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Dissolution Rate Behavior of Solid Cholesterol Preparations in Bile Acid Solutions

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Abstract □ Recent work in these laboratories showed that different preparations of cholesterol monohydrate and anhydrous cholesterol had different dissolution rates under the same conditions. A method was developed by which both the thermodynamic contribution (C_s) and the interfacial kinetic contribution (P) to dissolution could be determined from experimental data. The rotating-disk dissolution method was used with the Levich theory to assess the data. Dissolution rates were determined in solutions partially saturated with cholesterol. Dynamic solubilities were determined by plotting dissolution rates (J/A) *versus* bulk concentration (C_b) and extrapolating to zero J/A. By using a best-fit analysis, it was possible to determine solubility as well as the interfacial transport constant independently. When this method was used to study differences in dissolution behavior of different solid preparations of cholesterol, the differences could be accounted for primarily by variations in the interfacial transport constant rather than by solubility variations.

Keyphrases □ Cholesterol—dissolution rate behavior in bile acid solutions, comparison of solid preparations □ Dissolution—cholesterol, comparison of solid preparations in bile acid solutions □ Bile acids—cholesterol dissolution, comparison of solid preparations □ Gallstones—cholesterol dissolution, comparison of solid preparations in bile acid solutions

Most dissolution rate experiments have been interpreted satisfactorily by diffusion–convection models (1). However, recent studies (2, 3) of the dissolution rate behavior of cholesterol in bile acid solutions showed that interfacial resistance is often the dominant factor to mass transfer.

Preliminary studies in these laboratories showed that different preparations of solid cholesterol result in large variations in dissolution rate behavior. A method was needed to determine both the thermodynamic contribution and the kinetic contribution to dissolution from experimental data for different solid preparations.

The rotating-disk method was used with the accompanying Levich theory (4) to analyze results since it is well suited for quantifying the contributions of the diffusion–convection mass transfer resistance and the contributions of the interfacial barrier and/or solubilization kinetics in the diffusion layer to the overall kinetics. The purpose of this paper was to demonstrate how dissolution rate data can be analyzed when interfacial kinetics and the thermodynamic driving force for various solid phases of a substance vary simultaneously. The method was applied to the problem of cholesterol dissolution in bile acid solutions.

THEORETICAL

According to the Nernst diffusion layer theory, the total resistance for the transfer of a solute into the solution immediately adjacent to the solid surface and then into the bulk solution is given by h/D, a diffusion term. Both convection and diffusion are expected to be important in most dissolution situations. Therefore, for the rotating-disk method, the effective diffusion resistance (h/D) may be equated to an appropriate function that considers both processes. Thus, when both diffusion and convection are important, the mass flux as given by Levich (4) is:

$$J = 0.62AD^{2/3}\nu^{-1/6}\omega^{1/2}(C_s - C_b) \qquad \text{(Eq. 1)}$$

where:

A = apparent surface area of the pellet
D = diffusion coefficient
ν = kinematic viscosity
ω = angular velocity of rotation
C_s = saturation solubility
C_b = bulk concentration

The effective diffusion layer thickness is given as:

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

For the region of dissolution in which both diffusion and a first-order interfacial resistance are important, the flux as given by Levich (4) is:

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

where P is the permeability coefficient (interfacial transport constant).

In the case of pure bulk transport, the observed dissolution rate should be essentially equal to the calculated flux (Eq. 1). However, if the observed reaction rate is much lower than the calculated diffusion–convection flux, it may be concluded that dissolution is taking place in the interfacially controlled region, *i.e.*, $1/P$ has become rather large (Eq. 3).

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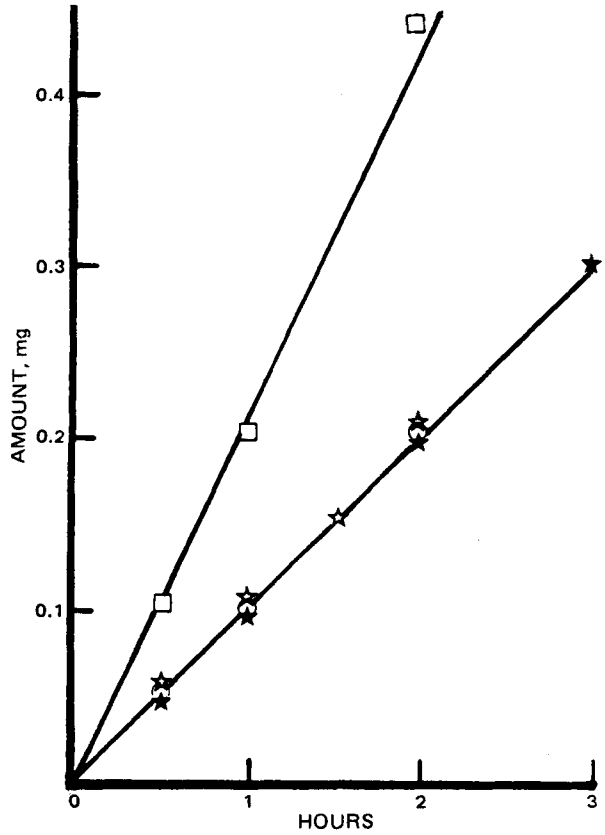

Figure 1—*Effect of pH on the dissolution rate of cholesterol in 5% sodium cholate solutions (pH was adjusted by addition of sodium hydroxide). Key:* □, *pH 7.1;* ★, *pH 7.4;* ○, *pH 8.02; and* ☆, *pH 11.08.*

EXPERIMENTAL

Solutions—Sodium cholate test solutions were prepared by titrating equivalent amounts of cholic acid[1] with sodium hydroxide. The solution pH then was adjusted to 8.0. While this value was not the physiological pH, it was at a region where J/A would experience minimum variability because of pH variations (Fig. 1). The solutions were allowed to equilibrate for at least 1 day before use.

Materials—Commercial cholesterol[2] was recrystallized three times from 95% ethanol. Radioactive cholesterol monohydrate was prepared by mixing [4-^{14}C]cholesterol[3] with a known amount of the recrystallized cholesterol at 60° (specific activity of ~10 μCi/g). The solutions then were allowed to stand undisturbed for 48 hr at room temperature. The cholesterol crystals were filtered and dried overnight *in vacuo*. The cholesterol monohydrate crystals were stored in the dark in a desiccator saturated with water vapor at room temperature.

Preparation of Solid Phases—Cholesterol samples that gave different dissolution rates under the same conditions were obtained. Two preparations of cholesterol monohydrate were obtained. One sample (I) was prepared by mixing 5 g of recrystallized cholesterol and 50 μCi of [4-^{14}C]cholesterol with 400 ml of 95% ethanol at 60°. Another sample (II) was prepared by mixing 10 g of recrystallized cholesterol and 100 μCi of [4-^{14}C]cholesterol with 400 ml of 95% ethanol at 60°. During the crystallization of I and II, the crystallization rates were significantly different. Crystals were first noticed ~12–15 and ~3–4 hr after dissolution[4] in I and II, respectively.

In addition, two nonhydrated forms of cholesterol were obtained by pretreatment of I. One sample (III) was prepared by melting a small amount of I at 160° for 5 min. Another preparation (IV) was obtained by heating I at 100° for 24 hr.

To check the hydrated nature of the two monohydrates and the nonhydrated nature of the melt and the heated sample, weight loss experiments were conducted on each solid form. For all of these preparations, ~1 g of the cholesterol was weighed into a vial and then heated at 100° for 15 min. After heating, the samples were reweighed. This process was repeated until the weight of the vial plus the cholesterol was constant. Any weight loss during this process was attributed to the loss of the solvation molecule (water).

For the two monohydrate samples, the loss in weight corresponded to 1.0 ± 0.3 mole of water for each mole of cholesterol. However, for the melt and the heated sample, no weight loss was detected after further heating. This finding demonstrates the monohydrate nature of the two hydrated forms and the anhydrous nature of the two heated preparations.

In addition to weight loss determination, it also was important to check for decomposition products. TLC plates were prepared and placed into tanks with chloroform–acetone (90:10) as the solvent (5). For all solid preparations, one spot with an R_f value of 0.62 was found.

Equilibrium Solubility Determination—The solubility of each cholesterol monohydrate sample was determined by adding an excess of radiolabeled cholesterol monohydrate to a few milliliters of solvent. The tube containing the slurry then was shaken by a wrist-action shaker in a water bath maintained at 37°. Samples were taken periodically, 10 ml of scintillation fluid was added to the sample, and the amount of cholesterol dissolved was calculated from the radioactivity determined in a liquid scintillation counter[5]. When the amount of dissolved cholesterol reached a constant level, this limiting value was used to calculate the equilibrium solubility (2).

Diffusion Coefficient Determination—The diffusion coefficient was determined in a small-volume diaphragm cell at 37°. The apparatus and the procedure were described previously (6). The apparatus consisted of two well-stirred reservoirs separated by a silver filter membrane. Both reservoirs contained 3.4 ml of the solvent medium, and each was stirred at 150 rpm with a magnetic bar in the bottom chamber and a paddle in the upper chamber. At the end of the run, the concentration in each reservoir was determined.

Method—For the initial portion of this investigation, the static-disk dissolution apparatus was employed (Fig. 2). Approximately 200 mg of the various solid preparations was compressed[6] into pellets [compression load of 1362 kg (3000 lb)] with a diameter of 1.27 cm. The pellets then were placed into the dissolution setup. Exactly 10 ml of the sodium cholate solution was added, and the system was stirred with a 150-rpm stirrer[7].

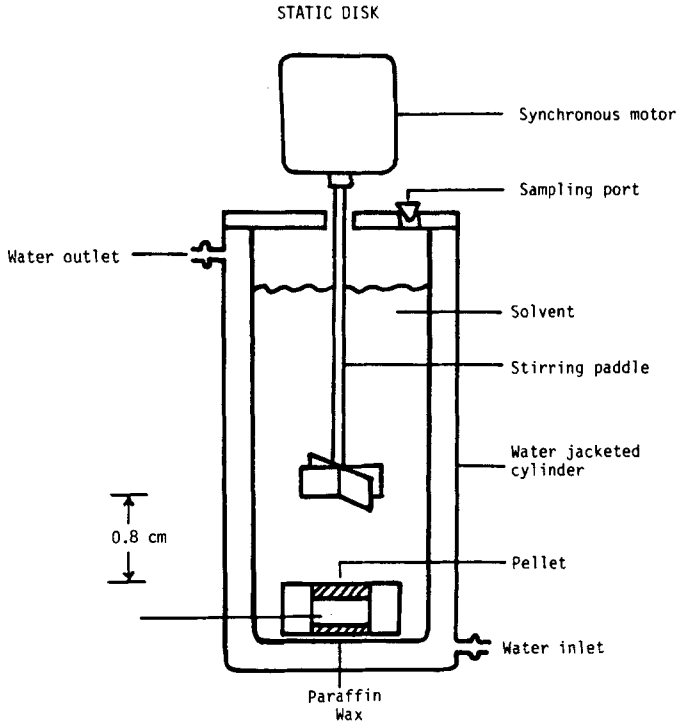

Figure 2—*Diagrammatic representation of the static-disk dissolution apparatus.*

[1] Weddell, London, England.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] New England Nuclear Corp., Boston, Mass.
[4] In later discussions, I will be referred to as normal cholesterol and II will be referred to as rapid cholesterol.
[5] Beckman Instruments, Irvine, Calif.
[6] Model B laboratory press, Fred Carver Inc., Summit, N.J.
[7] Model CA stirring motor, Hurst Manufacturing Co., Princeton, Ind.

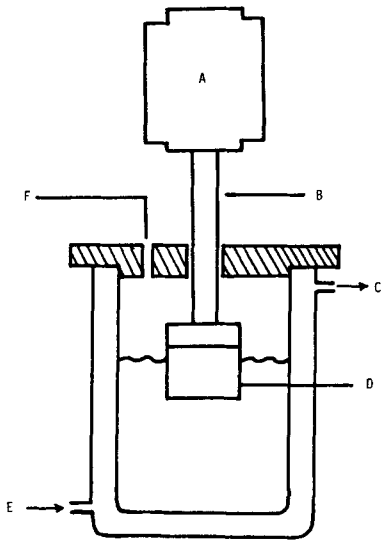

Figure 3—*Diagrammatic representation of the rotating-disk dissolution apparatus. Key: A, constant-speed motor; B, rotating shaft; C, water outlet; D, disk with pellet; E, water inlet; and F, sample port.*

Samples were taken at different times, and the amount dissolved was measured in a liquid scintillation counter.

For the more detailed analysis, the rotating-disk apparatus was used (Fig. 3). This apparatus consisted of three main parts: the outside water-jacketed beaker, the removable disk, and the controlled-rotating device[8]. Deviation of the experimental results from theory was assessed using benzoic acid as a model solute since benzoic acid dissolution is diffusion–convection controlled (6).

Fifty milliliters of solvent was pipetted into the water-jacketed beaker maintained at 37° for each dissolution run. The rotating disk containing the pellet then was centered at ~1 cm below the solvent level so that the distances from the disk wall to the inner surface of the beaker exceeded 0.5 cm. Samples were taken at appropriate times and assayed for the amount dissolved. All experiments were concluded early enough so that sink conditions were always maintained. Rotation speeds varied between 20 and 450 rpm. Partially saturated solutions of 5% sodium cholate and 0.1 *M* phosphate buffer at pH 8.0 were prepared by adding nonradioactive

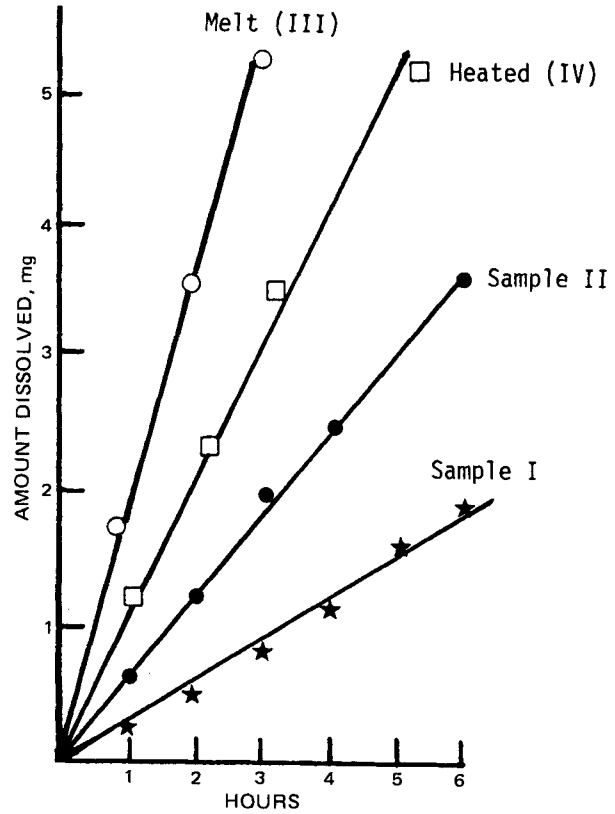

Figure 4—*Effect of the solid preparation on the dissolution rate in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00 in the static-disk dissolution apparatus.*

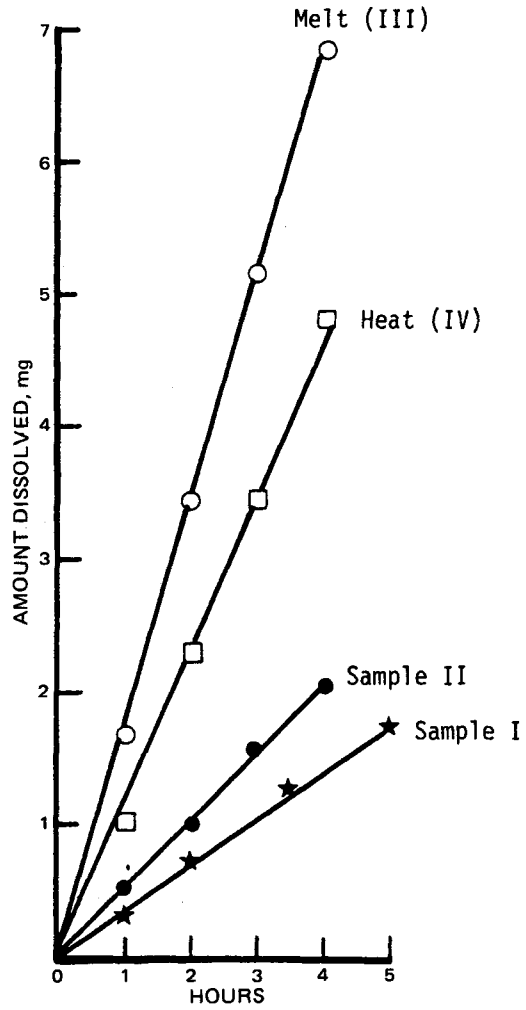

Figure 5—*Effect of the solid preparation on the dissolution rate in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00 in the rotating-disk apparatus.*

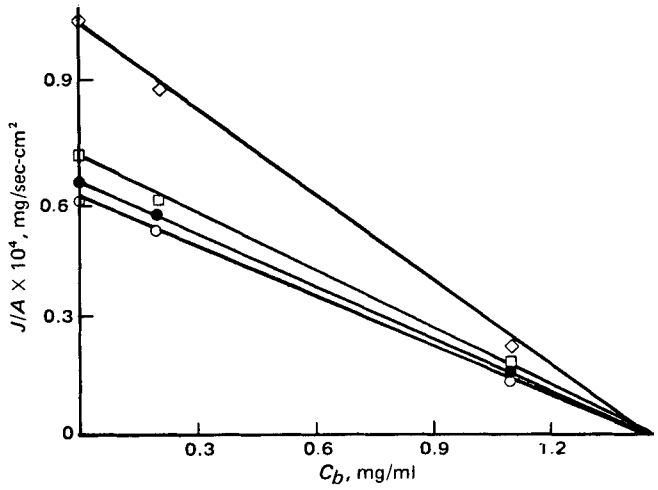

Figure 6—*Dissolution rate* versus *bulk concentration for the normal (I) pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key: ○, 20 rpm; ●, 50 rpm; □, 150 rpm; and ◇, 450 rpm. Each J/A value is an average of two experiments. Since the typical variations are ±5–~10% for these results, which are too small to be seen, only the average values are presented here and in Figs. 7–13.*

[8] Servodyne laboratory stirrer, Cole-Parmer Instrument Co., Chicago, Ill.

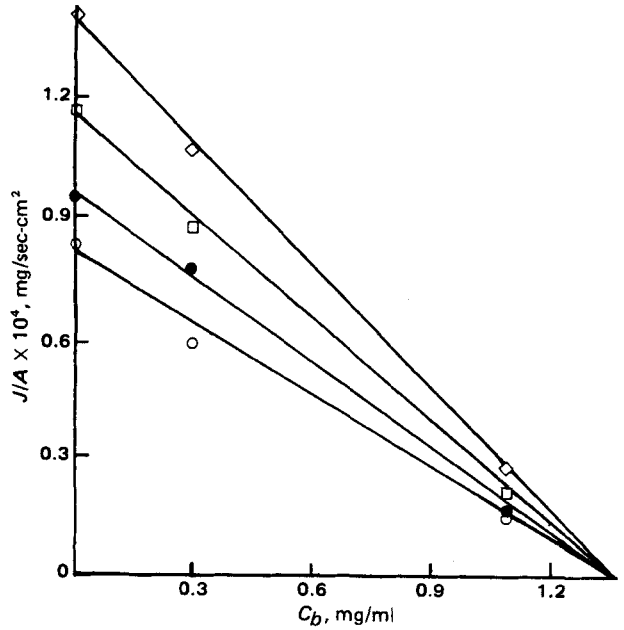

Figure 7—*Dissolution rate* versus *bulk concentration for the rapid (II) pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm.*

cholesterol to the solutions and allowing the cholesterol to dissolve completely. Bulk concentrations of cholesterol varied from 0 to ~1.0 mg/ml.

RESULTS AND DISCUSSION

As already stated, different solid preparations of cholesterol give different dissolution rates under the same conditions. These differences may be caused by variations in P and/or C_s (Eq. 3) between the different preparations. By varying C_b (bulk concentration) and ω (rotational speed), it was possible to determine C_s and P values for all solid preparations.

Since cholesterol dissolution is essentially surface controlled, $1/P$ is much larger than $1.612D^{-2/3}\nu^{1/6}\omega^{-1/2}$ (Eq. 3). Therefore, the actual value of D is not required for the present analysis. However, as an independent check, D was determined and compared to the value obtained in the later analysis.

Dissolution Results of Benzoic Acid—The results of benzoic acid dissolution were the same as those previously reported (6). Benzoic acid dissolution was linear at all rotation speeds. When these dissolution rates

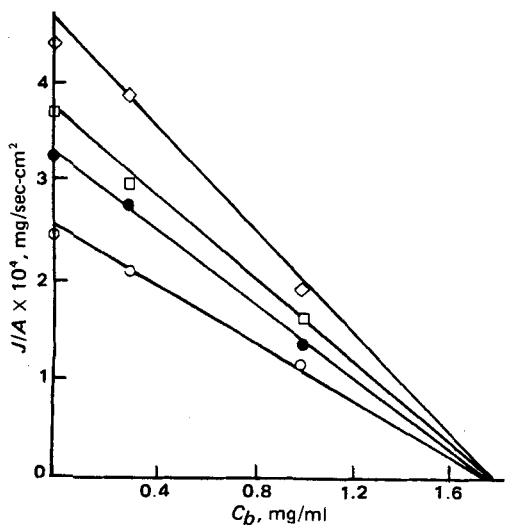

Figure 8—*Dissolution rate* versus *bulk concentration for the melt (III) pellets in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm.*

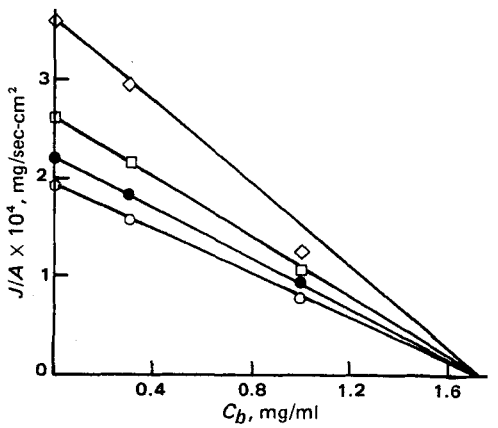

Figure 9—*Dissolution rate* versus *bulk concentration for the pellets (IV) prepared from I heated at 100° for 24 hr in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *20 rpm;* ●, *50 rpm;* □, *150 rpm; and* ◇, *450 rpm.*

were plotted *versus* the square root of the rotation speed, a linear relationship was obtained. This relationship was compared to the dissolution rates predicted by the Levich theory (4) (Eq. 1). The experimental dissolution rates were 15% lower than those predicted by the Levich theory. This discrepancy is consistent with the views of Gregory and Riddiford (7) regarding the performance of rotating-disk geometrics of the type used in the present study.

Dissolution of Cholesterol Solid Preparations Using Static-Disk Setup—Measurement of dissolution rates of the cholesterol solid phases showed significant differences (Fig. 4 and Table I). Sample II gave a dissolution rate that was ~70% higher than that of I. The heated preparation gave a dissolution rate ~3.6 times as great as did I, while the melt gave an increase in the dissolution rate by a factor of 5. The results of several pellets of each preparation were reproducible to ±10%. Because the differences in dissolution rates were significant, it was necessary to determine whether these differences were due to variations in C_s (solubility) or P (interfacial transport constant).

Dissolution of Cholesterol Solid Preparations in Rotating-Disk Setup—For each solid preparation, initial dissolution rates in the rotating-disk apparatus were obtained in the same manner as for the static-disk experiments. Figure 5 shows the results of some typical experiments for the various solid preparations in solutions of 5% sodium cholate and 0.1 M phosphate buffer at pH 8.0 with bulk cholesterol concentrations equal to zero. As was found with the static disk, these results were reproducible to ±10%. Plots such as these were prepared under all conditions to obtain the initial dissolution rates.

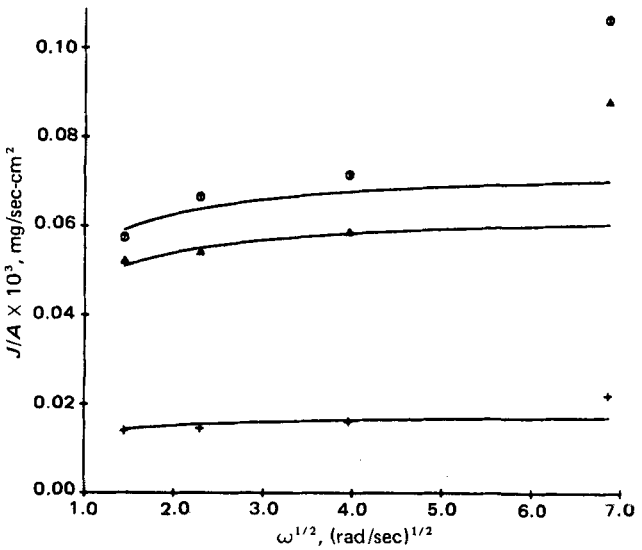

Figure 10—*Best-fit analysis of the normal pellets (I) in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key* (C_b *in milligrams per milliliter):* ○, *0.0 mg/ml;* ▲, *0.2 mg/ml; and* +, *1.1 mg/ml.*

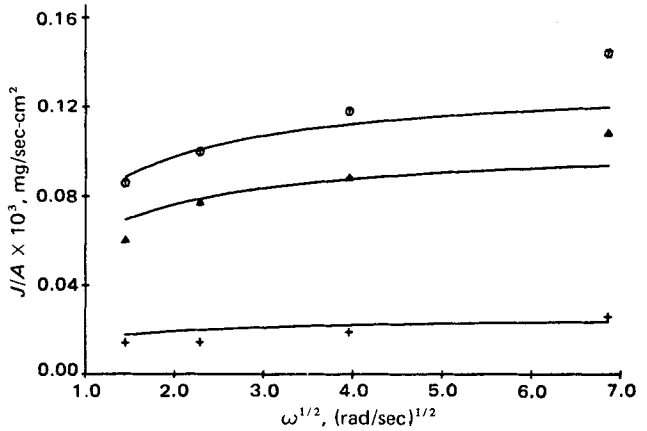

Figure 1—*Best-fit analysis of the rapid pellets (II) in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key* (C_b *in milligrams per milliliter):* ○, *0.0 mg/ml;* ▲, *0.3 mg/ml; and* +, *1.1 mg/ml.*

To determine the dynamic solubility (C_s), initial dissolution rates were determined and then plotted against C_b (bulk concentration). Figures 6–9 show the results of experiments carried out in 5% sodium cholate and 0.1 *M* phosphate buffer solutions at pH 8.0. The extrapolations of these data to zero J/A gave the dynamic solubilities.

As was predicted, Figs. 6–9 show a linear decrease in the cholesterol monohydrate dissolution rate as saturation was approached. This finding indicates that dissolution kinetics are proportional to the change in concentration (ΔC) over the entire saturation range. The dynamic solubilities for both cholesterol monohydrate samples were found by extrapolation to be ~1.40 mg/ml (Figs. 6 and 7). The C_s value for the two anhydrous preparations was ~1.75 mg/ml (Figs. 8 and 9).

To look at the data in a more meaningful way, plots of J/A *versus* $\omega^{1/2}$ were prepared. Figures 10–13 clearly show the important involvement of the interfacial barrier. If the interfacial barrier was not significant (*i.e.*, diffusion-controlled case), a plot of J/A *versus* $\omega^{1/2}$ would give a straight line through the origin. If the interfacial barrier is significant in dissolution, a slope of <1 and a deviation from linearity should be obtained.

From a least-squares best-fit analysis, using Eq. 3 with a 15% correction, it was possible to obtain values for C_s, P, and D, where P is the interfacial transport constant and D is the diffusivity. The calculated dissolution rates using the best-fit values from Eq. 3 were compared to the experimental dissolution rates (Figs. 10–13). A good fit between experiment and theory is seen. The points at 450 rpm ($\omega^{1/2} = 6.86$) were not weighted in this analysis.

Tables II–V summarize the results of the partial saturation, the least-squares analyses, and the solubility determined by the slurry method (2). The tables show good agreement among the C_s values obtained by all three methods for both I and II and nearly equivalent C_s values between the samples. However, the interfacial transport constant (P) differed by a factor of 1.90 between the samples. Therefore, the differences in the dissolution rates between I and II can be accounted for primarily by a difference in their P values.

The C_s value from the partial saturation experiments for the melt-prepared and heat-prepared pellets was ~1.75 mg/ml. This value is ~20% greater than the C_s value obtained from either monohydrate sample. Again, the big difference was seen in the interfacial transport constant (P). The P_{IV}:P_I ratio was 3.99, while the P_{III}:P_I ratio was 6.53. As was the case with I and II, the difference in the dissolution behavior between the anhydrous preparations can be accounted for primarily by a difference in P. The variations in the dissolution rates between the hydrated preparations and the nonhydrated preparations can be attributed to small changes in C_s and large changes in P.

Experimental determination of D (diffusivity) agreed well with the

Table I—Dissolution Rates of I–IV in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

Sample	$J/A \times 10^4$, mg/sec-cm²
I	0.73
II	1.24
III	3.60
IV	2.64

Table II—Summary of Partial Saturation Experiments and Best-Fit Analysis for Normal Cholesterol (I) Pellets in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s from Slurry, mg/ml	C_s from Extrapolation of Partial Saturation Data, mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.34	1.45	20	1.45	0.51	1.25
	1.45	50			
	1.45	150			
	1.42	450			

Table III—Summary of Partial Saturation Experiments and Best-Fit Analysis for II in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s from Slurry, mg/ml	C_s from Extrapolation of Partial Saturation Data, mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.33	1.38	20	1.37	0.96	1.15
	1.36	50			
	1.37	150			
	1.37	450			

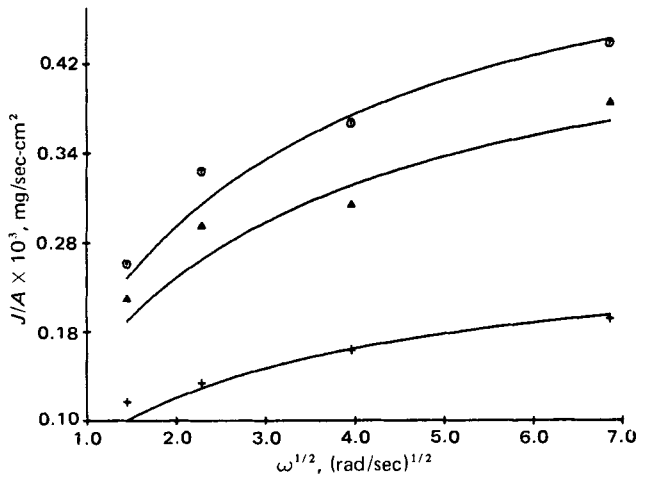

Figure 2—*Best-fit analysis of the melt pellets (III) in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key* (C_b *in milligrams per milliliter):* ○, *0.0 mg/ml;* ▲, *0.3 mg/ml; and* +, *1.0 mg/ml.*

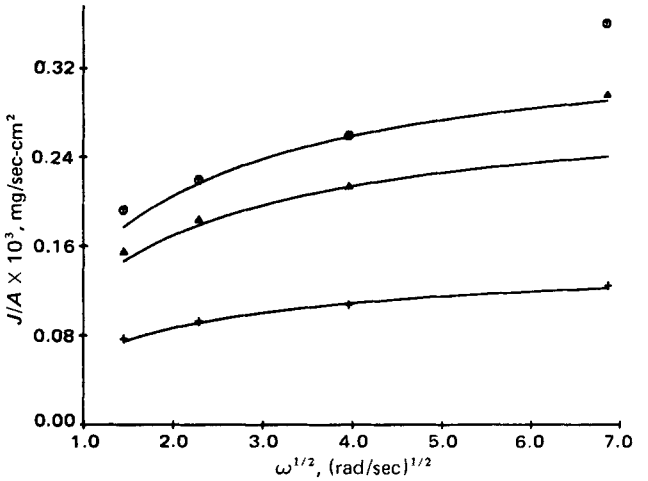

Figure 3—*Best-fit analysis of the pellets (IV) prepared from I heated at 100° for 24 hr in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key* (C_b *in milligrams per milliliter):* ○, *0.0 mg/ml;* ▲, *0.3 mg/ml; and* +, *1.0 mg/ml.*

Table IV—Summary of Partial Saturation Experiments and Best-Fit Analysis for III in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s from Extrapolation of Partial Saturation Data, mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.76	20	1.79	3.32	1.26
1.72	50			
1.76	150			
1.74	450			

Table V—Summary of Partial Saturation Experiments and Best-Fit Analysis for IV Prepared from I Heated at 100° for 24 hr in 5% Sodium Cholate and 0.1 *M* Phosphate Buffer at pH 8.00

C_s from Extrapolation of Partial Saturation Data, mg/ml	Revolutions per Minute	Best-Fit Analysis C_s, mg/ml	$P \times 10^4$, cm/sec	$D \times 10^6$, cm²/sec
1.74	20	1.73	2.03	1.26
1.74	50			
1.74	150			
1.73	450			

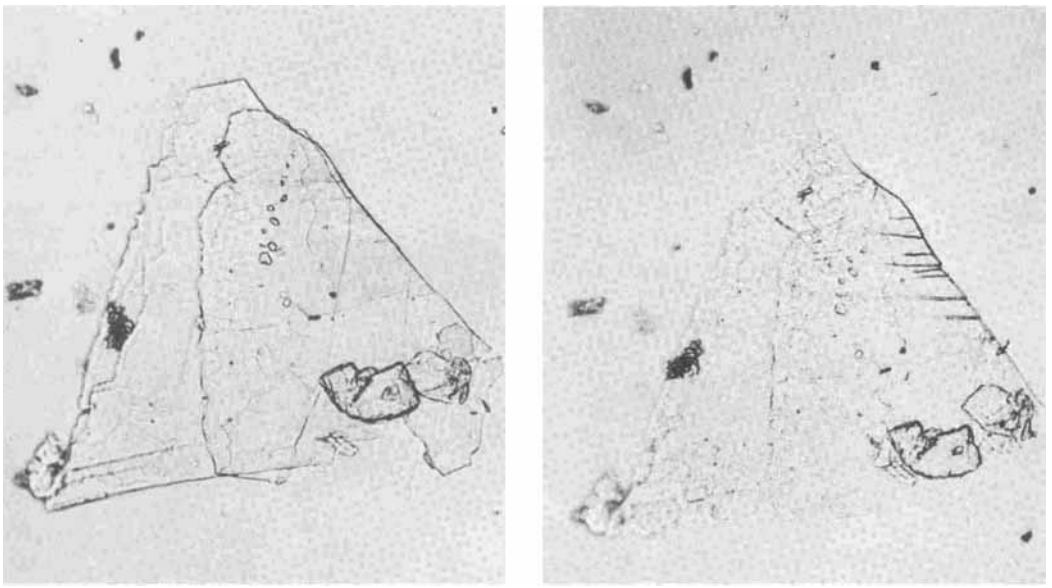

Figure 14—*Photographs of cholesterol monohydrate crystals (180×) in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Left:* T = *0 hr. Right:* T = *2 hr.*

values of D determined from the best-fit analysis. From the small-volume diaphragm cell, D was determined to be 1.52×10^{-6} cm²/sec; from the best-fit analysis, D was $\sim 1.25 \times 10^{-6}$ cm²/sec.

As already stated, the data at 450 rpm ($\omega^{1/2} = 6.86$) was not weighted in the best-fit analysis. The points at 450 rpm deviate substantially from the theoretical curve (Figs. 10–13). A possible explanation for this deviation can be found in the single-crystal experiments performed previously (8). Individual cholesterol crystals were mounted in a cell containing the bile acid solution, and the cell then was placed into a 37° chamber. Microscopic examination of the crystal under static conditions showed that surface etching occurred. Within 2 hr, a definite saw-tooth pattern developed along the edge of the crystal (Fig. 14). At 450 rpm in the rotating-disk experiments, the effective surface area might possibly have increased or some shearing of the peaks along the crystal surface may have occurred. This phenomenon, along with the normal micellar dissolution, could result in a positive deviation in the apparent dissolution rate from that expected from the theory.

These findings are consistent with those of Shefter and Higuchi (9). They showed that the dissolution behavior of hydrated and nonsolvated forms of cholesterol was indeed different. The initial dissolution rate was shown to be three times greater for the nonsolvated cholesterol as for the hydrated form at 25°. They also showed that the maximum concentration reached with the nonsolvated form was 1.4 times as great as the solubility of the hydrate at 25°.

Implications in Cholesterol Gallstone Research—Higuchi *et al.* (2, 3) proposed that cholesterol monohydrate pellets may be employed as a model for cholesterol gallstones in *in vitro* dissolution rate experiments. Borgren and Larsson (10) examined biliary calculi from humans immediately after removal. In all cases, the cholesterol present was cholesterol monohydrate. These investigators also showed that the monohydrate can lose water to give the anhydrous form. Other investigators (11, 12) found both forms of cholesterol in gallstones, but their studies were conducted on stones that had been removed several years earlier. The authors suggested that the anhydrous cholesterol was formed from the monohydrate and that cholesterol monohydrate was formed *in vivo*.

The present investigation demonstrated that large variations in results among studies may occur if the solid phase of cholesterol is not prepared in the same manner from study to study. There was approximately a fivefold difference between the highest (melt preparation) and lowest (I) rates observed. Even two preparations of the monohydrate (I and II) may show differences in pellet dissolution rates as great as 80%. Thus, the potential for problems should be recognized and the proper cautions exercised when comparing data from different studies (especially from different laboratories).

A rather subtle but important situation concerns the study of dissolution rate accelerators. The large difference in the dissolution rates between the melt (or the anhydrous preparation) and I was due mainly to the difference in P values rather than to a difference in solubility (C_s). Thus, for example, the P value for the anhydrous cholesterol pellet prepared by heating I was approximately four times larger than that for I itself. Thus, for the same hydrodynamic (convective–diffusion) situation, the anhydrous pellet can be up to four times less sensitive than a pellet of I to agents that may influence the interfacial kinetics.

From their studies, Tao *et al.* (13) concluded that convective diffusion rather than interfacial kinetics is the major determinant of the dissolution rate and that greatly accelerating the dissolution of cholesterol gallstones could be difficult. These investigators, however, prepared their cholesterol by evaporating a cholesterol–benzene solution to dryness and, consequently, their material was anhydrous cholesterol[9]. This procedure, at least in part, may account for their experimental results being closer to convective diffusion control than those results obtained by Higuchi *et al.* (2, 3).

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Variability in Dissolution Rates of Cholesterol Pellets in Bile Acid Solutions

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Abstract □ Large variations in dissolution rate behavior of cholesterol monohydrate pellets may result from small changes in experimental procedures. For example, when cholesterol monohydrate pellets were stored overnight prior to a dissolution run, the initial dissolution rates varied by more than a factor of 2. It is well known that cholesterol monohydrate is converted to anhydrous cholesterol; cholesterol may be unstable toward light, heat, and other radiation in the presence of air, leading to its decomposition. To determine the cause of the variable dissolution rates, experiments were conducted with pellets "aged" under various conditions. The data show that the probable cause of the variations is the pellet surface conversion of the monohydrate to anhydrous cholesterol, which may take place during pellet storage. The combined effects of temperature and humidity seem to be important. A uniform experimental procedure is needed if investigators hope to reproduce results within their own laboratories as well as reproduce the findings of others.

Keyphrases □ Cholesterol—dissolution rate behavior in bile acid solutions □ Dissolution—rate behavior of cholesterol in bile acid solutions □ Gallstones—cholesterol, dissolution rate behavior in bile acid solutions

Cholesterol monohydrate has been used as a model substance in gallstone dissolution research (1). In earlier work in this laboratory, it was noticed that small changes in the experimental procedure resulted in significant variations in the dissolution rate behavior of cholesterol pellets. In some experiments when the pellet was stored overnight at 35° prior to a dissolution run, the initial dissolution rate was two to three times greater than the dissolution rate of freshly made pellets. Since it is desirable to limit the variations to ~10–15% in many mechanistic studies on cholesterol dissolution kinetics (1–4), the cause of dissolution rate variability was investigated.

EXPERIMENTAL

Initially, the static-disk dissolution method was used, while the more quantitative rotating-disk dissolution method was used in the later analysis. The experimental procedures were described previously (5). All dissolution rate experiments were run at 37° in 5% sodium cholate and 0.1 *M* phosphate buffer at pH 8.00.

Pellets of radiolabeled cholesterol were prepared as described previously (5). Pellets placed in the dissolution cell without solvent for varying times at ambient (room) humidity or humidity of <100% at 35° will be referred to as aged pellets. The pellets kept at 100% humidity at ambient temperature for 24 hr will be referred to as normal pellets. This latter treatment was shown to give the same dissolution rate as that of freshly made pellets.

One hundred percent humidity containers were prepared by adding water to a desiccator until it completely filled the area under the plate. The other constant-humidity chambers were prepared by making saturated aqueous solutions (6) of 80% sodium bromide and of 40% calcium chloride which then were placed in an oven at 35°.

RESULTS AND DISCUSSION

Static-Disk Apparatus—When small changes in the normal experimental procedure were introduced, significant differences in dissolution behavior resulted (Fig. 1 and Table I). Figure 2 shows the effects of exposing the pellets to ambient humidity at 35° for varying periods (aging). The normally treated pellet and the pellet aged for 1 hr gave the same dissolution rate as well as the same dissolution pattern (linear). However, aging the pellet for 12 or 24 hr markedly increased the initial dissolution rate (Table II). After a few hours of dissolution, these pellets showed a definite curvature in their dissolution profiles.

Figure 3 shows the effects of varying the humidity as well as varying

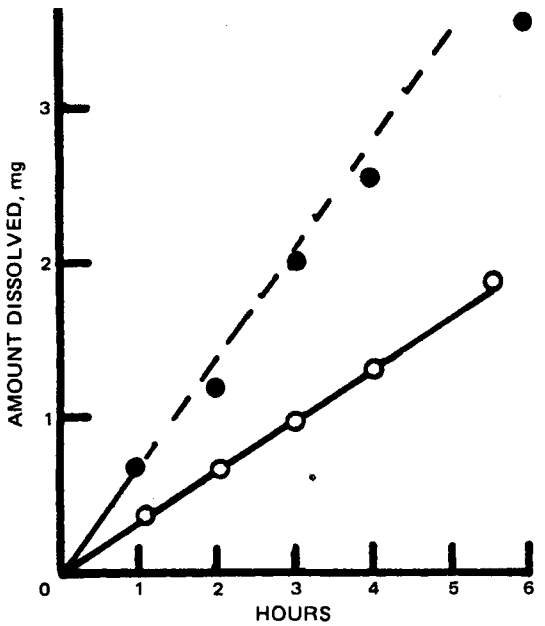

Figure 1—*Effect of pellet handling on the dissolution of cholesterol pellets at 150 rpm in 5% sodium cholate and 0.1* M *phosphate buffer at pH 8.00. Key:* ○, *normally prepared pellets; and* ●, *aged pellets.*

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