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[x] To whom inquiries should be directed.

Dissolution Kinetics of Cholesterol in Simulated Bile I: Influence of Bile Acid Type and Concentration, Bile Acid–Lecithin Ratio, and Added Electrolyte

K. H. KWAN [*], W. I. HIGUCHI [*x], A. M. MOLOKHIA [*], and A. F. HOFMANN [‡]

Abstract □ A physical model approach was utilized to investigate cholesterol monohydrate dissolution kinetics in simulated bile. The static pellet method and the Berthoud theory were employed to assess the contributions of the diffusion–convection mass transfer resistance and those of the interfacial resistance to the overall kinetics. For almost all situations studied, the interfacial resistance was the dominant rate-determining factor. The effects of four bile acids and their concentrations, the bile acid–lecithin ratio, and the added electrolytes and their concentrations on the interfacial resistance were examined. The results were correlated with those obtained with human bile samples, and the indications were that the kinetics of cholesterol dissolution in bile may be explainable on the basis of the principal bile acids, lecithin, and the electrolytes in the bile.

Keyphrases □ Cholesterol monohydrate—pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Dissolution kinetics—cholesterol monohydrate pellets in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Bile acids—effect of type and concentration on dissolution of cholesterol monohydrate pellets in simulated bile □ Lecithin—ratio to bile acid concentration, effect on dissolution kinetics of cholesterol monohydrate pellets in simulated bile □ Electrolytes—effect on dissolution kinetics of cholesterol monohydrate pellets in simulated bile □ Gallstones, model—cholesterol monohydrate pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes □ Steroids—cholesterol monohydrate pellets, dissolution kinetics in simulated bile, effect of bile acid type and concentration, ratio to lecithin, and added electrolytes

During the past decade, major advances have been made in understanding the cholesterol gallstone problem. But, until recently, the only treatment for cholesterol gallstones was surgery. Recent studies (1–4) demonstrated that oral administration of chenodeoxycholic acid, a naturally occurring bile acid, to patients with cholesterol gallstones decreased the relative concentration of cholesterol in bile and induced dissolution of stones in 6–36 months.

While much is known (5–8) about the thermodynamic factors governing cholesterol gallstone formation and dissolution *in vivo*, there is relatively little information on the kinetics of gallstone dissolution. Such studies could be important, since a relatively slow rate of dissolution of cholesterol gallstones was observed in several clinical studies. A theoretical treatment by Higuchi *et al.* (9) led to the proposal that *in vivo* dissolution of cholesterol gallstones occurred at rates much slower than anticipated when dissolution was solubility–diffusion controlled; therefore, the anomalously slow rates for gallstone dissolution observed previously (1) indicated that interfacial factors might be important *in vivo*.

Indeed, experimental studies (10, 11) showed that *in vitro* dissolution of cholesterol gallstones in simulated bile was dominated by an interfacial barrier at the crystal–solution interface. Subsequent dissolution rate experiments with model gallstones (compressed pellets of cholesterol monohydrate crystals) yielded comparable results and suggested that cholesterol monohydrate pellets were valid model gallstones in studies of cholesterol gallstone dissolution kinetics.

Analyses of biliary lipids in patients showing gallstone dissolution during chenodeoxycholic acid treatment (3) confirmed that desaturation of bile occurs in most instances. Preliminary experiments on the *in vitro* dissolution of cholesterol gallstones, as well as cholesterol monohydrate pellets in micellar bile acid solutions, showed (10, 11) that added lecithin significantly decreased the dissolution rate, even though its addition enhanced equilibrium cholesterol solubility. A review of the chemical composition of human gallbladder bile (12) along with these observations suggested that the major determinants of the dissolution rate process would be the bile acid type and its concentration, the bile acid–lecithin molar ratio, and the electrolytes and their concentrations. This paper reports a systematic study of the effects of these factors on

the kinetics of cholesterol monohydrate dissolution *in vitro* in simulated bile.

THEORETICAL

A physical model approach was utilized (9). The physical model provides a semiquantitative treatment of the kinetic data that should help identify possible rate-limiting situations and establish reasonable correlations between clinical and *in vitro* results.

Dissolution of a nonionic, inert solid involves (*a*) the contact of the solvent with the solid surface where (*b*) an interaction occurs, followed by (*c*) the disengagement of the solute molecule and its transport away from the interface into the bulk solution. Usually step *a* occurs instantaneously, and steps *b* and/or *c* are generally considered to be rate determining. The following equation was derived by Berthoud (13) to account for both the interfacial resistance and the diffusional resistance across the Nernst diffusion layer in a dissolution process:

$$J = \frac{A(C_s - C_b)}{R} = \frac{A(C_s - C_b)}{(h/D + 1/P)} \qquad \text{(Eq. 1)}$$

where:

J = dissolution rate
A = surface area of dissolving solid exposed to solution
C_s = concentration of solute in solution at saturation
C_b = concentration of solute in the bulk (under sink conditions, $C_b = 0$)
R = total resistance to dissolution
D = diffusion coefficient of solute in solution
h = Nernst diffusion layer thickness
P = effective permeability coefficient of the interfacial barrier

When interactions at the surface occur rapidly, $1/P$ becomes negligible, Eq. 1 reduces to the Nernst equation (14):

$$J = \frac{AD(C_s - C_b)}{h} \qquad \text{(Eq. 2)}$$

and the dissolution process becomes diffusion controlled. If surface interactions take place slowly, $1/P$ becomes much greater than h/D, in which case:

$$J = AP(C_s - C_b) \qquad \text{(Eq. 3)}$$

and the dissolution process becomes interfacial barrier controlled.

Both the Berthoud and the Nernst theories represent only semiempirical treatments of the dissolution process and assume a purely diffusional resistance, h/D, for the transfer of solid across the diffusion layer. However, in most dissolution situations, both convection and diffusion are expected to be important. Thus, for example, in the rotating-disk dissolution situation (15), it can be shown by the Levich treatment (16) that there may be a substantial contribution to mass transfer by convection, and the dissolution rate is given by:

$$J = \frac{A(C_s - C_b)}{(1.612D^{-2/3}\upsilon^{1/6}\omega^{-1/2}) + 1/P} \qquad \text{(Eq. 4)}$$

where υ is the kinematic viscosity of the solvent, and ω is the angular velocity of rotation. The corresponding effective diffusion layer thickness may be written as:

$$h = 1.612D^{1/3}\upsilon^{1/6}\omega^{-1/2} \qquad \text{(Eq. 5)}$$

In previous studies (17, 18), both Eqs. 1 and 4 were employed to analyze the experimental data on cholesterol monohydrate pellet dissolution in bile acid–lecithin solutions. With the rotating-disk technique, the rotation speed dependence predicted by Eq. 4 and the experimental results generally agreed well for a wide range of P values. This study established a general method for quantitatively determining the interfacial barrier transport coefficient, P, even when $1/P$ was comparable to the diffusional resistance term.

EXPERIMENTAL

Design and Considerations—The semiquantitative static pellet dissolution method (11) was chosen over the more quantitative rotating-disk method (15) since only one rotation speed is required with the static-pellet method. [With the rotating-disk method, dissolution rates must be determined at several rotation speeds to utilize the Levich (16) treatment.] The static-disk method required one-tenth the volume of the solvent system that would have been required with the rotating-disk method. Use of this more rapid method resulted in a great saving of chemicals and permitted the rapid, semiquantitative assessment of a large number of variables. Despite its semiempirical nature and its limitations, the static-pellet method accompanied by the Berthoud treatment is more than adequate for the present purposes.

Table I—Influence of Sodium Chloride Concentration on Solubility (C_s), Dissolution Rate (J/A), Total Resistance (R), and $1/P$ of Cholesterol Monohydrate in 116 mM Bile Acid–32 mM Lecithin Solutions Containing 0.01 M Phosphate Buffer at pH 7.4

Sodium Chloride, M	Parameter	I	II	III	IV
0	C_s[a]	3.37	3.36	2.95	3.15
	(J/A)[b] $\times 10^4$	0.0584	0.0314	0.168	0.022
	R[c] $\times 10^{-3}$	577	1070	176	1419
	$(1/P)$[c] $\times 10^{-3}$	574.7	1067.7	173.7	1416.7
0.10	C_s	4.07	3.96	3.00	3.34
	$(J/A) \times 10^4$	0.466	0.384	0.274	0.121
	$R \times 10^{-3}$	87.3	103	110	276
	$(1/P) \times 10^{-3}$	85.0	100.7	107.7	273.7
0.25	C_s	4.17	4.32	3.07	3.45
	$(J/A) \times 10^4$	1.64	1.10	0.685	0.200
	$R \times 10^{-3}$	25.4	39.3	44.8	173
	$(1/P) \times 10^{-3}$	23.1	37.0	42.5	170.7
0.50	C_s	—	—	3.20	3.69
	$(J/A) \times 10^4$	—	—	1.61	0.632
	$R \times 10^{-3}$	—	—	19.9	58.4
	$(1/P) \times 10^{-3}$	—	—	17.6	56.1

[a] In mg cm^{-3}. [b] In mg cm^{-2} sec^{-1}. [c] In sec cm^{-1}.

It was decided to utilize Eq. 1 in the following way with the static-pellet method. The parameters J and C_s were to be measured in all cases so that R could be calculated. To determine P, an h/D value for the static-pellet system was estimated that was expected to be applicable to all situations involving micellar cholesterol in the bile acid–lecithin solutions to within about a factor of two. It was based upon representative diffusivity measurements in various cholesterol–bile acid–lecithin solutions, an h determination using a benzoic acid pellet (19), and the assumption that h is proportional to $D^{1/3}$ (18). For most situations in this study, $1/P \gg h/D$ or $R \cong 1/P$. Therefore, even though the uncertainty in h/D is large, rather accurate P values could be obtained for the cases of interest.

With regard to the solvent compositions to be used, a review of the chemical composition of human gallbladder bile (12) suggested the following:

1. The conjugated bile acids, cholyltaurine, cholylglycine, chenodeoxycholyltaurine, and chenodeoxycholylglycine, constitute over 80% of the major bile acids in bile, and dissolution kinetics for each bile acid should be defined. Since sodium is the major cation in bile, solutions should be prepared with the sodium salt.
2. The bile acid to lecithin molar ratio in bile samples from normal subjects and gallstone patients varies over a wide range. Ratios of 2.72, 3.63, and 5.44 should be adequate for defining the influence of this variable over the physiological range.
3. The major inorganic ions in bile are Na^+, Ca^{2+}, and Cl^-. Sodium chloride is a 1:1 electrolyte with a monovalent cation, and calcium chloride represents a typical 2:1 electrolyte providing a divalent cation. For sodium chloride, concentrations from 0.1 to 0.5 M should adequately cover the range; for calcium chloride, 0.025–0.10 M appears reasonable on the basis of published data.
4. The bile acid–lecithin concentration level should be important since earlier studies (20) indicated that dissolution in the more dilute duodenal bile was much slower than in gallbladder bile. While 116 mM bile acid–32 mM lecithin represents a reasonable level of bile acid–lecithin concentration in gallbladder bile, a wider range is assumed for the often diluted duodenal bile, namely from 23.2 mM bile acid–6.4 mM lecithin to 46.4 mM bile acid–12.8 mM lecithin (21).
5. The pH of the solutions should be maintained at 7.40 using 0.01 M sodium phosphate buffer to simulate physiological pH as well as to ensure that all bile acids in solution are fully ionized species. The pKa's of all bile acids studied are less than 5.0 (22). Exploratory studies already showed that at pH $\geq$ 7.0, both J and C_s are constant for each of the four bile acids studied.

Materials—Commercial cholesterol[1] was recrystallized three times

[1] Eastman Kodak Co., Rochester, N.Y.

Table II—Influence of Sodium Chloride Concentration on Solubility (C_s), Dissolution Rate (J/A), Total Resistance (R), and $1/P$ of Cholesterol Monohydrate in 46.4 m*M* Bile Acid–12.8 m*M* Lecithin Solutions Containing 0.01 *M* Phosphate Buffer at pH 7.40

Sodium Chloride, *M*	Parameter	I	II	III	IV
0	C_s[a]	1.10	1.50	0.784	1.05
	(J/A)[b] $\times 10^4$	0.00752	0.00859	0.0360	0.00657
	R[c] $\times 10^{-3}$	1463	1746	218	1600
	$(1/P)$[c] $\times 10^{-3}$	1460.7	1743.7	215.7	1597.7
0.10	C_s	1.27	1.63	0.802	1.12
	$(J/A) \times 10^4$	0.077	0.0286	0.0382	0.0192
	$R \times 10^{-3}$	165	570	210	584
	$(1/P) \times 10^{-3}$	162.7	567.7	207.7	581.7
0.25	C_s	1.39	2.01	0.832	1.19
	$(J/A) \times 10^4$	0.243	0.322	0.105	0.0491
	$R \times 10^{-3}$	57.2	50.2	79.2	242
	$(1/P) \times 10^{-3}$	54.9	47.9	76.9	239.7
0.50	C_s	1.56	—	1.19	1.30
	$(J/A) \times 10^4$	0.996	—	0.274	0.0844
	$R \times 10^{-3}$	15.7	—	43.4	154
	$(1/P) \times 10^{-3}$	13.4	—	41.1	151.7

[a] In mg cm^{-3}. [b] In mg cm^{-2} sec^{-1}. [c] In sec cm^{-1}.

from 95% ethanol. Radioactive cholesterol monohydrate was prepared by mixing 5 g of the recrystallized cholesterol with 100 μCi of a benzene solution of 4-^{14}C-cholesterol[2] in 400 ml of 95% ethanol at 60°. This solution was filtered while hot, and the filtrate was allowed to stand for 48 hr at room temperature. Then the cholesterol monohydrate crystals were filtered and dried *in vacuo* for 24 hr. The crystals obtained were stored in the dark in a desiccator saturated with water vapor at room temperature.

NMR studies quantitatively confirmed the monohydrate nature of the crystals. TLC studies indicated the absence of any impurities (23). X-ray crystallography[3] studies indicated that they were indeed cholesterol monohydrate crystals and that they had a lattice system similar to that of cholesterol found in human biliary calculi (24). The monohydrate crystals will lose their water content readily on exposure to low humidity and light.

The sodium salts of chenodeoxycholyltaurine (I) and chenodeoxycholylglycine (II) were prepared by the method of Norman (25) with certain modifications (26). The sodium salts of cholyltaurine (III) and cholylglycine (IV) were prepared using the method of Norman (25) with certain modifications[4] (27). The purity of these compounds was checked and confirmed by TLC using a destructive detection method (28).

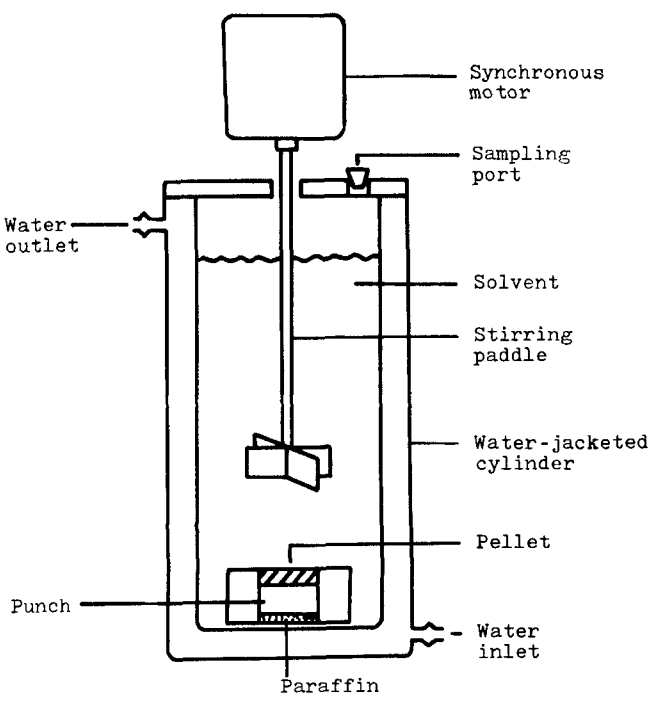

Figure 1—*Diagrammatic representation of the static-pellet dissolution apparatus.*

Egg lecithin was prepared from fresh egg yolks and subsequently stored according to the method of Singleton *et al.* (29). Chromatographically homogeneous lecithin, mol. wt. 771, was obtained. Monobasic sodium phosphate, dibasic sodium phosphate, sodium chloride, and calcium chloride[5] were analytical grade and were used as received.

Preparation of Solutions—All bile acid–lecithin solutions were prepared as follows. The sodium salt of the bile acid was dissolved in sufficient distilled water. The desired electrolyte, sodium chloride or calcium chloride, was added to the bile acid solution. For solutions containing no calcium chloride, 0.01 *M* sodium phosphate buffer at pH 7.40 was also added. Lecithin was dissolved separately in sufficient distilled water until a homogeneous colloidal suspension was obtained.

The lecithin suspension was then added to the bile acid–electrolyte solution and was diluted to the desired value with distilled water. The mixture was then shaken and allowed to stand overnight at 5° so that complete solubilization of lecithin was obtained. With sodium glycocholate, complete lecithin solubilization took more than 24 hr. The resulting clear solutions were then used for both dissolution rate and solubility determinations.

Dissolution Rate Determination—Pellets of ^{14}C-cholesterol monohydrate were prepared by directly compressing 100 mg of the material in a die, 1.27 cm (0.50 in.) i.d., under a force of 1362 kg (3000 lb) using a laboratory press[6]. The exposed surface area of the resulting pellets was 1.267 cm^2. The pellet was held firmly in a die by covering the bottom with melted paraffin. This die was then placed on the bottom of a water-jacketed cylinder, with the pellet facing a stirring paddle inserted at the top of the cylinder (Fig. 1). The stirring speed was maintained at 150 rpm during dissolution by a constant-speed motor[7].

Exactly 10 ml of the dissolution medium preequilibrated at 37° was added into the cylinder. Immediately, the first 0.50-ml sample was withdrawn using a pipet. Four other samples were taken at suitable time intervals. The ^{14}C-labeled samples were subsequently counted using a liquid scintillation counter[8], and the amount of cholesterol dissolved in the solvent was plotted against time.

Solubility Determination—The solubilities of cholesterol monohydrate in various solvent media were determined by introducing an excess of ^{14}C-cholesterol monohydrate, about 20 mg, into 2 ml of a solvent in a test tube. The tube was flushed with nitrogen, capped, and then shaken by a wrist-action shaker[9] in a water bath at 37°. After 4 days, a sample was taken and quickly filtered through a glass wool-wrapped, long tipped pipet preequilibrated at 37°. Exactly 0.2 ml of the filtrate was then assayed for cholesterol using a liquid scintillation counter[8].

More samples were taken every 2 days and assayed for cholesterol. The

[2] New England Nuclear Corp., Boston, Mass.
[3] Performed by Dr. C. Nordman, Department of Chemistry, University of Michigan, Ann Arbor, Mich.
[4] By A. F. Hofmann.
[5] Matheson, Coleman and Bell, Norwood, Ohio.
[6] Model B, Fred Carver Inc., Summit, N.J.
[7] Model CA, Hurst, Princeton, Ind.
[8] Model LS 200, Beckman Instruments, Fullerton, Calif.
[9] Burrell Corp., Pittsburgh, Pa.

Table III—Influence of Sodium Chloride Concentration on Solubility (C_s), Dissolution Rate (J/A), Total Resistance (R), and $1/P$ of Cholesterol Monohydrate in 23.2 mM Bile Acid–6.4 mM Lecithin Solutions Containing 0.01 M Phosphate Buffer at pH 7.40

Sodium Chloride, M	Parameter	I	II	III	IV
0.25	C_s[a]	0.952	0.864	0.664	0.814
	(J/A)[b] $\times 10^4$	0.0769	0.082	0.0342	0.0130
	R[c] $\times 10^{-3}$	123.8	105.2	194	626
	$(1/P)$[c] $\times 10^{-3}$	121.5	102.9	191.7	623.7
0.50	C_s	1.03	0.993	0.872	0.976
	$(J/A) \times 10^4$	0.343	0.411	0.0872	0.0565
	$R \times 10^{-3}$	30.0	24.2	100	173
	$(1/P) \times 10^{-3}$	27.7	21.9	97.7	170.7

[a] In mg cm^{-3}. [b] In mg cm^{-2} sec^{-1}. [c] In sec cm^{-1}.

solubility of cholesterol monohydrate in the medium was obtained when the concentration reached a constant level.

RESULTS

Figure 2 shows typical raw data obtained for the dissolution of cholesterol monohydrate in two solvent media. The dissolution rates were obtained by taking the slopes of the dissolution curves. The duplicate sets of data indicate the typical degree of reproducibility obtainable. For one solution, a standard deviation of 5% was obtained; for the other solution, a deviation close to 10% was obtained when the rates were much slower.

Figure 3 shows plots of amounts of cholesterol monohydrate solubilized in the same two solvent media (Fig. 2) as a function of time. Multiple samples were taken until a constant value for the equilibrium solubility was obtained for each solvent. Again, excellent reproducibility was obtained, with standard deviations of the equilibrium values lying within the range of 3–5%. Based on these solubility values, it may be estimated that at the end of the dissolution experiments (Fig. 2), the solvents become, at the most, around 15% saturated in cholesterol. Thus, sink conditions are maintained during dissolution, which justifies the use of the initial linear portions of plots of the type presented in Fig. 2 to calculate the dissolution rate for sink conditions, *i.e.*, $C_s \gg C_b$.

Figures 4–11 and Tables I–V summarize all results. The tabulated C_s and J/A values represent the average of at least two determinations. The value of R was calculated using Eq. 1 and assuming sink conditions, so that $C_b = 0$. The R values shown in the tables were calculated from the average C_s and J/A values.

As can be seen in Figs. 4–6 and Tables I–III, an increase in sodium chloride concentration resulted in a decrease in R for all four bile acids at all three bile acid concentrations. Studies at higher concentrations of sodium chloride were not possible for chenodeoxycholyltaurine (I) and chenodeoxycholylglycine (II) due to the salting out of the bile acids. For all three concentrations of bile acids, the R values of the chenodeoxycholate conjugates were generally lower than those of their cholate counterparts at all sodium chloride concentrations, except in the absence of sodium chloride. In general, differences among individual bile acids were significant.

A similar, but by far more remarkable, effect was observed with calcium chloride (Table IV). These systems were not buffered with 0.01 M phosphate, because calcium would precipitate as phosphates. However, the pH of the solutions remained at around 7.7 ± 0.15 during the dissolution rate and solubility determinations. No data were obtained for II, because this conjugate salted out in the presence of only a trace amount of calcium chloride. For the other three bile acids, an increase in calcium chloride concentration resulted in large reductions in R. At the same electrolyte concentrations, the effects of calcium chloride on R were greater than those of sodium chloride (Table I), the difference being eightfold for cholyltaurine (III) and 35-fold for cholylglycine (IV) on a concentration basis. Compound I precipitated out at higher calcium chloride concentrations.

In Figs. 7–10, R was plotted as a function of the bile acid–lecithin concentration at various sodium chloride concentrations for the four bile acids. In all cases, a smaller R was obtained at a higher bile acid–lecithin

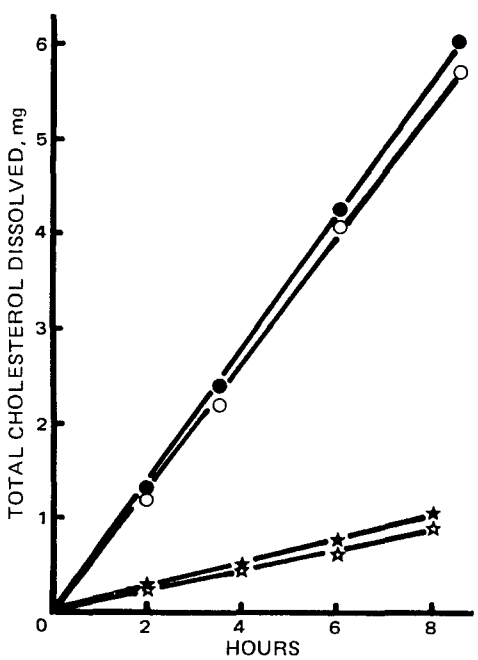

Figure 2—*Dissolution of cholesterol monohydrate at 37° in two solvent media. Key: ●, ○, in 116 m*M *cholyltaurine, 32 m*M *lecithin, 0.50* M *sodium chloride, and 0.01* M *phosphate buffer, pH 7.4; and ★, ☆, in 46.4 m*M *cholyltaurine, 12.8 m*M *lecithin, 0.50* M *sodium chloride, and 0.01* M *phosphate buffer, pH 7.4.*

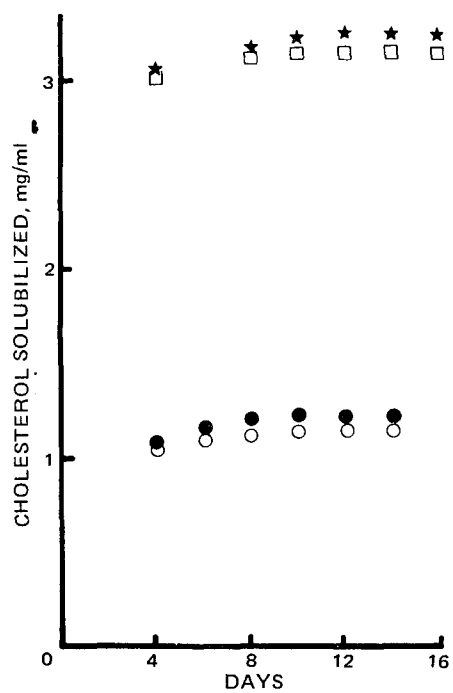

Figure 3—*Solubility of cholesterol monohydrate at 37° in two solvent media. Key: ★, □, in 116 m*M *cholyltaurine, 32 m*M *lecithin, 0.50* M *sodium chloride, and 0.01* M *phosphate buffer, pH 7.4; and ●, ○, in 46.4 m*M *cholyltaurine, 12.8 m*M *lecithin, 0.50* M *sodium chloride, and 0.01* M *phosphate buffer, pH 7.4.*

Table IV—Influence of Calcium Chloride Concentration on Solubility (C_s), Dissolution Rate (J/A), Total Resistance (R), and $1/P$ of Cholesterol Monohydrate in 116 mM Bile Acid–32 mM Lecithin Solutions

Bile Acid	Calcium Chloride, M	C_s, mg/cm^3	$(J/A) \times 10^4$, mg/cm^2 sec	$R \times 10^{-3}$, sec/cm	$(1/P) \times 10^{-3}$, sec/cm
I	0.0250	4.04	2.56	15.8	13.5
	0.050	4.35	5.37	8.10	5.80
III	0.050	3.34	1.64	20.4	18.1
	0.10	3.78	2.76	13.7	11.4
IV	0.050	3.69	3.27	11.3	9.0
	0.10	4.14	5.30	7.81	5.51

concentration. Again, the differences among bile acids were pronounced.

Figure 11 indicates that as the bile acid–lecithin molar ratio was increased from 2.72 to 5.44 with the lecithin concentration kept constant at 32 mM, a gradual reduction of R was obtained. Striking differences were again observed among bile acids. Extremely large R values were observed for III and IV at 0.25 M sodium chloride at the lowest bile acid–lecithin ratio of 2.72.

DISCUSSION

Surface-Controlled *versus* Diffusion-Controlled Kinetics—An evaluation of the magnitude of h/D (Eq. 1) permits the assessment of the relative importance of the two resistances involved in cholesterol dissolution kinetics. Representative cholesterol diffusivity determinations were carried out (18) over the range of conditions reported in the present study, and the D values were generally in the range of $1.0–1.5 \times 10^{-6}$ cm^2/sec. These values were in good agreement with those recently reported by Sehlin *et al.* (30) for similar situations. An h value of 62 μm was determined for the present system with benzoic acid as a reference compound (19). Then, by assuming the relationship of:

$$\frac{h_1}{h_2} = \left(\frac{D_1}{D_2}\right)^{1/3} \qquad \text{(Eq. 6)}$$

and using an average D value of 1.25×10^{-6} cm^2/sec for micellar cholesterol and $D = 14.0 \times 10^{-6}$ cm^2/sec for benzoic acid (11), the effective h for the cholesterol–micelle system was found to be 28.2 μm. Hence, an h/D value for the cholesterol–bile acid–lecithin system of 2.3×10^3 sec/cm may be estimated.

Thus, for most conditions (Tables I–V), $h/D \ll 1/P$. This finding underscores the earlier suggestion (9–11) that the interfacial barrier may indeed be the dominant rate-determining factor in cholesterol gallstone and cholesterol monohydrate dissolution.

The results of this study and similar investigations using human gallbladder bile (20) have great bearing on the issue of whether cholesterol gallstone dissolution rate accelerators have a place *in vivo*. Recent clinical studies (1–4) showed that cholesterol gallstones may be dissolved by the oral administration of chenodeoxycholic acid. In most instances, however, the treatment times were long, often extending beyond 12 months and sometimes to 36 months.

It was suggested earlier (9–11), on the basis of limited studies, that the dissolution of cholesterol gallstones may be controlled by an interfacial barrier rather than by a diffusion–convection barrier and, therefore, offers a very attractive "handle" in seeking out potential agents that may act as dissolution rate accelerators. It had been apparent for some time (31) that, if stone dissolution was primarily diffusion–convection controlled, a purely medical approach involving the use of an agent coadministered with chenodeoxycholic acid would not be promising. Since bile itself is such an efficient solubilizing agent, increases in dissolution rates based upon a solubility-enhancing principle were expected to offer at best around 50% increases under the most favorable conditions.

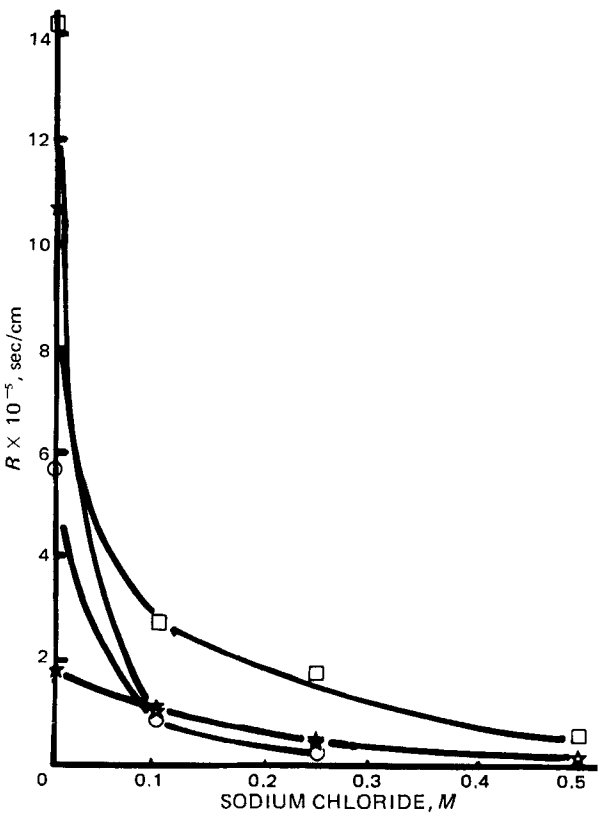

Figure 4—*Effect of sodium chloride on* R *in 116 m*M *bile acid, 32 m*M *lecithin, and 0.01* M *phosphate buffer, pH 7.4, at 37°. Key:* ○, *I;* ★, *II;* ☆, *III; and* □, *IV.*

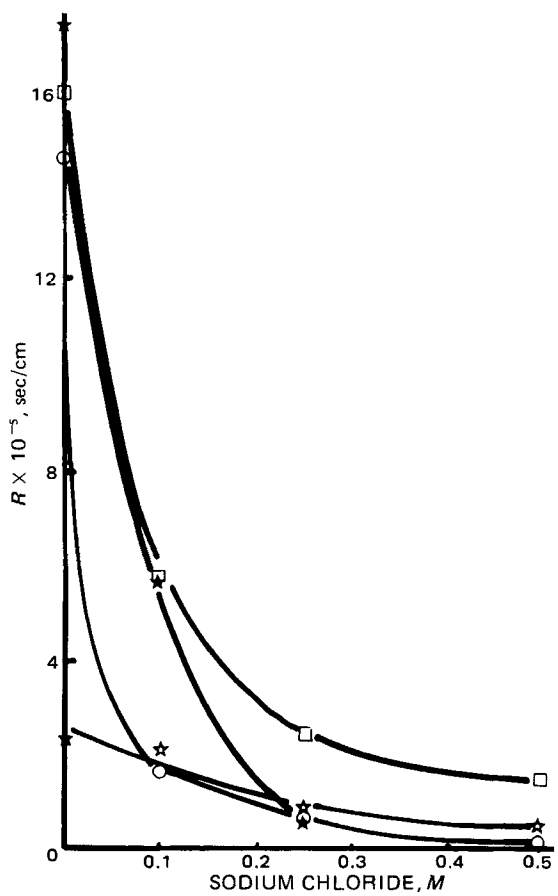

Figure 5—*Effect of sodium chloride on* R *in 46.4 m*M *bile acid, 12.8 m*M *lecithin, and 0.01* M *phosphate buffer, pH 7.4, at 37°. Key:* ○, *I;* ★, *II;* ☆, *III; and* □, *IV.*

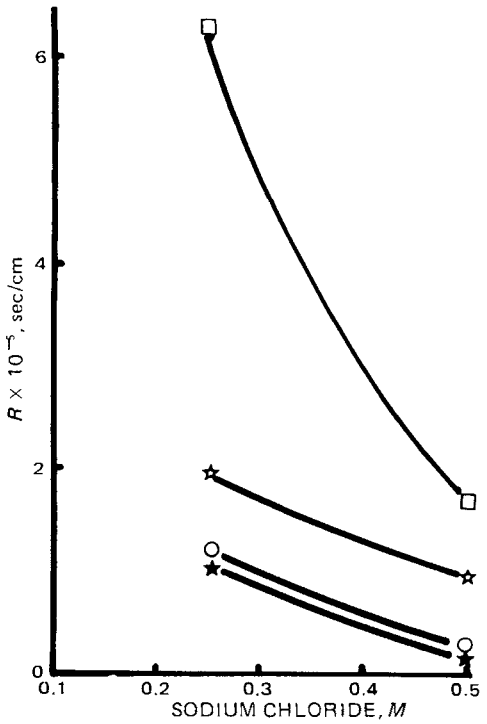

Figure 6—*Effect of sodium chloride on* R *in 23.2 m*M *bile acid, 6.4 m*M *lecithin, and 0.01* M *phosphate buffer, pH 7.4, at 37°. Key:* ○, *I;* ★, *II;* ☆, *III; and* □, *IV.*

The interfacial barrier and dissolution rate acceleration concepts based upon this premise were subsequently tested in a series of limited *in vitro* studies (10, 11) in which sodium cholate–lecithin and sodium taurocholate–lecithin were employed as synthetic biles. Under the prevailing *in vitro* hydrodynamic conditions, interfacial resistances up to around 20–50 times larger than diffusion–convection mass transfer resistances were present. Furthermore, a large number of compounds (32) were found to be effective dissolution accelerators and, in some cases, yielded dissolution rate increases up to 20–50 times the control rates.

Table V—Influence of Bile Acid Concentration on Solubility (C_s), Dissolution Rate (J/A), Total Resistance (R), and $1/P$ of Cholesterol Monohydrate in Solutions Containing 32 m*M* Lecithin, 0.25 *M* Sodium Chloride, and 0.01 *M* Phosphate Buffer at pH 7.40

Bile Acid	Parameter	Bile Acid–Lecithin Molar Ratio 2.72	3.63	5.44
I	C_s [a]	3.68	4.17	4.51
	(J/A) [b] $\times 10^4$	0.99	1.64	3.13
	R [c] $\times 10^{-3}$	37.2	25.4	14.4
	$(1/P)$ [c] $\times 10^{-3}$	34.9	23.1	12.1
II	C_s	3.71	4.32	4.80
	$(J/A) \times 10^4$	0.548	1.10	2.74
	$R \times 10^{-3}$	67.7	39.3	17.5
	$(1/P) \times 10^{-3}$	65.4	37.0	15.2
III	C_s	2.82	3.07	3.90
	$(J/A) \times 10^4$	0.182	0.685	1.93
	$R \times 10^{-3}$	155	44.8	20.2
	$(1/P) \times 10^{-3}$	152.7	42.5	17.9
IV	C_s	3.32	3.45	3.84
	$(J/A) \times 10^4$	0.0520	0.200	0.730
	$R \times 10^{-3}$	638	173	52.6
	$(1/P) \times 10^{-3}$	635.7	170.7	50.3

[a] In mg cm^{-3}. [b] In mg cm^{-2} sec^{-1}. [c] In sec cm^{-1}.

The major unsolved problem is the lack of understanding of the nature of the flow of bile in the human gallbladder. To relate past and present studies meaningfully to the question of whether the interfacial barrier can be *clinically* important requires an estimate of the time average value for *h, in vivo,* or an alternative estimate of the time average diffusion–

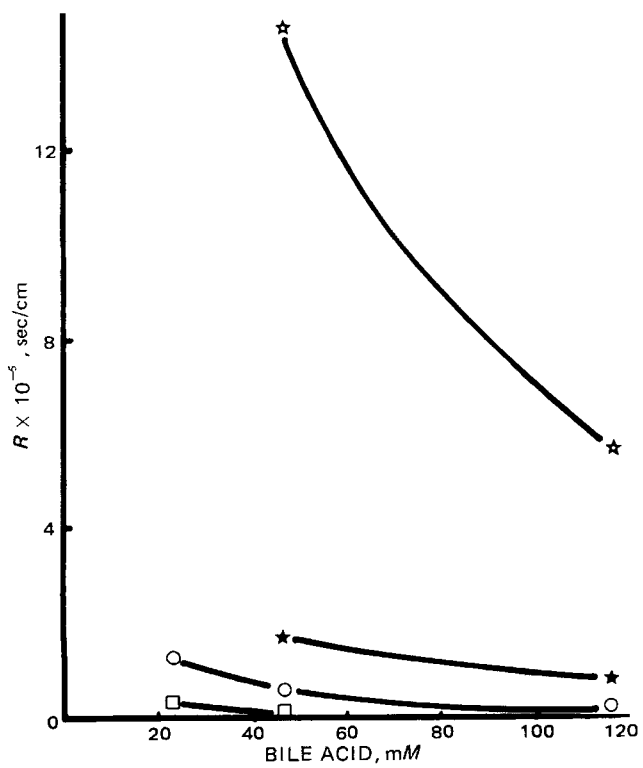

Figure 7—*Effect of I concentration on* R *at various sodium chloride concentrations with the bile acid–lecithin molar ratio kept constant at 3.63. Key:* ☆, *no sodium chloride;* ★, *0.10* M *sodium chloride;* ○, *0.25* M *sodium chloride; and* □, *0.50* M *sodium chloride.*

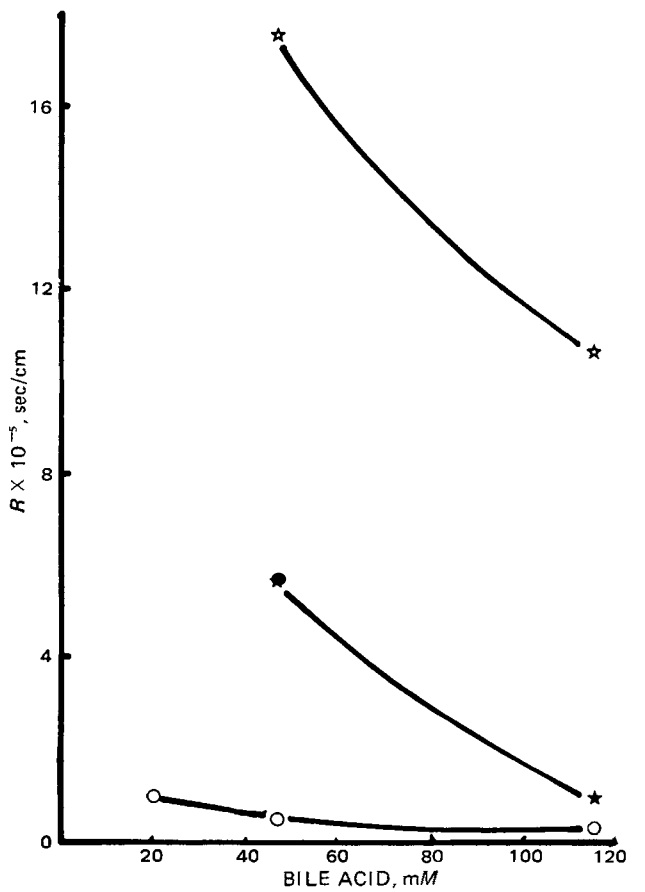

Figure 8—*Effect of II concentration on* R *at various sodium chloride concentrations with the bile acid–lecithin molar ratio kept constant at 3.63. Key:* ☆, *no sodium chloride;* ★, *0.10* M *sodium chloride; and* ○, *0.25* M *sodium chloride.*

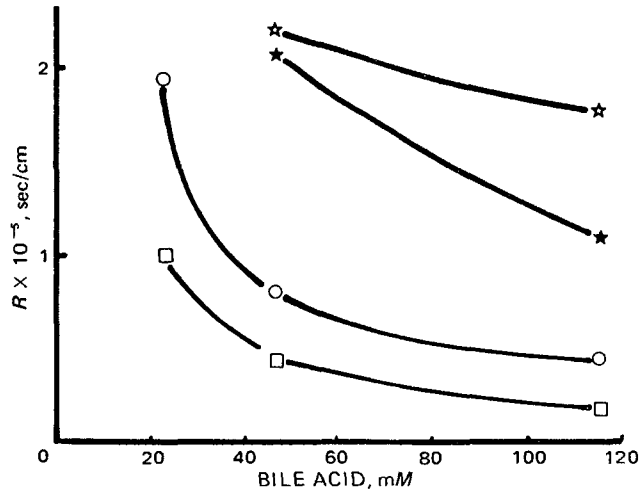

Figure 9—*Effect of III concentration on* R *at various sodium chloride concentrations with the bile acid–lecithin molar ratio kept constant at 3.63. Key:* ☆, *no sodium chloride;* ★, *0.10* M *sodium chloride;* ○, *0.25* M *sodium chloride; and* □, *0.50* M *sodium chloride.*

convection mass transfer coefficient as suggested, for example, by Tao *et al.* (31). The problem is difficult because of the irregularity of bile flow rates and general gallbladder dynamics.

Based upon what is generally considered to be the range of h values for natural (free) convection and/or slow bile flow, however, an estimate for the time average h may be proposed (9, 18, 33, 34). Previously (9, 18), an h value of around 200 μm was suggested as a reasonable upper limit, *i.e.*, for slow bile flow and/or free convection. Subsequently, Tao *et al.* (31), arguing that slow bile flow should result primarily from free convection, gave an estimate of the range for the mass transfer coefficient, K, for cholesterol stone dissolution in bile. Figure 3 of Ref. 31 shows that this range is $0 \leq K \cong 1.5 \times 10^{-4}$ cm/sec. Since:

$$K = \frac{D}{h} \qquad \text{(Eq. 7)}$$

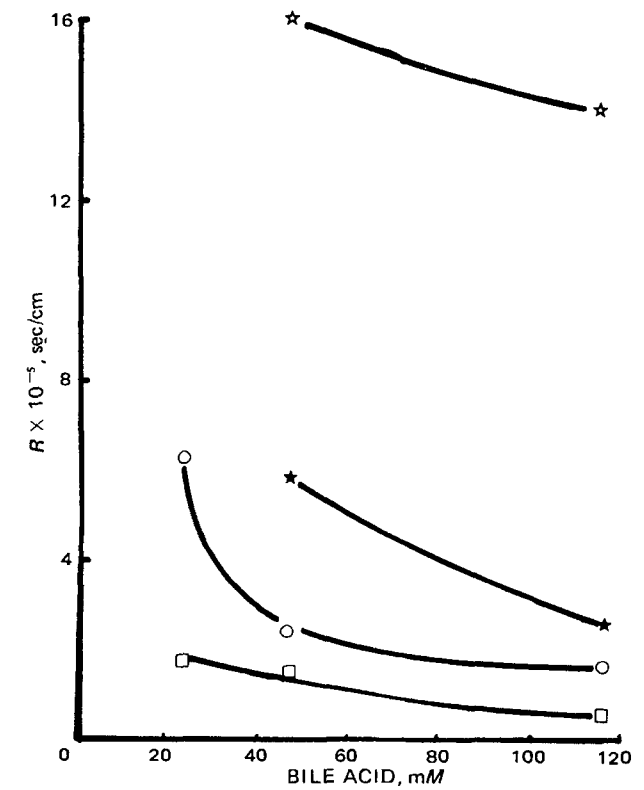

Figure 10—*Effect of IV concentration on* R *at various sodium chloride concentrations with the bile acid–lecithin ratio kept constant at 3.63. Key:* ☆, *no sodium chloride;* ★, *0.10* M *sodium chloride;* ○, *0.25* M *sodium chloride; and* □, *0.50* M *sodium chloride.*

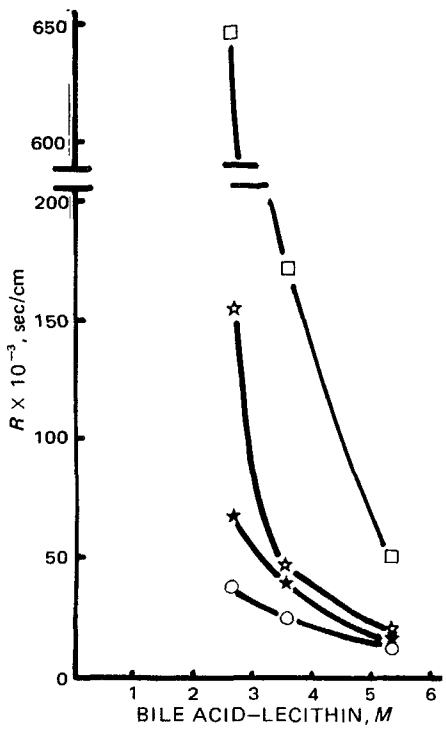

Figure 11—*Effect of bile acid–lecithin molar ratio on* R *for various bile acids in the presence of 0.25* M *sodium chloride and 0.01* M *phosphate buffer at pH 7.4 with the lecithin concentration kept constant at 32 m*M. *Key:* □, *IV;* ☆, *III;* ★, *II; and* ○, *I.*

when Eq. 2 is considered, with $D = 1.8 \times 10^{-6}$ cm²/sec, $\infty \gtrsim h \cong 120$ μm, therefore giving an independent assessment of what might be a reasonable range for h *in vivo*. Thus, an h value of around 200 μm should be a reasonable (or, at least, a reasonably liberal) estimate of the effective diffusion–convection barrier. This value would approximately correspond to the midpoint of the range for slow flow suggested by Tao *et al.* (31).

At this point, it is instructive to compare the proposed $(h/D)_{in\ vivo} = 1.6 \times 10^4$ sec/cm value (for $h = 200$ μm and $D = 1.25 \times 10^{-6}$ cm²/sec) with the $1/P$ values given in Tables I–V. While in some cases the values are comparable, $1/P \gg (h/D)_{in\ vivo}$ in many instances. Several aspects in this connection are noteworthy.

First, even at 0.10–0.25 *M* NaCl levels, which may be considered to be physiological, the $1/P$ values for the bile acid–lecithin ratio of 3.63 are two to 40 times larger than the diffusion–convection resistance estimate of 1.6×10^4 sec/cm (Tables I–III). This indicates the probable dominance of the interfacial resistance over the diffusion–convection resistance *in vivo* in certain situations.

The second noteworthy aspect is the situation involving the bile acid–lecithin ratio. At the high bile acid–lecithin ratio of 5.44, the interfacial resistance may only be comparable to the diffusion–convection resistance in the case of I and II and moderately larger for III and IV. However, at the bile acid–lecithin ratio of 2.72, the results indicate that the interfacial resistance may become the strongly dominating resistance in bile with $1/P$ values as high as 6×10^5 sec/cm. Apropos to this point are the recent experiments (20) employing human gallbladder bile taken from patients during surgery, where values for $1/P$ of around 3×10^5 sec/cm were found with bile from two patients having bile acid–lecithin ratios of 2.3 and 2.9. This result corresponds to a dominance of the interfacial resistance over the diffusion–convection resistance by a factor of 20. These very large $1/P$ values, which are believed to be clinically significant, may be contrasted with the smaller values ($2.5–5 \times 10^4$) found (20) with several specimens for which the bile acid–lecithin ratios were high—*viz.*, $\gtrsim 3.6$. When it is noted that about 30% of the 20 human bile samples investigated thus far in these laboratories showed bile acid–lecithin ratios $\cong 2.7$, one may infer that the interfacial resistance may be a substantial rate-determining factor in cholesterol stone dissolution in a large fraction of the population undergoing chenodeoxycholic acid treatment[10].

[10] Data on bile acid–lecithin ratios taken from published reports (35–37) were also considered. Analyses made of 66 human bile samples revealed that about 19 had bile acid–lecithin ratios $\gtrsim 2.7$, in good agreement with the present results.

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ˣ To whom inquiries should be directed.

Dissolution Rates of Model Gallstones in Human and Animal Biles and Importance of Interfacial Resistance

A. M. MOLOKHIA *, A. F. HOFMANN ‡, W. I. HIGUCHI *ˣ, M. TUCHINDA *, K. FELD *, S. PRAKONGPAN *, and R. G. DANZINGER §

Abstract □ Cholesterol monohydrate dissolution kinetics in human gallbladder bile were studied to determine the magnitudes of the *in vitro* dissolution rates, the rate resistances in human gallbladder bile, and the extent that the interfacial resistance is the rate-determining factor. Dissolution rate studies also were conducted using human duodenal bile and animal bile for comparison. The dissolution rate resistance, R, ranged from 10^4 sec/cm for chicken bile to 10^4–10^6 sec/cm for human bile. Interfacial resistance was the rate-determining factor for essentially all results. Where chemical composition data were obtained, the R values for the human bile samples were consistent with predictions made from the simulated bile studies. In two human gallbladder specimens having low bile acid–lecithin molar ratios (*i.e.*, 2.9 and 2.3), very high R values of 1.9×10^5 and 4.1×10^5 sec/cm were found. These values were in good agreement with the findings in the simulated bile studies and suggest that stone dissolution in patients with low bile acid–lecithin ratios may proceed very slowly, even when the bile is highly undersaturated with respect to cholesterol.

Keyphrases □ Cholesterol monohydrate—pellets, dissolution kinetics in human, animal, and simulated bile, effect of interfacial resistance □ Dissolution—kinetics, cholesterol monohydrate pellets in human, animal, and simulated bile, effect of interfacial resistance □ Gallstones, model—cholesterol monohydrate pellets, dissolution kinetics in human, animal, and simulated bile, effect of interfacial resistance □ Biles, various—dissolution kinetics of cholesterol monohydrate pellets in human, animal, and simulated bile, effect of interfacial resistance □ Steroids—cholesterol monohydrate pellets, dissolution kinetics in human, animal, and simulated bile, effect of interfacial resistance

Chenodeoxycholic acid is an effective agent for cholesterol gallstone dissolution in humans (1), but relatively lengthy treatment times are necessary. In a recent evaluation of 243 patients (1), treatment times of 8–24 months were required to obtain complete gallstone dissolution. Consequently, recent investigations (2–8) have been di-