

(8) B. F. Johnson, H. Greer, J. McCredie, C. Bye, and A. Fowle, *Lancet*, **1**, 1473 (1973).

(9) J. Khulmann, U. Abshagen, and N. Rietbrock, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **276**, 149 (1973).

(10) M. H. Gault, J. D. Charles, D. L. Sugden, and D. C. Kepkay, *J. Pharm. Pharmacol.*, **29**, 27 (1977).

(11) U. Abshagen, K. v. Bergmann, and N. Rietbrock, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **275**, 1 (1972).

(12) G. Kroneberg, *Arch. Exp. Pathol. Pharmakol.*, **237**, 222 (1959).

(13) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 149.

(14) D. Wells, B. Katzung, and F. H. Meyers, *J. Pharm. Pharmacol.*, **13**, 389 (1961).

(15) T. W. Smith, V. P. Butler, Jr., and E. Harber, *N. Engl. J. Med.*, **281**, 1212 (1969).

(16) M. C. Castle, *J. Chromatogr.*, **115**, 437 (1975).

(17) T. Sonobe, S. Hasumi, T. Yoshino, Y. Kabayashi, and H. Kawata, *Yakuzaigaku*, **37**, 215 (1977).

(18) L. A. Sternson and R. D. Shaffer, *J. Pharm. Sci.*, **67**, 327 (1978).

(19) A. T. Florence and E. G. Salole, *J. Pharm. Pharmacol.*, **28**, 637 (1976).

(20) S. A. H. Khalil, *J. Pharm. Pharmacol., Suppl.*, **28**, 6 P (1976).

(21) A. T. Florence, E. G. Salole, and J. B. Stenlake, *J. Pharm. Pharmacol.*, **26**, 481 (1974).

(22) S. A. H. Khalil, *ibid.*, **26**, 961 (1974).

(23) D. D. Brown and R. P. Juhl, *N. Engl. J. Med.*, **295**, 1034 (1976).

ACKNOWLEDGMENTS

Presented at the 97th Japanese Pharmaceutical Conference, Tokyo, Japan, April 1977.

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.[1] The cholesterol mono-

0022-3549/80/0400-0413$01.00/0

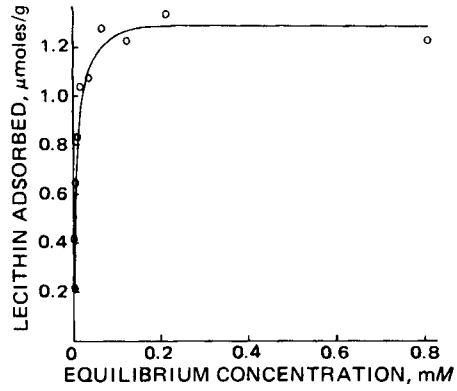

Figure 1—*Lecithin adsorption by cholesterol monohydrate in pH 7.4 tromethamine buffer with a constant cholate–lecithin molar ratio of 3.8.*

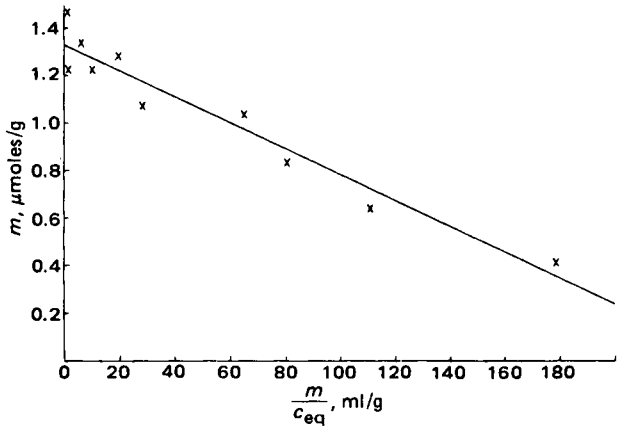

Figure 2—*Langmuir plot of lecithin adsorption in pH 7.4 tromethamine buffer with a constant cholate–lecithin molar ratio of 3.8.*

hydrate dissolution rate in artificial bile is ~20 times as slow as that predicted for diffusion-controlled dissolution.

The possibility arises that lecithin adsorbs onto the cholesterol surface and forms a condensed layer that restrains dissolution of cholesterol and thus acts as the interfacial barrier. The present study examined the *in vitro* adsorption of lecithin onto cholesterol and the effect of cholate concentration on binding.

EXPERIMENTAL

Materials—Commercial cholesterol[1] was ground in a mortar and stored in the dark in a desiccator saturated with water vapor. Differential scanning calorimetry[2] confirmed the hydrate nature of the crystals. The surface area of the cholesterol monohydrate powder was determined by an air permeability method (5).

Radioactive lecithin was prepared by mixing 10 mg of egg lecithin[3] in chloroform–methanol with 2 μCi of L-α-distearoyl[1-^{14}C]phosphatidylcholine[4] in benzene–ethanol. Before use, TLC confirmed the purity of the egg lecithin to be >99%, and the radiochemical purity of distearoylphosphatidylcholine was >95%.

The mixture was dried in an argon atmosphere and dissolved in 5 ml of pH 7.4, 0.1 *M* tromethamine buffer[5] containing 0.05 *M* NaCl and 4.6 m*M* sodium cholate[5]. The lecithin concentration was determined by measuring the phosphorus content by a method described previously (6).

Benzalkonium chloride Ph.Nord.63, lysolecithin[5], and dioctyl sodium sulfosuccinate[6] were used as received. Octadecyltrimethylammonium chloride[7] was dried *in vacuo*.

Adsorption Measurements—Cholesterol monohydrate, 0.50 g, was introduced into 2 ml of incubation medium in centrifuge tubes. The incubation medium was pH 7.4, 0.1 *M* tromethamine buffer containing 0.05 *M* NaCl with varying amounts of radioactive lecithin and sodium cholate. The tubes were filled with argon before closing. The powder was dispersed in the incubation medium by sonication in a bath-type instrument for 10 min.

After incubation in a circulating shaker at room temperature for 4 hr, the tubes were centrifugated. The supernate, 100 μl, was added to 5 ml of scintillation fluid[8], and the radioactivity was counted[9]. The amount of lecithin that was adsorbed was the difference between the initial and the equilibrium concentrations of the incubation medium. The effect of different substances on the adsorption of lecithin was measured by adding the substances at equilibrium, incubating for 4 hr, and remeasuring the radioactivity in the supernate.

[1] Merck, Darmstadt, West Germany.
[2] Perkin-Elmer DSC-1B.
[3] Lipid Products Ltd. (England).
[4] Applied Science Laboratories.
[5] Sigma Chemical Co.
[6] Aerosol OT, Cyanamid.
[7] Arquad 18/50, Akzo Chemie (United Kingdom).
[8] Pico-Fluor 15, Packard.
[9] Beckman LS-250.

RESULTS AND DISCUSSION

Adsorption at Constant Cholate–Lecithin Ratio—The adsorption of lecithin by cholesterol monohydrate from a medium with a cholate–lecithin molar ratio of 3.8 is shown in Figs. 1 and 2. The molar ratio of 3.8 is within the size range of physiological bile (4).

Figure 1 shows a plot of the amount of lecithin adsorbed per gram of cholesterol *versus* the equilibrium lecithin concentration. Since the isotherm becomes linear at low concentrations and shows a limiting value, the binding can be described by the Langmuir adsorption equation:

$$m = b\frac{Kc_{eq}}{1 + Kc_{eq}} \qquad \text{(Eq. 1)}$$

where m is the amount adsorbed, c_{eq} is the equilibrium concentration, and b and K are constants. By transformation of Eq. 1, the following expression is derived:

$$m = b - \frac{1}{K}\left(\frac{m}{c_{eq}}\right) \qquad \text{(Eq. 2)}$$

from which the constants can be determined. At sufficiently high concentrations, m approached the limiting value, b. Thus, b is a measure of the adsorbent capacity, and K is the adsorption intensity.

In Fig. 2, the results were plotted according to Eq. 2; the best straight line through the experimental data was calculated by the least-squares method. The values of b and K were determined from the intercept and the reciprocal of the regression line slope. The b value was 1.34 μmoles/g, and K was 1.8×10^5 liters/mole.

The effective area occupied by a lecithin molecule in a pure lecithin monolayer is 96 Å^2; but in mixtures with cholesterol (38 Å^2/molecule), the area occupied by a lecithin molecule is reduced, depending on the amount of cholesterol built into the layer (7). For example, when there are two or three lecithin molecules for one molecule of cholesterol, 1 m^2 is occupied by 1.67 or 1.76 μmoles of lecithin, respectively. A monolayer formed entirely of lecithin is occupied by 1.72 μmoles of lecithin/m^2.

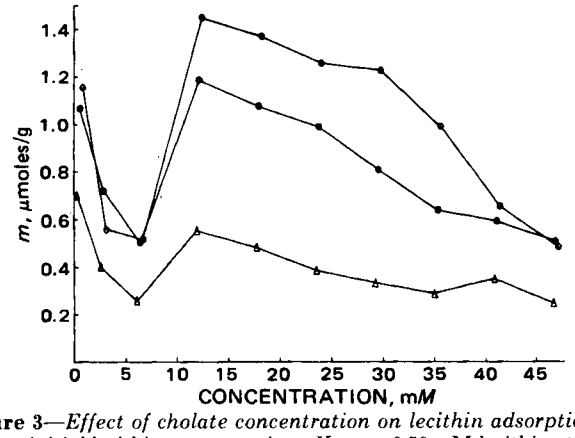

Figure 3—*Effect of cholate concentration on lecithin adsorption at various initial lecithin concentrations. Key:* ○, *0.50 mM lecithin;* ●, *0.38 mM lecithin; and* △, *0.18 mM lecithin.*

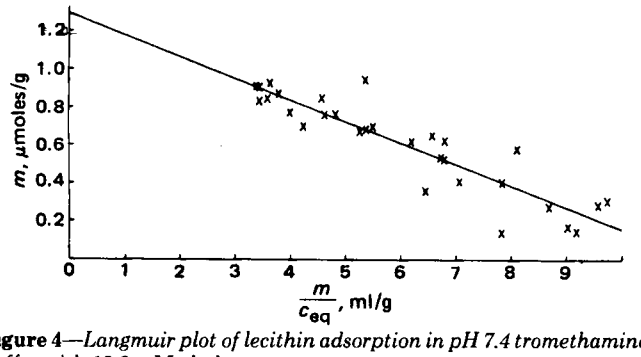

Figure 4—*Langmuir plot of lecithin adsorption in pH 7.4 tromethamine buffer with 12.2 mM cholate.*

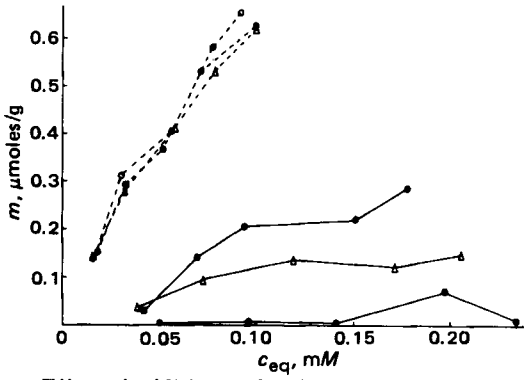

Figure 5—*Effect of additive on lecithin adsorption in pH 7.4 tromethamine buffer with 12.2 mM cholate. Key: - - -, no additive; ○, 1% octadecyltrimethylammonium chloride; △, 1% benzalkonium chloride; and ●, 1% dioctyl sodium sulfosuccinate.*

The surface area of the cholesterol powder was determined to be 0.4 m^2/g, which gives the experimental adsorptive capacity of 3.3 μmoles of lecithin/m^2 of cholesterol. Thus, the results are consistent with formation of a lecithin bilayer or a mixed bilayer.

A hydrophobic layer composed of lecithin or lecithin and cholesterol possibly could form the interfacial barrier for cholesterol dissolution in bile. A study conducted with liposomes showed that lecithin mixed with cholesterol forms condensed layers with reduced permeability (8).

Effect of Bile Salt Concentration—To investigate the effect of the bile salt concentration on adsorption, incubation was performed with cholate concentrations in the 0.05–50 m*M* range and at different initial lecithin concentrations. The results in Fig. 3 show that no simple correlation exists between cholate concentration and lecithin adsorption. The profiles showing a minimum at about 5 m*M* cholate, and decreased adsorption at concentrations greater than ~12 m*M* suggest that different mechanisms are involved.

A possible explanation of the minimum can be derived from the work of Shankland (9), who found that stable mixed micelles are formed only when the solution contains enough cholate to give the critical micelle concentration, which was 5 m*M* sodium cholate. This finding means that adsorption is highly dependent on how cholate molecules are associated in the solution. Since the detergent action of bile salts influences the structural integrity of liposomes (10), a similar effect on the adsorptive layer is expected at the higher cholate concentrations.

Adsorption experiments were carried out with a constant cholate concentration of 12.2 m*M*. The results are plotted in Fig. 4, and the constants of Eq. 2 were determined to be $b = 1.29$ μmoles/g and $K = 8.8 \times 10^3$ liters/mole. The result shows a low adsorption intensity but the same adsorption capacity as in the experiment with a constant cholate–lecithin molar ratio.

Desorption—Different substances were added to the incubation media to examine their effect on the adsorptive layer. Figure 5 shows the adsorption of lecithin from a 12.2 m*M* cholate solution measured before and after the addition of 1% octadecyltrimethylammonium chloride, benzalkonium chloride, or dioctyl sodium sulfosuccinate. These detergents caused desorption, especially dioctyl sodium sulfosuccinate, which prevented the formation of an adsorptive layer. Lysolecithin at 1% had no effect on adsorption.

If the adsorptive layer of lecithin is the interfacial barrier of dissolution, substances that cause desorption of lecithin may act as dissolution accelerators. Kwan *et al.* (11) found that amines and quaternary ammonium compounds were effective cholesterol dissolution accelerators.

The interfacial barrier is believed to be clinically important in gallstone dissolution (3). The observation of an adsorptive lecithin layer may contribute to the understanding of dissolution kinetics. The type of bile acid, the total bile acid concentration, and the lecithin concentration influence dissolution (12). These parameters also affect the formation of an adsorptive layer like the factors that increase the dissolution rate decrease adsorption. Preliminary experiments show that substitution of cholate with chenodeoxycholate leads to less lecithin adsorption onto cholesterol. The significance of the adsorptive layer in cholesterol dissolution as well as of the adsorption behavior under physiological conditions with various conjugated bile acids is not known.

By elucidating the type and structure of the rate-limiting barrier and the factors influencing it for dissolution, it may be possible to search for substances that reduce or destroy the barrier and thereby accelerate dissolution.

REFERENCES

(1) W. I. Higuchi, S. Prakongpan, and F. Young, *J. Pharm. Sci.*, **62**, 945 (1973).

(2) S. Prakongpan, W. I. Higuchi, K. H. Kwan, and A. M. Molokhia, *ibid.*, **65**, 685 (1976).

(3) K. H. Kwan, W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann, *ibid.*, **66**, 1094 (1977).

(4) A. M. Molokhia, A. F. Hofmann, W. I. Higuchi, M. Tuchinda, K. Feld, S. Prakongpan, and R. G. Danzinger, *ibid.*, **66**, 1101 (1977).

(5) N. Möller, *Dan. Tidsskr. Farm.*, **46**, 47 (1972).

(6) C. W. F. McClare, *Anal. Biochem.*, **39**, 527 (1971).

(7) D. G. Dervichian, *Prog. Biophys. Mol. Biol.*, **14**, 263 (1964).

(8) R. A. Demel, S. C. Kinsky, C. B. Kinsky, and L. L. M. van Deenen, *Biochim. Biophys. Acta*, **150**, 655 (1968).

(9) W. Shankland, *Chem. Phys. Lipids*, **4**, 109 (1970).

(10) M. H. Richards and C. R. Gardner, *Biochim. Biophys. Acta*, **543**, 508 (1978).

(11) K. H. Kwan, W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann, *J. Pharm. Sci.*, **66**, 1105 (1977).

(12) K. H. Kwan, W. I. Higuchi, and A. F. Hofmann, *ibid.*, **67**, 1711 (1978).