(3) A. Tsuji, O. Kubo, E. Miyamoto, and T. Yamana, *J. Pharm. Sci.*, **66**, 1675 (1977).
(4) A. E. Bird and A. C. Marshall, *J. Chromatogr.*, **63**, 313 (1971).
(5) G. L. Biagi, A. M. Barbaro, M. C. Guerra, and M. F. Gamba, *ibid.*, **44**, 195 (1969).
(6) H. Juncher and F. Raaschou, *Antibiot. Med. Clin. Ther.*, **4**, 497 (1957).
(7) C. G. McCarthy and M. Finland, *N. Engl. J. Med.*, **263**, 315 (1960).
(8) I. M. Rollo and D. M. Burley, *Br. Med. J.*, **13**, 76 (1962).
(9) C. M. Kunin, *Antimicrob. Agents Chemother.*, **1966**, 1025.
(10) G. N. Rolinson and R. Sutherland, in "Advances in Pharmacology and Chemotherapy," vol. 11, G. Garrattini, A. Goldin, F. Hawking, and I. P. Kopin, Eds., Academic, New York, N.Y., 1973, p. 151.
(11) I. M. Rollo, *Can. J. Physiol. Pharmacol.*, **50**, 986 (1972).
(12) D. Perrier and M. Gibaldi, *J. Pharm. Sci.*, **62**, 1486 (1973).
(13) S. C. Penzotti, Jr., and J. W. Poole, *ibid.*, **63**, 1803 (1974).
(14) M. Yasuhara, Y. Miyoshi, T. Kimura, S. Muranishi, and H. Sezaki, *Chem. Pharm. Bull.*, **25**, 675 (1977).
(15) A. Tsuji, E. Miyamoto, I. Kagami, H. Sakaguchi, and T. Yamana, *J. Pharm. Sci.*, **67**, 1701 (1978).
(16) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
(17) H. Bundgaard and K. Ilver, *J. Pharm. Pharmacol.*, **24**, 790 (1972).
(18) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 413 (1964).
(19) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).
(20) J. S. Robertson and B. W. Madsen, *ibid.*, **63**, 234 (1974).
(21) J. B. Houston, D. G. Upshall, and J. W. Bridges, *J. Pharmacol. Exp. Ther.*, **195**, 67 (1975).
(22) G. E. Schumacher and J. B. Nagwekar, *J. Pharm. Sci.*, **63**, 240 (1974).
(23) S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill, New York, N.Y., 1941.
(24) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 421 (1964).
(25) K. Kakemi, T. Arita, R. Hori, R. Konishi, K. Nishimura, H. Matsui, and T. Nishimura, *ibid.*, **17**, 255 (1969).
(26) J. W. Bridges, J. B. Houston, M. J. Humphrey, W. E. Lindup, D. V. Parke, J. S. Shillingford, and D. G. Upshall, *J. Pharm. Pharmacol.*, **28**, 117 (1976).
(27) C. A. M. Hogben, D. J. Tocco, B. B. Brodie, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **125**, 275 (1959).
(28) D. Winne, *J. Pharmacokinet. Biopharm.*, **5**, 53 (1977).
(29) S. Furusawa, K. Okumura, and H. Sezaki, *J. Pharm. Pharmacol.*, **24**, 272 (1971).
(30) H. Nogami and T. Matsuzawa, *Chem. Pharm. Bull.*, **9**, 532 (1961).
(31) W. G. Crouthamel, G. H. Tan, L. W. Dittert, and J. T. Doluisio, *J. Pharm. Sci.*, **60**, 1160 (1971).
(32) J. G. Wagner and A. J. Sedman, *J. Pharmacokinet. Biopharm.*, **1**, 23 (1973).
(33) A. Suzuki, W. I. Higuchi, and N. F. H. Ho, *J. Pharm. Sci.*, **59**, 644 (1970).
(34) *Ibid.*, **59**, 651 (1970).
(35) N. H. F. Ho and W. I. Higuchi, *J. Pharm. Sci.*, **63**, 686 (1974).
(36) V. F. Smolen, *ibid.*, **62**, 77 (1973).
(37) K. R. M. Vora, W. I. Higuchi, and N. F. H. Ho, *ibid.*, **61**, 1785 (1972).

ACKNOWLEDGMENTS

Presented in part at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Japan, April 1977.

The authors acknowledge the gifts of penicillins from Banyu Pharmaceutical Co., Meiji Seika Kaisha, and Takeda Chemical Industries. They thank Dr. A. Suzuki for helpful discussions and Miss E. Kosugi for assistance.

Dissolution Kinetics of Cholesterol in Simulated Bile II: Influence of Simulated Bile Composition

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

Received November 18, 1977, from the **College of Pharmacy, University of Michigan, Ann Arbor, MI 48109*, and the ‡*University Hospital, Department of Medicine, Division of Gastroenterology, University of California at San Diego, San Diego, CA 92103.* Accepted for publication April 26, 1978.

Abstract □ Normal human gallbladder bile and gallbladder bile of patients undergoing chenodeoxycholic acid therapy were simulated by using appropriate combinations of taurine and glycine conjugates of cholic, chenodeoxycholic, and deoxycholic acids. Also, the total bile acid concentration and the total bile acid to lecithin ratio were varied over physiological ranges. Dissolution rates of cholesterol monohydrate pellets (model gallstone) in these solutions were 90–99% interfacially controlled. Even under conditions favorable for dissolution, *i.e.*, high bile acid concentration and high bile acid to lecithin ratio, the interfacial resistances were extremely large. These results are of the same order of magnitude as those found in the limited studies with actual gallbladder bile and suggest that the bile acids, lecithin, and the electrolytes are the primary determinants of the interfacial resistance for cholesterol dissolution. Furthermore, the kinetics of dissolution were always much faster with the chenodeoxycholic acid-rich compositions than with the corresponding normal compositions. This finding suggests, therefore, that in addition to desaturating bile with respect to cholesterol, the feeding of chenodeoxycholic acid further facilitates cholesterol gallstone dissolution by reducing the interfacial resistance of the process.

Keyphrases □ Cholesterol—dissolution kinetics in simulated bile, effect of bile composition □ Dissolution kinetics—cholesterol in simulated bile, effect of bile composition □ Bile composition—effect on dissolution kinetics of cholesterol in simulated bile

Recent studies (1–7) on the dissolution of human cholesterol gallstones and cholesterol monohydrate pellets in bile acid–lecithin solutions and in human gallbladder bile indicated that:

1. The dissolution of both cholesterol gallstones and cholesterol monohydrate pellets (model gallstones) was interfacially controlled rather than diffusion–solubility controlled, providing a possible explanation for the rather slow rate of stone dissolution *in vivo* (8).

2. The interfacial resistance to dissolution was a function of the composition of the simulated bile solution—*viz.*, the bile acid type and concentration, the bile acid to lecithin ratio, and the electrolyte type and concentration.

3. The magnitudes of the interfacial resistances in both the simulated bile solutions and the human gallbladder biles were indeed comparable.

0022-3549/78/1200-1711$01.00/0

Table I—Bile Acid Compositions of Normal Human Gallbladder Bile (N) and Gallbladder Bile of Patients Undergoing Chenodeoxycholic Acid Therapy (C)

Type of Bile	Mole Percent					
	I	II	III	IV	V	VI
N	10	30	5	15	10	30
C	15	75	1	4	1	4

4. The kinetics of cholesterol gallstone dissolution in bile may be explainable on the basis of the principal bile acids, lecithin, and the electrolytes in the particular bile.

In the present study, an attempt was made to simulate normal human gallbladder bile, which has been shown to dissolve cholesterol stones very slowly (2, 7), as well as the gallbladder bile of patients undergoing chenodeoxycholic acid therapy for the dissolution of cholesterol gallstones (8–12). The following parameters were varied: the composition of the six principal bile acids, the total concentration of these bile acids, the lecithin concentration, and, hence, the total bile acid to lecithin ratio. A wide range of conditions was simulated with regard to these parameters to cover the likely situations in both populations. All simulated solutions contained 0.10 *M* sodium chloride since the concentration of this predominant bile electrolyte seems to remain rather constant in human bile. In addition, the solutions were maintained at pH 7.40 at 37° with 0.01 *M* phosphate buffer.

The rate of dissolution per unit area, J/A, and the equilibrium solubility, C_s, of cholesterol monohydrate in these simulated solutions were determined experimentally, and the total resistance to dissolution, R, was calculated using (6, 7):

$$J/A = \frac{C_s}{R} \qquad \text{(Eq. 1)}$$

for sink conditions. In Eq. 1, R represents the sum of the diffusional resistance, h/D, and the interfacial resistance, $1/P$, where h is the Nernst effective diffusion layer thickness, D is the diffusion coefficient of micelle-solubilized cholesterol in solution, and P is the effective permeability coefficient of the solid–solution interface (1–7). In the present consideration, however, since $1/P$ is much greater than h/D in all situations, $R \cong 1/P$ = interfacial resistance.

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 indicated the absence of any impurities (13). X-ray crystallography[3] indicated that they were cholesterol monohydrate crystals and that they had a lattice system similar to that of cholesterol found in human biliary calculi (14). These monohydrate crystals lose their water content readily on exposure to low humidity and light.

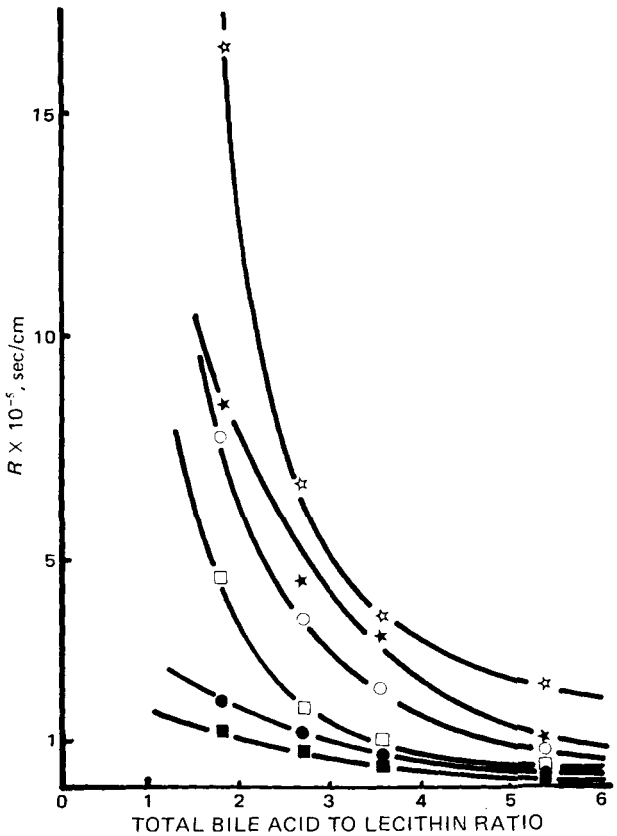

Figure 1—*Profile of* R *dependence on [total bile acid] to [lecithin] ratio at various total bile acid concentrations for simulated normal gallbladder bile (open symbols) and simulated gallbladder bile of patients undergoing chenodeoxycholic acid therapy (closed symbols). Key (concentration of bile acid):* ☆, ★, *46.4 m*M; ○,●, *116.0 m*M; *and* □,■, *174.0 m*M.

The sodium salts of chenodeoxycholyltaurine (I) and chenodeoxycholylglycine (II) were prepared by the method of Norman (15) with certain modifications (16). The sodium salts of deoxycholyltaurine[4] (III) and deoxycholylglycine[4] (IV) were used as received. The sodium salts of cholyltaurine (V) and cholylglycine (VI) were prepared using the method of Norman (15) with certain modifications by Pope (17) and Hofmann[5]. The purity of these compounds was checked and confirmed by TLC using a destructive detection method (18).

Egg lecithin was prepared from fresh egg yolks and subsequently stored according to the method of Singleton *et al.* (19). Chromatographically homogeneous lecithin (mol. wt. ≅ 771) was obtained. Monobasic sodium phosphate, dibasic sodium phosphate, and sodium chloride[6] were analytical grade and were used as received.

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 i.d., under a force of 1360.8 kg with a laboratory press[7]. 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 (6). The stirring speed was maintained at 150 rpm during dissolution by a constant-speed motor[8].

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 with a

[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] Calbiochem, Los Angeles, Calif.
[5] A. F. Hofmann, unpublished data.
[6] Matheson, Coleman and Bell, Norwood, Ohio.
[7] Model B, Fred Carver Inc., Summit, N.J.
[8] Model CA, Hurst, Princeton, Ind.

Table II—Influence of Total Bile Acid Concentration and [Bile Acid] to [Lecithin] Ratio on Solubility, C_s, Dissolution Rate, J/A, and Resistance, R, for Simulated Normal Gallbladder Bile

Total [Bile Acid], mM	Total [Lecithin], mM	[Bile Acid] to [Lecithin] Ratio	$J/A \times 10^4$, mg/cm^2/sec	C_s, mg/ml	$R \times 10^{-3}$, sec/cm
174	96	1.81	0.219	10.27	469
174	64	2.72	0.470	7.65	163
174	48	3.63	0.548	5.86	107
174	32	5.44	0.914	4.55	50
116	64	1.81	0.0914	7.16	783
116	42.6	2.72	0.128	4.80	375
116	32	3.63	0.164	3.60	220
116	21.3	5.44	0.296	2.50	85
46.4	25.6	1.81	0.0146	2.43	1664
46.4	17.0	2.72	0.0301	2.04	678
46.4	12.8	3.63	0.0438	1.70	388
46.4	8.53	5.44	0.0532	1.24	233

Table III—Influence of Total Bile Acid Concentration and [Bile Acid] to [Lecithin] Ratio on Solubility, C_s, Dissolution Rate, J/A, and Resistance, R, for Simulated Gallbladder Bile of Patients Undergoing Chenodeoxycholic Acid Treatment

Total [Bile Acid], mM	Total [Lecithin], mM	[Bile Acid] to [Lecithin] Ratio	$J/A \times 10^4$, mg/cm^2/sec	C_s, mg/ml	$R \times 10^{-3}$, sec/cm
174	96	1.81	0.726	9.07	125
174	64	2.72	0.824	6.59	80
174	48	3.63	1.37	5.71	41.7
174	32	5.44	1.64	4.40	26.8
116	64	1.81	0.367	6.98	190
116	42.6	2.72	0.411	4.56	111
116	32	3.63	0.548	3.67	67.0
116	21.3	5.44	0.685	2.82	41.2
46.4	25.6	1.81	0.0274	2.30	839
46.4	17.0	2.72	0.0392	1.81	462
46.4	12.8	3.63	0.0477	1.63	342
46.4	8.53	5.44	0.107	1.06	99.1

Table IV—Comparison of Experimental and Estimated R Values for Human Gallbladder Bile Samples (7)

Bile Sample	Total [Bile Acid], mM	Total [Lecithin], mM	[Bile Acid] to [Lecithin] Ratio	$R \times 10^{-3a}$, sec/cm	$R \times 10^{-3b}$, sec/cm
1	194.1	41.3	4.70	26.6	55
2	133.7	24.9	5.38	30.6	75
3	237.2	64.9	3.65	40.1	35
4	206.5	46.3	4.46	24.5	55

[a] Determined from $R = AC_s/J$. [b] Estimated from Fig. 1.

liquid scintillation counter[9], 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 amount of ^{14}C-cholesterol monohydrate of about 20 mg into 2 ml of a solvent in a test tube. The tube was then flushed with nitrogen, capped, and shaken by a wrist-action shaker[10] 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 with a liquid scintillation counter. More samples were taken every 2 days and assayed for cholesterol. The solubility of cholesterol monohydrate in the medium was obtained when the concentration reached a constant level.

RESULTS AND DISCUSSION

The average bile acid compositions in normal human gallbladder bile (20) and in gallbladder bile of patients undergoing chenodeoxycholic acid therapy (10) are presented in Table I. The selected ranges of the total bile acid concentration, the lecithin concentration, and, hence, the bile acid to lecithin ratio were representative of over 100 human bile samples analyzed in several published reports (21–24).

Tables II and III and Fig. 1 summarize the dissolution kinetic data obtained with the simulated bile solutions. The R values were about 20–700 times the diffusion–convection resistance previously reported for similar systems[11] (Tables II and III); *i.e.*, the dissolution rates in these solutions were essentially 90–99% interfacially controlled. The magnitudes of these R values seemed to be comparable to those found in the situations where only one bile acid was present in solution (6). In addition, as shown previously with the individual bile acids (6), R decreased with an increasing bile acid concentration and an increasing bile acid to lecithin ratio. While this result occurred for both compositions of bile acids, the corresponding R values for the chenodeoxycholic acid-rich solutions were consistently lower than those with normal compositions (Fig. 1).

Even under the most favorable conditions with regard to the composition of the bile, *i.e.*, high bile acid concentration, high bile acid to lecithin ratio, and high chenodeoxycholate concentration, the R values were much larger than the diffusion–convection resistance. This result confirms the hypothesis (2, 5–7) that the interfacial resistance may indeed be an important rate-determining factor in cholesterol stone dissolution *in vivo*. Moreover, in the region where the bile acid concentration and the bile acid to lecithin ratio are low, the observation that three- to fourfold smaller R values were found with the chenodeoxycholic acid-rich compositions may have the following clinical implications:

1. Even if the oral administration of cholic acid had been as effective as chenodeoxycholic acid in desaturating bile with respect to cholesterol, from the kinetic viewpoint it would not have been as effective as cheno-

[9] Model LS 200, Beckman Instruments, Southfield, Mich.
[10] Burrell Corp., Pittsburgh, Pa.

[11] The h/D value for these systems $\cong 2.3 \times 10^3$ sec/cm (6).

deoxycholic acid.

2. In addition to its primary effect on cholesterol desaturation, the feeding of chenodeoxycholic acid also appears to promote stone dissolution by lowering the interfacial resistance in bile, thereby facilitating the kinetics of dissolution as well.

Recent clinical efforts (25) indicated that the oral administration of ursodeoxycholic acid may also be effective in promoting cholesterol gallstone dissolution but without some of the side effects attributed to chenodeoxycholic acid. Therefore, the dissolution kinetics in simulated bile acid mixtures should be studied using the ursodeoxycholic conjugates (glycine and taurine) and the results from these studies should be used to assess the relative effectiveness of ursodeoxycholic acid and chenodeoxycholic acid in speeding up the kinetics of stone dissolution.

At low bile acid concentrations and/or low bile acid to lecithin ratios, the R values are extremely large (Table III). These large R values are believed to be clinically significant since, in many instances, necessary treatment periods for significant stone dissolution have been very long, often extending beyond 12 months. In some cases, little or no dissolution was found (8–12) even after such long times. In view of this fact, it was suggested previously (26, 27) that dissolution rate accelerators might have therapeutic value in effecting more rapid stone dissolution when administered simultaneously with chenodeoxycholic acid.

Table IV shows some preliminary dissolution data on human gallbladder bile reported earlier (7). Based on the total bile acid concentration and the bile acid to lecithin ratio, a rough estimate of R was obtained for each bile sample by extrapolation and interpolation of the curves in Fig. 1. The magnitudes of these R values are reasonably comparable to the experimental values found in the present studies. Since the bile acid composition and the electrolyte content of these samples are not known, a better estimation of R based on the simulated solution data is not possible at this time. Nonetheless, the results suggest that the bile acids, lecithin, and the electrolytes are the primary determinants of interfacial resistance in cholesterol dissolution.

REFERENCES

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

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

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

(4) W. I. Higuchi, M. Surpuriya, S. Prakongpan, and F. Young, *J. Pharm. Sci.*, **62,** 695 (1973).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(20) A. F. Hofmann and D. M. Small, *Ann. Rev. Med.*, **18,** 333 (1967).

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

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

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

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

(25) I. Makino, K. Shinozaki, and K. Yoshino, *Jpn. J. Gastroenterol.*, **72,** 690 (1975).

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

(27) K. H. Kwan, W. I. Higuchi, A. M. Molokhia, and A. F. Hofmann, *ibid.*, **66,** 1105 (1977).

ACKNOWLEDGMENTS

Supported by Grants AM 16694 and AM 16770 from the National Institutes of Health.