

Accelerating Gallstone Dissolution

(cholelithiasis/bile/detergent/mass transfer/kinetics)

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ABSTRACT The dissolution rates of cholesterol in model bile salt solutions are controlled by diffusion in slowly flowing bile and by interfacial kinetics in rapidly flowing bile. At low flow, dissolution varies with the square root of bile flow and can be predicted, *a priori*, from existing correlations of mass transfer. At high bile flow, dissolution is independent of bile flow and is probably dominated by the rate of micelle adsorption. These results show that cholesterol gallstone dissolution, a potential nonsurgical therapy for cholelithiasis, can be accelerated little in slow bile, but more significantly in rapidly flowing bile.

In this paper, we identify the mechanisms responsible for medically induced cholesterol gallstone dissolution in bile. Gallstone dissolution is a possible nonsurgical therapy for cholelithiasis. Such dissolution has already been achieved by the oral administration of chenodeoxycholic acid (1-3). While few in number, the successes to date are of sufficient promise to justify much more comprehensive studies. One major disadvantage of current treatments is that they are too slow; the time required to dissolve a stone of 1 cm³ volume is over a year (1). Accelerating this dissolution rate would make this treatment much more attractive.

Understanding dissolution has been hampered by inexact definitions of what "stone dissolution" is. Two different processes can be involved: fragmentation, the removal of small chunks of the stone; and true dissolution, the solubilization of a few cholesterol molecules at a time. The rates of these two processes are radically different. Fragmentation by, for example, ultrasound, can be rapid, but may be very difficult to achieve *in vivo*. On the other hand, true dissolution is often slow. Stones can be fragmented, but not dissolved, in bile supersaturated with cholesterol. Since the current nonsurgical therapies for gallbladder stones are consistent with dissolution, we report here studies of dissolution which provide a scientific basis for anticipating when gallstone dissolution can be accelerated.

THEORY

Mechanisms of dissolution are most easily discussed in terms of mass transfer rates (4). In these terms, the flux of dissolving cholesterol j is given as:

$$j = K\Delta c_1 \quad [1]$$

where Δc_1 is the cholesterol concentration at saturation minus that in the surrounding bile. The overall mass transfer coefficient K is a function of the system's properties, such as the viscosity of the bile, the diffusion of the micelles, and the nature of the stone's surface. More explicitly, the way in which K is affected by these properties is shown by the schematic drawing in Fig. 1. The overall mechanism involves

three steps. The first step depends on diffusion of the micelle to the cholesterol surface. In the second step, cholesterol is detached from the surface and is incorporated into a micelle. The third step involves diffusion away from the surface of the micelle containing cholesterol.

Each of the steps in this mechanism has a characteristic velocity k_i . The slowest velocity influences the overall dissolution rate most. Alternatively, one may characterize this mechanism by three electrical resistors in series, each of which is characterized by a resistance $1/k_i$. The largest resistance then dominates the overall dissolution rate. Since the three resistances are in series, the overall resistance $1/K$ is

$$\frac{1}{K} = \frac{1}{k_1} + \frac{1}{k_2} + \frac{1}{k_3} \quad [2]$$

Strictly speaking, the term $1/k_3$ should be replaced by the term $1/K_2k_3$, where K_2 is the equilibrium constant for the forward and reverse reactions in the second step. However, we have found that the experimental values of K_2 for a wide variety of chemical systems, including this one, are close to unity. Why this is so is discussed in detail elsewhere (J. C. Tao, A. M. Chan, E. L. Cussler, and D. F. Evans, manuscript to be submitted).

We can measure K using Eq. 1; we know how each k_i changes under different experimental conditions. Thus, measurements of K under different conditions allow identification of the dominant k_i and hence in the overall mechanism for dissolution. The k_i 's have some general properties which merit further discussion. At rapid bile flow, k_1 and k_3 are large, and k_2 then dominates K . The value of k_2 is independent of bile flow because it is determined by a sequence of chemical changes at the stone's surface. This value is difficult to deter-

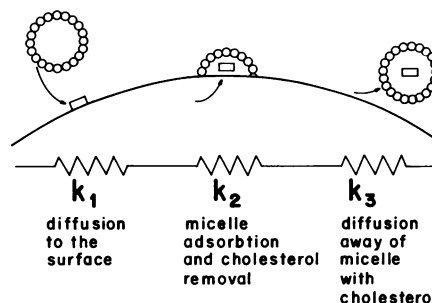


FIG. 1. Postulated mechanism for cholesterol dissolution. The dissolution rate equals the cholesterol concentration difference between the surface and the bulk divided by the total resistance ($1/k_1 + 1/k_2 + 1/k_3$).

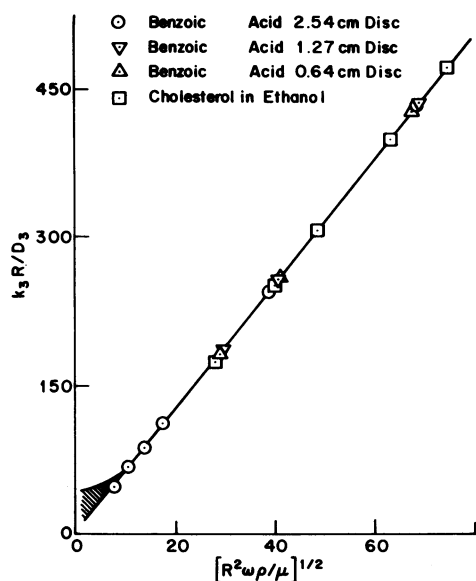


FIG. 2. Dissolution kinetics with the spinning disc. The data agree with the theoretically predicted line only at fast rotation.

mine independently, but can generally be obtained from dissolution studies at high flow.

The chemical changes that contribute to k_2 are commonly idealized in terms of three steps: adsorption, in this case of bile salt and lecithin; surface reaction, in which cholesterol moves into an adsorbed micelle; and desorption, in which the cholesterol-containing micelle detaches from the surface. This idealization, which is used with great success in heterogeneous catalysis (5), has the advantage of giving a coherent physical picture of the events occurring at the surface. This is superior to treating k_2 as an unknown, adjustable parameter, i.e., an "interfacial barrier," in which all our ignorance is buried.

At slow bile flow, k_1 and k_3 are very small. In the case of gallstone dissolution, they become much more important than k_2 in determining the overall dissolution rate and K . This means that diffusion in solution is more important than the events on the stone's surface, and that increasing bile flow increases the rate steps 1 and 3 in Fig. 1, and hence the dissolution rate.

In contrast with k_2 , k_1 and k_3 can be measured independently or calculated from experiments on other systems. For steady bile flow around a spherical stone, k_1 and k_3 can be calculated from (6):

$$\frac{k_3 d_s}{D_i} = 2. + 0.6 \left(\frac{d_s u \rho}{\mu} \right)^{1/2} \left(\frac{\mu}{\rho D_i} \right)^{1/3} \quad [3]$$

where d_s is the stone diameter; D_i is the diffusion coefficient characteristic of step "i," and u , ρ , and μ are the bile's velocity, density, and viscosity, respectively. The first quantity in parenthesis in Eq. 3, the Reynolds number, is characteristic of forced convection, when the bile flow is externally generated. For free convection, when the bile flow results from the density gradients caused by dissolution, the corresponding relation is (6):

$$\frac{k_3 d_s}{D_i} = 2. + 0.6 \left(\frac{d_s \rho g \Delta \rho}{\mu^2} \right)^{1/4} \left(\frac{\mu}{\rho D_i} \right)^{1/3} \quad [4]$$

where g is the acceleration due to gravity and $\Delta \rho$ is the density

change caused by dissolution. The first quantity in parentheses in Eq. 4, the Grashof number, has replaced the Reynolds number. Similar equations are available for other geometries, and are of great utility in processes like extraction and distillation.

EXPERIMENTAL

Materials. The model bile solutions used in these experiments contained various amounts of bile salts, lecithin, cholesterol, and sodium chloride. Sodium taurocholate (NK Labs) and sodium cholate (Mann) were better than 99 and 95% pure, respectively. In both these samples, thin-layer chromatography showed that the only additional impurities were other bile salts. There were no measurable amounts of phospholipids or of small electrolytes. Lecithin was prepared from egg yolks, paralleling the method of Small *et al.* (7) The sample, which gave only one spot by thin-layer chromatography, had a molecular weight of 755 as determined by vapor phase osmometry. The lecithin was stored at 0° in 100% ethanol solution. ¹⁴C-tagged cholesterol (New England Nuclear Corp) was combined with untagged cholesterol in benzene solution and then evaporated to constant weight.

Solutions were prepared as described previously (7). The pH of these solutions was adjusted to 8 except as indicated. Discs and spheres of radioactive cholesterol were made in a pellet press (8). The real gallstones used were white or pale yellow spheres containing more than 90% cholesterol. Dissolution was determined by liquid scintillation counting with a Packard Tri-Carb 3320 counter or by change in stone mass.

Apparatus. We found that three different experimental methods were required in these studies. The first method, used for rapidly flowing bile, was a spinning disc of cholesterol immersed in a large volume of bile solution. When the dissolution is diffusion controlled, i.e., when k_1 and k_3 are much less than k_2 ,

$$\frac{kR}{D} = f(t) + 0.6 \left(\frac{\omega R^2 \rho}{\mu} \right)^{1/2} \left(\frac{\mu}{\rho D} \right)^{1/3} \quad [5]$$

where R is the radius of the spinning disc; ω is its rotation speed; and $f(t)$ is a complex function of time (9). This function, which is not completely known, is experimentally unimportant at high flow but can not be ignored at low flow. Its neglect has seriously hampered earlier studies. The range of validity of Eq. 5 is shown in Fig. 2. The data shown include studies of the dissolution in water of benzoic acid. These dissolution rates, which were measured by titration, are known to be diffusion controlled (10). In other words, the interfacial kinetics of benzoic acid are much more rapid than the diffusion. The measurements include different disc sizes and different spinning speeds. When $(R^2\omega\rho/\mu)$ is above 100, the data fit Eq. 5, so the apparatus can be used to explore dissolution mechanisms in this region. Similar results are obtained for cholesterol dissolving in ethanol.

The second experimental method used a cholesterol sphere or a real gallstone suspended in bile. For studies at high flow, the sphere was suspended in a tube through which bile was steadily pumped at a known rate, and the results were analyzed with Eq. 3 (11). For studies at low flow where free convection is important, the sphere is suspended in an unstirred beaker of bile. Since the only flow in this case is that generated by the dissolution, the results were analyzed with Eq. 4.

The third experimental method (12) consisted of a cholesterol slab mounted at the bottom of a cylindrical volume of bile solution. This volume is bounded by identical holes in four flat plates, which are initially aligned but can be slid apart without causing flow in the vertical direction. This apparatus was used only for studies in stagnant bile where even free convection was absent. Both the second and the third methods were checked by studying the dissolution of benzoic acid. We again obtained results consistent with the known diffusion-controlled dissolution of benzoic acid (8).

RESULTS AND DISCUSSION

Gallstone dissolution will be a much more attractive non-surgical therapy for cholelithiasis if it can be accelerated. Whether this acceleration is feasible depends on the mechanisms which limit the dissolution rate. Which mechanism is most important depends in turn on the state of the surrounding bile, and thus more specifically on the bile velocity and the bile composition. Our results, which are the first to elucidate the mechanisms involved, are clearest if we initially discuss the variation of dissolution rates with the physical chemical factors, and then explain how these chemical factors produce physiological effects.

Chemical Factors. We first consider the influence of bile velocity on the rate of cholesterol dissolution. Typical results are shown in Fig. 3. The dissolution rates are reported as overall mass transfer coefficients K . The K include the effect of a partially saturated solution, i.e., they are essentially the dissolution rate per area per amount of unsaturation, as shown by Eq. 1. The velocities are reported as dimensionless variables, i.e., as the Grashöf number for free convection experiments and as the Reynolds number for forced convection experiments. Equal values of these dimensionless variables indicate an equal effect of bile velocity, viscosity and density on the dissolution rate.

The dissolution rates shown in Fig. 3 start at a small but finite value at zero bile velocity, then rise linearly with the square root of velocity, and finally are constant at high bile velocity. Thus the results are most conveniently described in terms of three regions: stagnant bile, slow bile flow, and fast bile flow. Previous studies have been hampered because these regions have not been adequately defined (13-15). As a result, the different mechanisms involved have not been delineated.

Stagnant Bile. The first region is that of stagnant bile, where the Reynolds and Grashöf numbers are zero. In this region, the mechanism controlling dissolution depends only on diffusion. The stagnant region is important because it establishes an experimental limit in agreement with that predicted by the theory given above. For example, the experimental value for K in stagnant 10% sodium taurocholate is 0.15×10^{-4} cm/sec; that predicted from Eqs. 1-3 is 0.14×10^{-4} cm/sec.

Slow Bile Flow. The second region in Fig. 3 is that of slow bile flow. All values of K in this region fall on the same straight line, independent of bile salt concentrations and temperatures studied. Cholesterol dissolution in this region of slow flow is determined by the properties of the bile salt-cholesterol solution, and is not affected by the nature of the cholesterol-micelle reaction on the surface. In other words, steps 1 and 3 in Fig. 1 are more important than step 2. The proof of this statement is the agreement of the experimental points and the

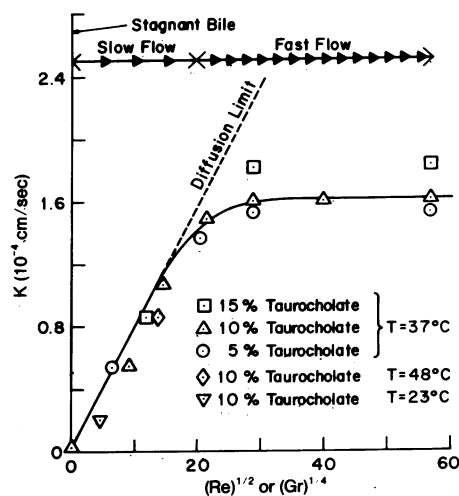


FIG. 3. Dissolution of cholesterol as a function of fluid velocity. Values at different bile salt compositions and at different temperatures fall on the same line at low flow, but not at high flow.

dotted line, which is calculated *a priori* from solution properties and Eqs. 1, 2, and 4. There are no adjustable parameters like the "unstirred layer thickness" in this calculation.

The key variables in determining dissolution rates are the diffusion coefficient of cholesterol, which we have measured independently (11), and the bile flow. The flow in this region results primarily from free convection, engendered by the dissolution process itself. The solution comprised of bile salt and newly dissolved cholesterol has a different density than a solution of the same concentration of bile salt; this causes flow. Density differences less than 10^{-3} g/cm³ can cause significant flows, dominating changes in the Grashöf number. Since these flows will occur *in vivo*, this region is physiologically important.

The effect of lecithin on the rate of cholesterol dissolution in slowly flowing bile is shown in Fig. 4. The decrease of dissolution rate on the addition of lecithin, which has also been observed by others (13-15), is counter to that expected from the increased solubility of cholesterol caused by the addition of lecithin. No single step controls dissolution in this region. About half of the observed decrease can be predicted from changes in solution properties, particularly decreases in the diffusion coefficient in step 1 (11). However, the remaining portion of this decrease depends on the chemical changes at the surface of the stone, i.e., on step 2. The nature of these

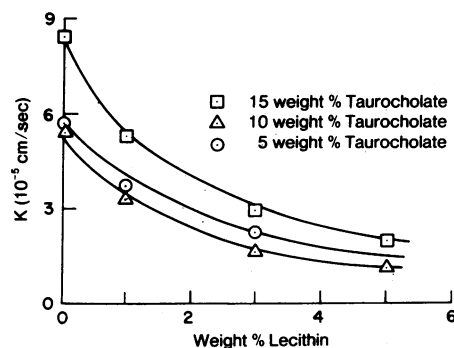


FIG. 4. Lecithin reduces dissolution rates at slow bile flow. About half of this reduction is caused by decreased diffusion.

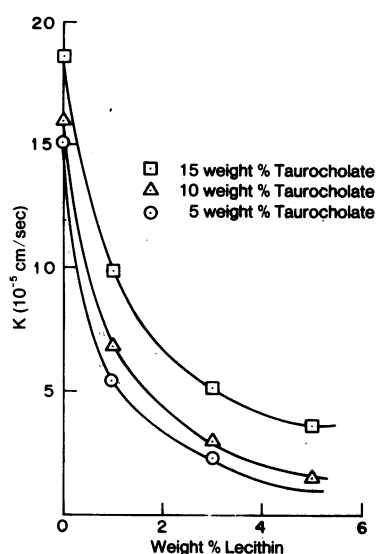


FIG. 5. Lecithin reduces dissolution rates at fast bile flow. This decrease probably results from slower micelle adsorption.

changes is easier to see when the bile flow is high and only step 2 is important.

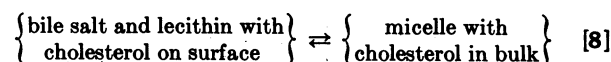
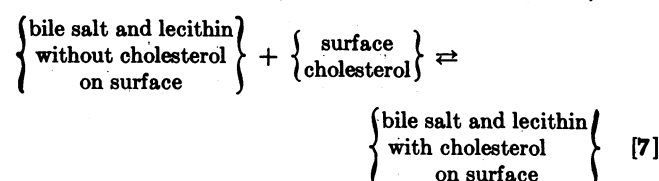
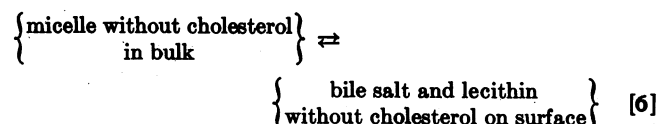
Since detergents have been reported to increase cholesterol dissolution at high bile flow (15), we measured their effect at low bile flow, i.e., under conditions of free convection. The dissolution rate in 10% sodium taurocholate is 80 ng/cm²·sec; when 0.1 wt % benzalkonium chloride is added to this same system, the rate is 110 ng/cm²·sec (8). Sodium chloride, which also has a large effect at high flow as shown below, also has little effect at low flow: the rate is 90 ng/cm²·sec in 0.3 M NaCl (8). The small effect of additives is a direct consequence of the fact that dissolution can not be faster than the diffusion-controlled limit, the dotted line in Fig. 3.

Fast Bile Flow. The third region in Fig. 3 is that of high bile flow. Values of K in this region are independent of bile flow, i.e., of Reynolds and Grashof numbers. They increase with temperature and decrease with bile salt concentration, in contrast with the values in the second region. This altered variation of K , so completely different from that in slow bile flow, results because a different mechanism now controls the dissolution. More specifically, this behavior depends on the nature of the cholesterol-micelle reaction on the surface, and is consistent with step 2 in Fig. 1 occurring much more slowly than steps 1 and 3. This region will be physiologically im-

portant if bile flow is sufficiently high in the gallbladder; specific examples are given later in the paper.

At high flow, values of K and hence of k_2 increase linearly with bile salt concentration but drop sharply with the addition of lecithin, as shown in Fig. 5. Cholesterol is more soluble in bile salt-lecithin solutions than in solutions of bile salt alone, so that consideration of solubility alone would suggest *increases* in dissolution rate. The *decreases* actually observed illustrate the importance of kinetic factors like those summarized above.

These decreases can be understood more completely by considering step 2 in more detail. As suggested above, this step can be idealized as three chemical reactions on the surface of the stone:



Rate equations giving the variation of k_2 with variables like bile salt concentration can be developed from Eqs. 6–8 (5, 8). These equations predict a linear variation of k_2 with bile salt concentration only if the reaction in Eq. 6 proceeds much more slowly than the others. Thus micelle adsorption is apparently the dominant factor in determining k_2 .

The suggestion that micelle adsorption determines k_2 is also consistent with the effect of added lecithin, added salt, and added cationic detergents. Adding lecithin increases micelle size, and so should make micelle adsorption slower and more difficult. Adding salt and cationic detergent would be expected to decrease the charge density on the micelle, and hence increase the adsorption rate and hence k_2 . Adding salts (9) and cationic detergents (15) can in fact increase k_2 . For example, dissolution at high flow in 10% sodium taurocholate and 1 M sodium chloride is 60% faster than in the bile salt alone. However, these increases can not exceed the diffusion controlled limit.

TABLE 1. Examples of kinetics of gallstone dissolution

Bile velocity (cm/sec)	Stone diameter (cm)	Dissolution rate (10 ⁻⁸ g·cm ⁻² ·sec ⁻¹)	Controlling resistances	Remarks
0.0	0.1	4.1	Diffusion	Very difficult to increase rate
	1.0	0.41		
0.01	0.1	7.0	Diffusion	Velocities from free convection; difficult to increase rate
	1.0	3.5		
0.1	0.1	8.5	Diffusion and Interfacial	All three resistances become important
	1.0	7.0		
1.0	1.0	10.0	Interfacial	Rate no longer varies with bile velocity; larger increase possible
	1.0	9.0		

Physiological Implications. Whether cholesterol gallstone dissolution can be significantly increased depends on which factors control the process. The effect of these factors is shown by the calculation of dissolution as a function of flow and stone size in Table 1, based on bile of 10% sodium taurocholate and 3% lecithin (8). The dissolution rates will be half as fast in bile saturated 50% with cholesterol, and only one-tenth as fast in bile saturated 90% (compare Eq. 1).

We can make a more direct comparison between our *in vitro* results and the *in vivo* dissolution studies with chenodeoxycholic acid if we assume that bile velocity is dominated by free convection. This means that the gallstone dissolution is diffusion controlled, i.e., that there is no significant "interfacial barrier." In other words, the velocity caused by filling is slower than that caused by free convection, and the velocity engendered by emptying is too brief to be significant.

Under these conditions, the overall mass transfer coefficient K in a solution of 10 wt % sodium taurocholate and 3 wt % lecithin is about 2×10^{-5} cm/sec (compare Fig. 4). For a spherical gallstone, a mass balance yields (16)

$$\text{change in stone radius per time} = K\Delta C/\rho_c \quad [9]$$

where ρ_c ($= 1.06$ g/cm³), the density of cholesterol. If this bile is 70% saturated with cholesterol, then ΔC is about 10^{-3} g/cm³. As a result,

$$\begin{aligned} \text{change in stone radius per time} &= 2 \times 10^{-8} \text{ cm/sec} \\ &= 0.6 \text{ cm/year.} \quad [10] \end{aligned}$$

The change in stone radius observed *in vivo* is about 0.3 cm/year. This agreement is surprisingly good. The difference between the estimated and experimental results might easily result from higher saturation or higher bile viscosity. Thus in contrast with earlier studies (17), our results suggest that

gallstone dissolution is diffusion controlled. If this is correct, greatly accelerating gallstone dissolution will be difficult.

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