REFERENCES

(1) R. G. Danzinger, A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle, *N. Engl. J. Med.*, **286**, 1 (1972).

(2) J. L. Thistle and A. F. Hofmann, *ibid.*, **289**, 655 (1973).

(3) R. G. Danzinger, A. F. Hofmann, J. L. Thistle, and L. J. Schoenfield, *J. Clin. Invest.*, **52**, 2809 (1973).

(4) G. D. Bell, B. Whitney, and R. H. Dowling, *Lancet*, **2**, 1213 (1972).

(5) W. H. Admirand and D. M. Small, *J. Clin. Invest.*, **47**, 1043 (1968).

(6) D. M. Small, *Adv. Intern. Med.*, **16**, 243 (1970).

(7) H. Dam and F. G. Hegardt, *Z. Ernaehrungswiss.*, **10**, 239 (1971).

(8) D. H. Gregory, Z. R. Vlahcevic, and L. Swell, *Am. J. Dig. Dis.*, **19**, 268 (1974).

(9) W. I. Higuchi, F. Sjuib, D. Mufson, A. P. Simonelli, and A. F. Hofmann, *J. Pharm. Sci.*, **62**, 942 (1973).

(10) W. I. Higuchi, S. Prakongpan, V. Surpuriya, and F. Young, *Science*, **178**, 633 (1972).

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

(12) "Geigy Scientific Tables," 7th ed., Geigy Pharmaceuticals, Ardsley, N.Y., 1973, pp. 653–656.

(13) A. Berthoud, *J. Chim. Phys.*, **10**, 624 (1912).

(14) W. Nernst and E. Brunner, *Z. Phys. Chem.*, **47**, 52 (1904).

(15) D. P. Gregory and A. C. Riddiford, *J. Chem. Soc.*, **1956**, 3756.

(16) V. G. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962.

(17) S. Prakongpan, W. I. Higuchi, K. H. Kwan, and A. M. Molokhia, *J. Pharm. Sci.*, **65**, 685 (1976).

(18) S. Prakongpan, Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1974.

(19) C. V. King and S. S. Brodie, *J. Am. Chem. Soc.*, **59**, 1375 (1957).

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

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

(22) P. P. Nair and D. Kritchevsky, "The Bile Acids," vol. 1, Plenum, New York, N.Y., 1971.

(23) A. T. James and L. J. Morris, "New Biochemical Separations," Van Nostrand, London, England, 1964, chap. 10.

(24) H. Bogren and K. Larsson, *Biochim. Biophys. Acta*, **75**, 65 (1963).

(25) A. Norman, *Ark. Kemi*, **8**, 331 (1955).

(26) A. F. Hofmann, *Acta Chem. Scand.*, **17**, 173 (1963).

(27) J. L. Pope, *J. Lipid Res.*, **8**, 146 (1967).

(28) A. F. Hofmann, *ibid.*, **3**, 127 (1962).

(29) W. S. Singleton, M. S. Gray, M. L. Brown, and J. L. White, *J. Am. Oil Chem. Soc.*, **42**, 53 (1965).

(30) R. C. Sehlin, E. L. Cussler, and D. F. Evans, *Biochim. Biophys. Acta*, **388**, 385 (1975).

(31) J. C. Tao, E. L. Cussler, and D. F. Evans, *Proc. Natl. Acad. Sci. USA*, **71**, 3917 (1974).

(32) W. I. Higuchi, S. Prakongpan, and F. Young, *J. Pharm. Sci.*, **62**, 1207 (1973).

(33) W. E. Ranz and W. R. Marshall, *Chem. Eng. Progr.*, **48**, 146 (1952).

(34) A. P. Simonelli, D. R. Flanagan, and W. I. Higuchi, *J. Pharm. Sci.*, **57**, 1629 (1968).

(35) H. Dam, I. Kruse, H. E. Kallehauge, O. E. Hartkopp, and M. K. Jensen, *Scand. J. Clin. Lab. Invest.*, **18**, 385 (1966).

(36) Z. R. Vlahcevic, C. C. Bell, and L. Swell, *Gastroenterology*, **59**, 62 (1970).

(37) D. M. Small and S. Rapo, *N. Engl. J. Med.*, **283**, 53 (1970).

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

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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-

Table I—Effect of Centrifugation on Human Gallbladder Bile Lipids

Bile Sample	Before Centrifugation: Bile Acids, μmoles/ml	Lecithin, μmoles/ml	Bile Acid/ Lecithin Ratio	Cholesterol, μmoles/ml	After Centrifugation: Bile Acids, μmoles/ml	Lecithin, μmoles/ml	Bile Acid/ Lecithin Ratio	Cholesterol, μmoles/ml
1	195.0	57.1	3.4	9.6	194.1	41.3	4.7	6.1
2	95.4	36.8	2.6	13.4	77.2	34.4	2.3	6.4
3	232.4	80.9	2.9	35.5	219.0	82.9	2.6	13.8

rected toward a better understanding of the kinetics of cholesterol gallstone dissolution and of methods for accelerating stone dissolution in patients under chenodeoxycholic acid therapy and in other situations, *e.g.*, the dissolution of retained common duct stones.

The physical model approach by Higuchi *et al.* (2) suggested that the dissolution of cholesterol gallstones *in vivo* may be rate limited by surface resistance to micellar solubilization rather than by the conventional diffusion–convection mass transfer of cholesterol molecules from the stone surface into the bile media. Further investigations (3–7) showed that *in vitro* cholesterol dissolution in simulated bile solutions was 20–100 times slower than predicted by the diffusion–convection-controlled mechanism. That the rate-limiting step for dissolution could be a surface reaction also was supported by the finding (6, 7) that the addition of a small amount of a dissolution rate accelerator for cholesterol, such as benzalkonium chloride, dramatically enhanced the dissolution process to a point where the rates were almost identical to those predicted by the physical model for "diffusion-controlled" kinetics.

The primary purpose of this study was to examine the cholesterol monohydrate dissolution kinetics in human gallbladder bile to determine the magnitudes of the *in vitro* dissolution rates and the rate resistances for cholesterol monohydrate pellets in human gallbladder bile compared to simulated bile. Dissolution rate experiments also were conducted using human bile samples obtained by duodenal drainage as well as gallbladder bile from several animals.

EXPERIMENTAL

Materials—*Bile*—Human gallbladder bile was collected by aspiration from patients undergoing cholecystectomy and was frozen. Before the experiments, the human gallbladder bile samples were thawed and centrifuged[1] for 30 min at 100,000×*g*, and the supernates were used. One sample was taken from a patient receiving chenodeoxycholic acid therapy; the others were from regular patients. Human duodenal bile was collected by duodenal aspiration after cholecystokinin injection from patients who were receiving chenodeoxycholic acid for about 1 year. The bile was then filtered through a 0.22-μm filter[2] and frozen prior to use.

Baboon gallbladder bile[3] from control animals and from animals fed with chenodeoxycholic acid, dog gallbladder bile[4], and chicken gallbladder bile[5] were filtered through 0.22-μm filters[2] and frozen prior to use.

Cholesterol—4-^{14}C-Cholesterol monohydrate was recrystallized from 95% ethanol (3). For the dissolution experiments, about 100 mg of cholesterol monohydrate crystals was compressed[6] in a 1.27-cm diameter die under a pressure of 1362 kg (3000 lb).

General Design Considerations—To achieve the primary goals of this study, it would have been ideal to experiment with freshly collected human gallbladder bile. However, for two important reasons, fresh bile was not used.

First, most specimens of human gallbladder bile under normal conditions are nearly saturated or supersaturated with cholesterol. Therefore, dissolution rate experiments would be either impossible or practically unsuitable unless the cholesterol levels were lowered significantly by some means without seriously altering the other bile constituents.

Second, the bile specimens had to be shipped several hundred miles by air and some means for preservation was needed. Freezing of the bile, thawing, and then centrifuging prior to the experiments solved both of these problems. Invariably, the cholesterol levels of the supernates were significantly lowered while the phospholipid and bile acid levels were altered little or none at all by this procedure. Also, the transporting of the bile was convenient and routine. Bile samples were received frozen and were transferred immediately to a freezer for storage until the experiment.

Because of the relatively small amounts (~15 ml) of human gallbladder bile available from each patient and because future plans include evaluating patient-to-patient variations in the cholesterol dissolution rate properties of bile, a smaller dissolution apparatus than used previously (3) was designed. It consisted of similar components, *i.e.*, a stirrer attached to a constant-speed motor (150 rpm), a water-jacketed beaker, and a die holding the compressed cholesterol monohydrate pellet. The hydrodynamic conditions were assessed from the dissolution rate of a benzoic acid pellet in 0.01 *N* hydrochloric acid solution, which is known to be a diffusion–convection-controlled process (9).

Effect of Centrifugation on Human Gallbladder Bile Lipids—Three human gallbladder bile samples were thawed for 1 hr at 37°, and then each was divided into two portions. One was immediately centrifuged[1] for 30 min at 100,000×*g*, and then 1.0 ml of the supernate was pipetted into 9 ml of 2-propanol for lipid analysis. The second portion was agitated on a wrist-action shaker[7] at 37° for 24 hr to disperse any insoluble matter. Then 1.0 ml of the mixture was pipetted into 9 ml of 2-propanol for analysis.

Solubility Determination—About 30 mg of ^{14}C-cholesterol monohydrate crystals was added to 2 ml of bile in a 15-ml firmly stoppered test tube, and the suspension was continuously agitated in a wrist-action shaker[7]. Samples of 0.1 ml were taken after 3 days and every other day thereafter until the cholesterol analysis indicated a constant solubility. Each sample was filtered through glass wool before analysis in a liquid scintillation counter[8]. These data represented the differences between the actual solubility, C_s, and the initial concentration of cholesterol, C_b. In addition, both C_b and C_s for cholesterol were determined for a number of samples by the colorimetric method of Sperry and Webb (10).

Dissolution Rate Determination—Exactly 10 ml[9] of bile was used for each run, and the cholesterol dissolution experiments were carried out as described previously (3), except that 0.2-ml samples were taken for determining the total amount dissolved at each time interval by means of liquid scintillation counting.

Diffusivity Measurements—The small diaphragm cell employed previously (3, 7) was used for the determination of cholesterol diffusivity in human duodenal and dog gallbladder bile samples.

Analysis of Biliary Lipids—Total bile acids were determined using an automated steroid dehydrogenase procedure (11). Phospholipids were determined colorimetrically (12) using an ethanol–ether solution of bile. Total cholesterol was determined colorimetrically after saponification and extraction into hexane using the method of Abell *et al.* (13). The solubilities of cholesterol in several bile samples were calculated based on their bile acid and lecithin contents (14) using the polynomial equations derived by Thomas and Hofmann (15).

[1] Model L3-40 ultracentrifuge, Beckman Instruments, Fullerton, Calif.
[2] Millipore Filter Corp., Bedford, Mass.
[3] Dr. C. K. McScherry, New York Hospital, Cornell Medical Center, New York, N.Y.
[4] Pel Freez Biologicals Inc., Rogers, Ark.
[5] Eastern Poultry Co., Detroit, Mich.
[6] Model B lab press, Fred Carver Inc., Summit, N.J.
[7] Burrel Corp., Pittsburgh, Pa.
[8] Beckman Instruments, Fullerton, Calif.
[9] The volume was 5 ml for baboon bile and the human gallbladder bile of the chenodeoxycholic acid-treated patient.

Table II—Comparison of the Cholesterol Solubility Determined Experimentally and the Value Calculated from the Polynomial Equation of Thomas and Hofmann (15)

Bile Sample	Bile Acids, μmoles/ml	Lecithin, μmoles/ml	Bile Acid/ Lecithin Ratio	Cholesterol C_s, μmoles/ml (Colorimetry)	Cholesterol Equivalent C_s, μmoles/ml (Calculated)
1	194.1	41.3	4.7	12.9	13.2
2	133.7	24.9	5.4	9.7	8.2
3	237.2	64.9	3.7	18.1	19.8
4	85.4	21.3	4.0	5.2	6.6

THEORETICAL

According to the Nernst diffusion theory (16), the dissolution rate of a solid solute into a liquid solvent is given by:

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

where J is the rate of dissolution, A is the area of solid–liquid contact, D is the diffusion coefficient, h is the effective diffusion layer thickness, and C_s and C_b are as previously defined.

While the factor h/D is a purely diffusional resistance in the Nernst theory, it is generally recognized (7) that, when applied to dissolution rate situations such as in this work, this factor actually represents contributions from both diffusion and convection.

For the case where the resistance to the dissolution process is associated with interactions at the crystal surface, the dissolution rate can be expressed as (2):

$$J/A = P(C_s - C_b) \quad \text{(Eq. 2)}$$

with $1/P$ representing the interfacial resistance, and P is the effective permeability coefficient across the interfacial barrier.

When both resistances—the diffusion–convection resistance and the interfacial resistance—are important, the overall dissolution rate can be given as (17):

$$J/A = \frac{C_s - C_b}{\frac{h}{D} + \frac{1}{P}} \quad \text{(Eq. 3)}$$

or:

$$J/A = \frac{C_s - C_b}{R} \quad \text{(Eq. 4)}$$

and:

$$R = \frac{h}{D} + \frac{1}{P} \quad \text{(Eq. 5)}$$

where R is the total resistance to dissolution.

When $1/P$ is negligible, Eq. 3 reduces to Eq. 1. On the other hand, when the dissolution rate is controlled by the interfacial barrier, $1/P$ is large compared to h/D and Eq. 3 reduces to Eq. 2.

In a given dissolution apparatus in which the hydrodynamics remain constant, the effective diffusion–convection resistance, h/D, for a compressed pellet dissolving in a solvent can be estimated independently. The diffusion coefficient, D, can be measured directly using a diffusion cell. The effective diffusion layer thickness, h, may be determined for a benzoic acid pellet dissolving in 0.01 N hydrochloric acid, and the corresponding h value for the particular system under investigation can then be deduced from $h \propto D^n$, where n varies from zero to one-third[10]. As will be seen in this study, the choice of the n value does not make much difference because the resistance for cholesterol dissolution in human and animal bile is always on the high side where the h/D contribution to R is relatively small.

Thus, for each dissolution experiment, if the resistance, R, calculated from the experimental results of J/A, C_s, and C_b with Eq. 4 approaches the estimated h/D value, the dissolution process is said to be fast and controlled by diffusion across the diffusion layer. If, on the other hand, the R value is significantly larger than h/D, the interfacial resistance may be important and the dissolution process is said to be slower than a diffusion-controlled process by a factor $R/(h/D)$.

[10] According to the Levich theory (18) and recent investigations with the rotating-disk dissolution apparatus (7), an h dependency on $D^{1/3}$ is obeyed. Also, for a static disk dissolution apparatus similar to the one used in this study, Prakongpan (19) recently showed that $h \propto D^{1/3}$ is a better approximation than $h \propto D^0$.

RESULTS

Effect of Centrifugation on Bile Lipids—Table I shows the effect of centrifugation on the total bile acids, lecithin, and cholesterol contents of three human gallbladder bile samples after freezing and thawing. While the changes in the bile acids and lecithin contents were relatively small, if any, the cholesterol contents were reduced significantly. The bile acid–lecithin ratios after centrifugation (Table I) remained within the physiological range.

Hydrodynamics and h/D Value for Micellar Cholesterol in Dissolution Apparatus—The baseline dissolution experiments with benzoic acid showed a linear relationship between the amount dissolved and time with a slope, J, equal to 1.23×10^{-2} mg/sec. By using Eq. 1 and substituting for the experimentally determined values of $J/A = 9.7 \times 10^{-3}$ mg/cm^2/sec, $C_s = 4.7$ mg/cm^3, and $D = 1.4 \times 10^{-5}$ cm^2/sec, an h value of 6.8×10^{-3} cm was obtained.

Because of the availability of only relatively small amounts of the human gallbladder bile and because the h/D term generally contributed little to the total resistance, R, for most situations, the diffusivity experiments with micellar cholesterol were carried out with only a few representative samples. A diffusivity value of $(1.0 \pm 0.3) \times 10^{-6}$ cm^2/sec was found with several human duodenal and dog bile specimens. This value was used to estimate the diffusion–convection resistance, h/D, for all bile samples tested. When using Eq. 6 with $n = 1/3$ and the benzoic acid dissolution data, an effective diffusion layer thickness of 2.82×10^{-3} cm was obtained for the cholesterol–bile micellar system. This value corresponds to an h/D value of 2.82×10^3 sec/cm.

Cholesterol Monohydrate Dissolution in Human Gallbladder Bile—Table II compares the cholesterol solubilities, C_s, determined colorimetrically for some bile samples with the values calculated as described previously (15) from the Hegardt and Dam (14) relationship based on the bile acid and lecithin contents of these bile specimens. The experimental and calculated values were in good agreement.

Table III summarizes the dissolution rates, solubilities, and resistances to dissolution of cholesterol monohydrate in 10 human gallbladder bile samples. For the three samples in which the cholesterol monohydrate values of $(C_s - C_b)$ were determined both colorimetrically and by the ^{14}C-analysis, good agreement between the two techniques was obtained. The R values, calculated using Eq. 4, ranged from 10×10^3 to 400×10^3 sec/cm. Bile Sample 8, obtained from a patient receiving chenodeoxycholic acid therapy, showed an R value of 40.3×10^3 sec/cm, which was in the range for the normals studied in this investigation.

As discussed, the h/D value was 2.82×10^3 sec/cm for these experiments. Thus, $(h/D) \ll (1/P)$ for essentially all R values given in Table III. Therefore, the interfacial resistance factor dominated the dissolution kinetics with all tested samples.

Cholesterol Monohydrate Dissolution in Human Duodenal Bile—Table IV shows the results of cholesterol monohydrate dissolution in two human duodenal bile samples. The dissolution rates were much lower and the R values were much larger with the duodenal bile than with the gallbladder bile. Experiments with other human duodenal bile samples gave R values that were always at least as large as those shown in Table IV. The addition of 1.25% benzalkonium chloride to the two samples increased the $(C_s - C_b)$ value up to sixfold and decreased R by about 100-fold.

Cholesterol Monohydrate Dissolution in Animal Bile—The data presented in Table V show that the resistance to cholesterol monohydrate dissolution was the highest in baboon bile and the lowest in chicken bile. The bile collected from chenodeoxycholic acid-fed baboons showed about the same resistance as that of the bile collected from normal baboons.

The two dog gallbladder bile samples examined gave almost the same resistance values. While the addition of 1.25% benzalkonium chloride to Sample I showed no effect on cholesterol solubility, a threefold decrease in the resistance was observed. The effect of dilution on cholesterol dissolution was studied by diluting an aliquot of Sample I with an equal

Table III—Dissolution Rates, Solubilities, and Transport Resistances of Cholesterol Monohydrate in Human Gallbladder Bile

Sample	Bile Acid/ Lecithin Ratio	$(J/A) \times 10^4$, mg/cm²/sec	Cholesterol after Centrifuging, C_b, μmoles/ml (Colorimetry)	Solubility, C_s, μmoles/ml (Colorimetry)	$C_s - C_b$, μmoles/ml (Radiometry)	$C_s - C_b$, μmoles/ml (Colorimetry)	$R^a \times 10^{-3}$, sec/cm
1	4.70	1.23	3.88	12.9	8.5	9.02	26.63
2	—	0.05	—	—	0.623	—	48.2
3	—	0.38	3.88	—	5.2	—	52.84
4	5.38	0.44	4.20	9.7	3.45	5.5	30.59
5	3.65	1.13	7.95	18.1	11.75	10.15	40.13
6	4.00	0.391	—	5.2	1.00	—	9.87
7	4.46	1.42	7.75	—	9.02	—	24.50
8[b]	3.71	0.27	—	—	2.84	—	40.30
9	2.29	0.3125	—	—	14.53	—	187.8
10	2.93	0.0684	—	—	6.98	—	412.3

[a] Calculated from Eq. 4 and using $(C_s - C_b)$ values determined by radiolabeling. [b] Patient was receiving chenodeoxycholic acid.

volume of double-distilled water and by using the resulting mixture for cholesterol dissolution and solubility determinations. The dissolution rate in the diluted sample was about 11 times slower than that in the original sample due to a decrease in the cholesterol solubility and an increase in the resistance to dissolution.

Chicken gallbladder bile offered only a modest resistance to cholesterol dissolution of 11.5×10^3 sec/cm.

DISCUSSION

These results, the first of their kind, provide the first insights regarding the possible importance of the interfacial resistance in cholesterol gallstone dissolution in real bile. These data, together with results obtained for chemically defined "synthetic bile," should permit the assessment of whether the kinetics of gallstone dissolution may be explained and predicted on the basis of the bile acids, lecithin, and the electrolytes in the bile.

Correlation with Studies in Synthetic Bile—The ranges of the R values found in this study with human and animal bile samples were consistent with those found by Kwan *et al.* (20) with the synthetic systems. The R values for the human gallbladder biles were in the range of $25\text{–}400 \times 10^3$ sec/cm. These values were within about a factor of two of predictions (20) based upon the R values for cholesterol monohydrate dissolution in lecithin solutions of taurocholate, glycocholate, taurochenodeoxycholate, and glycochenodeoxycholate at various electrolyte concentrations. Thus, while more experiments under a wider range of conditions are required, the indications so far are that the kinetics of cholesterol gallstone dissolution may be explainable on the basis of the principal bile acids, lecithin, and the electrolytes in any particular bile.

The unusually high R values found with human gallbladder bile Samples 9 and 10 are particularly noteworthy. The significant difference between these samples and the others was the bile acid–lecithin ratio. Both samples had rather low ratios—*viz.*, 2.3 and 2.9. Although other factors, such as the electrolytes and the bile acid concentration, must be considered, the much higher R values were consistent with the trends found by Kwan *et al.* (20) with the chemically defined synthetic systems.

The human duodenally aspirated biles (Table IV) gave R values much larger than those of most gallbladder bile samples in Table III. The bile acid and lecithin concentrations and the bile acid–lecithin ratios were not determined for these samples. According to the results of Kwan *et al.* (20) for chemically defined media, however, both of these variables may significantly influence the R values. Other evidence (21) confirms that the duodenally aspirated bile is indeed more dilute (two to four times) than gallbladder bile, and the difference in R values could well be explained by the bile acid and lecithin levels as seen with the synthetic systems (20). As shown in Table V, a twofold dilution with dog bile yielded a fourfold increase in R. Clearly, however, more studies are needed to answer this question.

The results with the animal biles were also consistent with the ranges of R values observed (20) for the synthetic systems. Again, in the absence of chemical analysis data, more cannot be said at this time since several variables may be at work simultaneously.

Possible *In Vivo* Implications—The R values of $25\text{–}400 \times 10^3$ sec/cm given in Table III correspond to 10–200 times the diffusion–convection resistance for cholesterol monohydrate dissolution in the present *in vitro* experiments. Therefore, it can be said that the rates are 90–99% "surface reaction controlled" for this situation. A clinically important question is what the effect of diffusion–convection resistance might be *in vivo*. Recent assessments (20) of this problem suggest that the order of magnitude of the time average $(h/D)_{in\ vivo}$ may be 16×10^3 sec/cm. This would mean that R values of $25\text{–}400 \times 10^3$ sec/cm represent 2–25 times the diffusion–convection resistance factor. Thus, gallstone dissolution acceleration of two- to 25-fold might be maximally achievable for these cases *in vivo*.

The effectiveness of the cholesterol gallstone accelerator benzalkonium chloride is demonstrated in Table IV. The addition of 1.25% of this compound in human duodenal bile taken from patients receiving chenodeoxycholic acid treatment showed a 500-fold acceleration in rate and about a 100-fold decrease in R. The observed R value of around 4×10^3 sec/cm was within a factor of two of purely diffusion–convection-controlled kinetics for these *in vitro* experiments.

Table IV—Dissolution Rates, Solubilities, and Transport Resistances of Cholesterol Monohydrate in Human Duodenal Biles

Bile Sample[a]	$(J/A) \times 10^4$, mg/cm²/sec	$\Delta C(C_s - C_b)$, mg/ml	$R \times 10^{-3}$, sec/cm
I	0.0191	0.91	483
II	0.0124	0.46	330
I plus 1.25% benzalkonium chloride	9.7	4.20	4.3
II plus 1.25% benzalkonium chloride	7.9	2.85	3.6

[a] Bile pooled from patients after about 1 year of chenodeoxycholic acid therapy.

Table V—Dissolution Rates, Solubilities, and Transport Resistances of Cholesterol Monohydrate in Animal Bile

Animal Bile	$(J/A) \times 10^4$, mg/cm²/sec	$\Delta C(C_s - C_b)$, mg/ml	$R \times 10^{-3}$, sec/cm
Control baboon	0.072	0.74	102.8
Chenodeoxycholic acid-treated baboon	0.248	2.24	90.3
Dog I	2.71	7.60	28
Dog II	1.15	3.09	27
Dog I plus 1.25% benzalkonium chloride	9.20	7.80	8.5
Dog I, 2× dilution	0.24	2.80	120
Chicken	2.88	3.30	11.5

REFERENCES

(1) A. F. Hofmann and G. Paumgartner, "Chenodeoxycholic Acid Therapy of Gallstones," Progress Report 1975, Schattauer Verlag, Stuttgart, West Germany, 1975.

(2) W. I. Higuchi, F. Sjuib, D. Mufson, A. P. Simonelli, and A. F. Hofmann, *J. Pharm. Sci.*, **62**, 942 (1973).

(3) W. I. Higuchi, S. Prakongpan, and F. Young, *ibid.*, **62**, 945 (1973).

(4) W. I. Higuchi, S. Prakongpan, V. Surpuriya, and F. Young,

Science, **178**, 633 (1972).
(5) W. I. Higuchi, V. Surpuriya, S. Prakongpan, and F. Young, *J. Pharm. Sci.*, **62**, 695 (1973).
(6) W. I. Higuchi, S. Prakongpan, and F. Young, *ibid.*, **62**, 1207 (1973).
(7) S. Prakongpan, W. I. Higuchi, K. H. Kwan, and A. M. Molokhia, *ibid.*, **65**, 685 (1976).
(8) A. M. Molokhia, W. I. Higuchi, and A. F. Hofmann, *ibid.*, **64**, 2029 (1975).
(9) C. V. King and S. S. Brodie, *J. Am. Chem. Soc.*, **59**, 1375 (1937).
(10) W. M. Sperry and M. Webb, *J. Biol. Chem.*, **187**, 97 (1950).
(11) R. H. Palmer, in "Methods in Enzymology," vol. 15, S. P. Colowick and N. O. Kaplan, Eds., Academic, New York, N.Y., 1969, p. 280.
(12) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).
(13) L. L. Abell, B. B. Levy, B. B. Brodie, and F. E. Kendall, *ibid.*, **195**, 357 (1952).
(14) F. Hegardt and H. Dam, *Z. Ernaehrungswiss.*, **10**, 223 (1971).
(15) P. J. Thomas and A. F. Hofmann, *Gastroenterology*, **65**, 698 (1973).
(16) W. Nernst and E. Brunner, *Z. Phys. Chem.*, **47**, 52 (1904).
(17) A. Berthoud, *J. Chim. Phys.*, **10**, 624 (1912).
(18) V. G. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962.
(19) S. Prakongpan, Ph.D. dissertation, University of Michigan, Ann Arbor, Mich., 1974.
(20) K. H. Kwan, W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann, *J. Pharm. Sci.*, **66**, 1094 (1977).
(21) H. Dam, I. Kruse, I. Prange, H. E. Kallehauge, H. J. Fenger, and M. K. Jensen, *Z. Ernaehrungswiss.*, **10**, 160 (1971).

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Cholesterol Gallstone Dissolution Rate Accelerators I: Exploratory Investigations

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Abstract □ Various compounds that might function as cholesterol gallstone dissolution accelerators were studied. The dissolution rates of cholesterol monohydrate pellets in synthetic bile (116 m*M* sodium cholate–32 m*M* lecithin) containing the agent at various concentration levels were determined. In the absence of any dissolution rate accelerator, the dissolution kinetics for cholesterol previously were found to be interfacial resistance controlled, and the rates were around 20 times less than the diffusion-controlled rates in the present experiments. Primary, secondary, and tertiary amines and quaternary ammonium compounds were effective accelerators. When the alkyl chain lengths were long enough and/or when the agent concentrations were high enough, the dissolution rates generally approached diffusion-controlled rates. Steroidal amines generally had good activity. Anionic and nonionic surfactants had little or negative activity.

Keyphrases □ Cholesterol monohydrate—pellets, dissolution rate in synthetic bile, effect of various nitrogen-containing compounds and surfactants □ Dissolution rate—cholesterol monohydrate pellets, effect of various nitrogen-containing compounds and surfactants □ Gallstones, model—cholesterol monohydrate pellets, dissolution rate in synthetic bile, effect of various nitrogen-containing compounds and surfactants □ Bile, synthetic—dissolution rate of cholesterol monohydrate pellets, effect of various nitrogen-containing compounds and surfactants □ Amines and ammonium salts, various—effect on dissolution rate of cholesterol monohydrate pellets in synthetic bile □ Surfactants, various—effect on dissolution rate of cholesterol monohydrate pellets in synthetic bile □ Steroids—cholesterol monohydrate pellets, dissolution rate in synthetic bile, effect of various nitrogen-containing compounds and surfactants

Recent investigations (1–7) showed that a substantial interfacial resistance (or interfacial barrier) is associated with the *in vitro* dissolution of cholesterol gallstones and cholesterol monohydrate pellets in human and simulated bile (bile acid–lecithin solutions). The magnitudes of these transport resistances may be large enough (6, 7) to affect significantly the rate of cholesterol stone dissolution *in vivo*. Accordingly, rate accelerators might have therapeutic value in decreasing the time required for total gallstone dissolution in patients receiving chenodeoxycholic acid.

The purposes of this study were to explore the kinds of compounds that might function as cholesterol gallstone dissolution accelerators and to begin to define structure–activity relationships.

EXPERIMENTAL

Materials—Commercial cholesterol[1] was recrystallized three times 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 (8). X-ray crystallography[3] studies indicated that the crystals were indeed cholesterol monohydrate crystals and that they had a lattice system similar to that of cholesterol found in human biliary calculi (9). If exposed to low humidity and light, these crystals lose their water content readily.

Sodium cholate[4] and the amines[1,4,5,6] were analytical grade and were

[1] Eastman Kodak Co., Rochester, N.Y.
[2] New England Nuclear Corp., Boston, Mass.
[3] Performed by Dr. C. Nordman, Department of Chemistry, University of Michigan, Ann Arbor, Mich.
[4] Schwarz Mann, Orangeburg, N.Y.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Lonza Inc., Fair Lawn, N.J.