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* To whom inquiries should be directed.

Cholesterol Gallstone Dissolution Rate Accelerators I: Exploratory Investigations

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

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.

Table I—Effect of Primary Amines (34 m*M*) on Dissolution Rate of Cholesterol Monohydrate in 116 m*M* Sodium Cholate–32 m*M* Lecithin Solution Buffered with 0.10 *M* Phosphate at pH 7.40 and 37°

Compound	$(J/A) \times 10^4$, mg/cm² sec	C_s, mg/ml	$D \times 10^6$, cm²/sec	$R = \left(\frac{h^a}{D} + \frac{1}{P}\right) \times 10^{-3}$, sec/cm	$(h/D) \times 10^{-3}$, sec/cm	$(1/P) \times 10^{-3}$, sec/cm
—	0.96	3.00	1.50	31.5	1.84	29.66
Ia	4.77	3.20	1.50	6.71	1.84	4.87
Ib	5.98	3.70	1.44	6.19	1.92	4.27
Ic	5.35	3.52	1.41	6.58	1.96	4.62
Id	7.40	4.25	1.35	5.74	2.04	3.70
Ie	12.75	4.93	1.29	3.87	2.14	1.73

[a] h = 27.6μm for the prevailing hydrodynamic conditions.

used as received. Egg lecithin was prepared by the method of Small *et al.* (10), except that commercial egg lecithin of purified grade[4] was used as the starting material. The cholylamine[7], 20,25-diazacholesterol hydrochloride[7], and 3-amino-7α,12α-dihydroxy-5β-cholanoic acid[8] (12) were used as received.

Cholylcholamine was synthesized[9] as described by Lack and Weiner (11). 6-Aminohexanoic acid[1], sodium *N*-lauroyl sarcosinate[10], polysorbate 80[11], sodium lauryl sulfate[5], and sodium laurate[5] were analytical grade and were used as received.

Methods—The procedures adopted for the determination of the cholesterol monohydrate solubility, dissolution rate, and diffusivity in the solvent media were described previously (1). In this study, a synthetic bile system of 116 m*M* sodium cholate and 32 m*M* lecithin in 100 m*M* phosphate buffer, pH 7.4, was used. This system is believed to simulate the average human gallbladder bile concentrations of total bile acids and lecithin as well as the pH.

The experimental data are presented as J/A, the dissolution rate per unit surface area; C_s, the equilibrium solubility; and R, the total resistance to cholesterol dissolution calculated from the following equation:

$$\frac{J}{A} \approx \frac{C_s - C_b}{R} \qquad \text{(Eq. 1)}$$

with C_b, the initial cholesterol concentration in the bulk, equal to zero.

Equation 1 was derived by Berthoud (13), and R was given as:

$$R = \frac{h}{D} + \frac{1}{P} \qquad \text{(Eq. 2)}$$

where h is the effective diffusion layer thickness, D is the diffusivity, and P is the effective permeability coefficient of the interfacial barrier. The terms h/D and $1/P$ represent the contribution to the total resistance from the diffusion–convection and the interfacial barriers, respectively.

RESULTS

Straight-chain aliphatic primary (Ia–Ie), secondary (IIa and IIb), and tertiary (IIIa–IIIc) amines; quaternary ammonium salts (IVa–IVd, Va, and Vb); steroidal amines (VIa–VId); long chain nitrogen-containing compounds [6-aminohexanoic acid (VIIa) and sodium *N*-lauroyl sarcosinate (VIIb)]; one nonionic surfactant (polysorbate 80); and two anionic surfactants (sodium lauryl sulfate and sodium laurate) were screened for acceleration activity. Table I shows the effects on cholesterol dissolution rate, solubility, and dissolution resistance when primary alkylamines were added to the synthetic bile system. The addition of 34 m*M* *n*-hexylamine (Ia) to the sodium cholate–lecithin solution lowered the total resistance, R, by a factor of about 5. Increasing the number of carbon atoms of the amine and keeping the concentration constant decreased R and $1/P$. However, from C_6 to C_9 (Ia–Ic), these decreases with increasing chain length were rather modest—*viz.*, 10–20% in R per carbon atom. A much larger effect upon R was observed for the C_{10} (Id) and C_{12} (Ie) amines.

With the addition of 34 m*M* of the amine, only moderate increases in the solubility were observed in all cases. The addition of the amines to the synthetic bile system had little or no effect on the diffusivity of cholesterol. Thus, the reduction in the total resistance to cholesterol dissolution in the presence of these amines could be attributed primarily to the reduction in the interfacial resistance ($1/P$), with the diffusion–convection resistance remaining almost constant.

Tables II and III show the concentration effect of *n*-octylamine (Ib) and *n*-dodecylamine (Ie), respectively, on cholesterol dissolution. The R values decreased with increasing amine concentration. The effect on $1/P$ began to level off at concentrations above 30 m*M*.

Table IV shows the effect of the presence of 34 m*M* of some secondary and tertiary amines and quaternary ammonium salts. The resistance to

$C_nH_{2n+1}NH_2$

Ia: n = 6
Ib: n = 8
Ic: n = 9
Id: n = 10
Ie: n = 12

$C_nH_{2n+1}NH(CH_3)$

IIa: n = 8
IIb: n = 12

$C_nH_{2n+1}N(CH_3)_2$

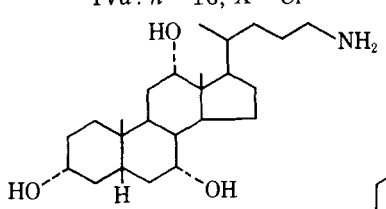
IIIa: n = 8
IIIb: n = 10
IIIc: n = 12

$C_nH_{2n+1}N^+(CH_3)_3$ X^-

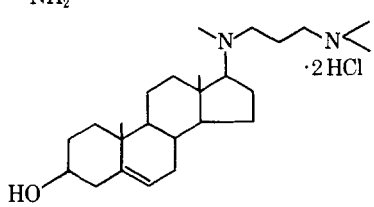
IVa: n = 12, X = Br
IVb: n = 12, X = Cl
IVc: n = 16, X = Br
IVd: n = 16, X = Cl

$(C_nH_{2n+1})_2N^+(CH_3)_2$ Cl^-

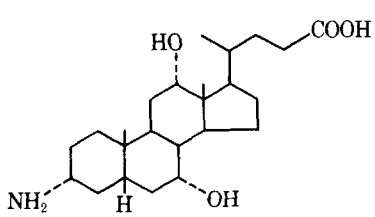
Va: n = 8
Vb: n = 10



VIa



VIb



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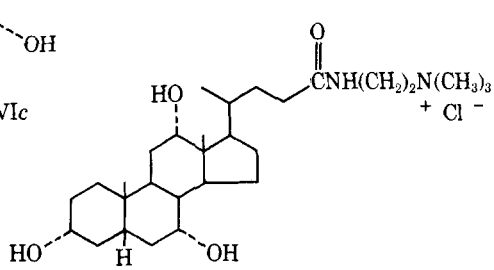
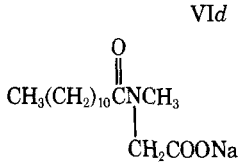


VId



VIIb

[7] Supplied by Dr. R. Counsell, Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Mich.
[8] Supplied by Dr. A. S. Jones, Sheffield University.
[9] D. T. E. Belobaba, Mayo Clinic.
[10] Proctor and Gamble Co., Cincinnati, Ohio.
[11] Tween 80, Sargent-Welch Scientific Co., Detroit, Mich.

Table II—Effect of Concentration of I*b* on Cholesterol Monohydrate Dissolution in 116 m*M* Sodium Cholate–32 m*M* Lecithin Solution Buffered with 0.10 *M* Phosphate at pH 7.40 and 37°

Concentration, m*M*	$(J/A) \times 10^4$, mg/cm² sec	C_s, mg/ml	$D \times 10^6$, cm²/sec	$R = \left(\frac{h}{D} + \frac{1}{P}\right) \times 10^{-3}$, sec/cm	$(h/D) \times 10^{-3}$, sec/cm	$(1/P) \times 10^{-3}$, sec/cm
—	0.96	3.00	1.50	31.5	1.84	29.66
3.61	1.22	3.10	1.30	25.4	2.12	23.28
13.23	3.05	3.23	1.40	10.6	1.97	8.63
21.64	5.48	3.60	1.40	6.60	1.97	4.63
34.0	5.98	3.70	1.44	6.19	1.92	4.27

Table III—Effect of Concentration of I*e* on Cholesterol Monohydrate Dissolution in 116 m*M* Sodium Cholate–32 m*M* Lecithin Solution Buffered with 0.10 *M* Phosphate at pH 7.40 and 37°

Concentration, m*M*	$(J/A) \times 10^4$, mg/cm² sec	C_s, mg/ml	$D \times 10^6$, cm²/sec	$R = \left(\frac{h}{D} + \frac{1}{P}\right) \times 10^{-3}$, sec/cm	$(h/D) \times 10^{-3}$, sec/cm	$(1/P) \times 10^{-3}$, sec/cm
—	0.96	3.00	1.50	31.5	1.84	29.66
2.88	0.92	3.08	1.30	33.5	2.12	31.38
9.38	2.49	3.11	1.30	12.50	2.12	10.38
34.0	12.75	4.93	1.29	3.87	2.14	1.73
84.31	19.90	6.33	1.25	3.18	2.21	0.97

Table IV—Effect of 34 m*M* of Secondary and Tertiary Amines and Quaternary Ammonium Salts on Dissolution

Compound	$(J/A) \times 10^4$, mg/cm² sec	C_s, mg/ml	$D \times 10^6$, cm²/sec	$R = \left(\frac{h}{D} + \frac{1}{P}\right) \times 10^{-3}$, sec/cm	$(h/D) \times 10^{-3}$, sec/cm	$(1/P) \times 10^{-3}$, sec/cm
—	0.96	3.00	1.50	31.5	1.84	29.66
II*a*	5.26	3.25	1.42	6.18	1.94	4.24
II*b*	7.13	3.85	1.37	5.40	2.01	3.39
III*a*	3.39	3.43	1.42	10.12	1.94	8.18
III*b*	4.12	3.98	1.38	9.65	2.00	7.65
III*c*	4.98	4.47	1.32	8.98	2.09	6.89
IV*a*	5.48	4.87	1.54	8.89	1.79	7.10
IV*b*	3.32	4.50	1.52	13.55	1.82	11.73
IV*c*	5.41	5.15	1.50	9.52	1.84	7.68
IV*d*	5.42	5.15	1.50	9.50	1.84	7.66
V*a*	19.34	6.45	1.50	3.33	1.84	1.49
V*b*	22.00	7.23	1.50	3.30	1.84	1.46

cholesterol dissolution changed with the number of methyl groups as well as with the number of carbon atoms in the long side chains. For the *n*-alkylamines, increasing the number of methyl groups on the amine nitrogen increased the resistance; *i.e.*, for cholesterol dissolution acceleration, the following order of effectiveness was observed: primary > secondary > tertiary > quaternary. However, V*a* was a more effective accelerator than IV*d*. Thus, the number of side chains may be more important than the number of carbon atoms in determining the effectiveness of these accelerators.

Other nitrogen-containing compounds as well as several anionic and nonionic surfactants (Table V) were ineffective as cholesterol dissolution rate accelerators. With the anionic surfactants, a retardation effect was actually observed.

The steroid derivatives were effective even at relatively low concentrations (Table VI). Compounds VI*a* and VI*b* reduced the total resistance by almost a factor of 5 at around 10 m*M*. Due to the limited availability of these compounds, studies at higher concentrations were not possible.

Table V—Effect of Some Nitrogen-Containing Compounds and Nonionic and Anionic Surfactants on Dissolution

Compound and Concentration	$(J/A) \times 10^4$, mg/cm² sec	C_s, mg/ml	$R = \left(\frac{h}{D} + \frac{1}{P}\right) \times 10^{-3}$, sec/cm
—	0.96	3.00	31.5
VII*a*, 32 m*M*	0.61	2.10	35.4
VII*b*, 34 m*M*	1.24	3.50	28.2
Polysorbate 80, 1.25%	0.27	1.35	50.0
Sodium lauryl sulfate, 34 m*M*	0.066	1.65	250
Sodium laurate, 34 m*M*	0.044	2.84	645

DISCUSSION

Together with the limited earlier results (4, 5) for benzalkonium chloride and cetylpyridinium chloride, these results represent the first assessment of the nature of compounds that may act as cholesterol gallstone dissolution accelerators. The primary, secondary, and tertiary amines and the quaternary ammonium salts all appear to be effective. Chain length and/or the degree of hydrophobicity also seem to be important, as do steric or structural features other than pure hydrophobicity as evidenced by the fact that the dioctyl quaternary (V*a*) was more effective than the monohexadecyl quaternary (IV*d*).

Surface activity *per se* does not appear to be important, since the nonionic and the anionic surfactants showed little or negative accelerator activity in these tests. Indirectly, however, surface activity may be important.

The data presented in Tables I–VI are complicated by the fact that

Table VI—Effect of Steroidal Amines on Cholesterol Monohydrate Dissolution in 116 m*M* Sodium Cholate–32 m*M* Lecithin Solution Buffered with 0.10 *M* Phosphate at pH 7.40 and 37°

Compound	Concentration, m*M*	C_s, mg/ml	$(J/A) \times 10^4$, mg/cm²/sec	$R \times 10^{-3}$, sec/cm
VI*a*	0	3.00	0.96	31.5
	5.29	3.00	2.31	13.0
	9.86	3.26	5.08	6.42
VI*b*	2.69	3.00	1.37	21.9
	5.29	3.06	2.19	14.0
	8.05	3.19	3.29	9.70
VI*c*	8.24	3.05	1.83	16.67
VI*d*	10.0	3.56	1.84	19.35

the accelerator in question may be bound to varying degrees to the micelles and other components of the synthetic bile. Thus, meaningful structure–activity relationships can only be considered when the "free" concentration, or the thermodynamic activity, of the accelerator is known. Further progress in this area depends on conducting studies in which the "intrinsic" activities of these agents can be determined.

A method based on the dialysis rate principle for determining the free accelerator concentration in simulated bile is being developed and appears feasible (14). It appears, for example, that the actual (or the intrinsic) difference between *n*-hexylamine and *n*-octylamine in the cholic acid–lecithin media is closer to a factor of 10 or 15 than to the marginal difference seen in Table I. As such data become available, one may begin to understand the action of these accelerators at the molecular level.

Of course, the search for suitable adjuvants for gallstone dissolution in humans *via* the oral route will require additional studies on the absorption, biliary excretion, metabolism, and toxicity of these compounds. While this fact may appear to make this approach impractical or very difficult, one needs only to note that VI*b*, 20,25-diazacholesterol, an established hypocholesterolemic agent, is orally absorbed in humans (15, 16) and in other animal species. Studies also showed (17) that significant percentages of high molecular weight quaternary ammonium cations such as dibenzyldimethylammonium, tribenzylmethylammonium, and benzomethamine are excreted in quantity in rat, rabbit, and human bile when given intravenously. Therefore, compounds such as the steroidal amines and the bile acid derivatives containing the amine nitrogen or the quaternary nitrogen functional groups may offer promise for this approach.

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ˣ To whom inquiries should be directed.

Spectrophotometric Determination of Cephapirin, a Cephalosporin Antibacterial

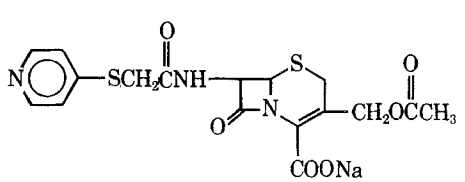
J. E. BODNAR, W. G. EVANS, and D. L. MAYS ˣ

Abstract □ A simple and specific method for the quantitative determination of cephapirin, a cephalosporin antibacterial, in finished bulk and dosage forms is reported. The method is based on reproducible degradation under controlled conditions to an unidentified species, which is measured spectrophotometrically at 375 nm. The procedure can be performed manually on a short series of samples in about 15 min or can be automated for large runs. Precursors and related substances show minimal interference. The coefficient of variation of the automated system is about 1% within days and 1.3% among days.

Keyphrases □ Cephapirin—spectrophotometric analysis, bulk drug and pharmaceutical formulations □ Spectrophotometry—analysis, cephapirin, bulk drug and pharmaceutical formulations □ Antibacterials—cephapirin, spectrophotometric analysis, bulk drug and pharmaceutical formulations

Cephapirin sodium (I), a new cephalosporin antibacterial for parenteral use, has the advantage of being less painful and better tolerated than cephalothin (1). The two methods of analysis officially approved for certification are the microbiological agar-plate diffusion method and the colorimetric hydroxylamine method (2). However, this paper reports a more rapid and selective spectrophotometric method for convenient and accurate product control of finished bulk and dosage forms. The method has been automated to accommodate large numbers of stability samples. Products arising through hydrolysis of the acetyl function show little or no interference. The method is selective for only a few cephalosporins.

Manual and automated procedures, the effects of several operating conditions, and validation of the method are presented.



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