

A Model for Gallbladder Function and Cholesterol Gallstone Formation*

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Abstract. An analysis of fluid mechanics and diffusion in the gallbladder predicts that there is a thin layer of high bile-constituent concentration near the gallbladder wall. Cholesterol may precipitate in this layer, even when the average cholesterol concentration in the gallbladder is below saturation. The amount precipitated increases with time, with increased average cholesterol concentration, and with decreased average lecithin concentration. If the gallbladder does not empty completely, the precipitated cholesterol particles may grow over many cycles of gall bladder filling and emptying. The analysis explains why cholesterol-gallstone formation is not correlated with bile-constituent concentration alone, why a flaccid, noncontractile, gallbladder has a greater chance of forming gallstones, why small stones are frequently found near the gallbladder wall, and why stones may be found in only one limb of a double gallbladder.

Cholesterol-gallstone formation is a major medical problem in Western society, affecting almost a quarter of the population. Research concerning the cause of gallstones has been largely limited to studies of the biochemical composition of bile. An excess of cholesterol relative to the concentration of bile acid and lecithin, leading to cholesterol saturation and precipitation, is central to current theories of gallstone formation,^{1,2} and has been shown experimentally.

Explanations based on saturation alone implicitly assume that bile is a homogeneous liquid containing mainly bile acid salts, lecithin, and cholesterol. When the cholesterol concentration exceeds saturation, it precipitates. Hence, any factor that increases cholesterol concentration should increase the probability of stone formation. Supersaturation of cholesterol without precipitation seems unlikely, since abundant nucleation sites, such as bilirubinate proteins, are present in bile.³

However, such explanations of gallstone formation in humans do not account for four important facts:

(1) There is no concentration difference between centrifuged gallbladder bile samples of normal patients and those with gallstones. For example, normal residents of Cleveland, Ohio, have the same average concentrations of gallbladder bile constituents as American Indians in Lawton, Oklahoma, of whom 85% get gallstones.⁴ There is not, in all instances, a simple relationship between cholesterol, lecithin, and bile acid concentration and the finding of gallstones in the

gallbladder. Dam *et al.*⁵ report cases where for comparable lecithin and bile acid concentrations in gallbladder bile, individuals with *lower* cholesterol concentrations (in centrifuged bile) were found to have gallstones.

(2) The ability of the gallbladder to concentrate, and subsequently expel, bile is important in normal gallbladder function. For example, if the vagus nerve is cut as part of an operation, the gallbladder becomes flaccid and the probability of stones increases.⁶ Failure to concentrate bile is a frequent clinical finding associated with gallstones observed on x-ray examinations.

(3) Small gallstones or cholesterol crystals are frequently found in groups along the wall of the gallbladder where liquid flow is particularly large.⁷

(4) Cholesterol gallstones may be found in only one limb of double gallbladders.⁸

These facts may be explained by a large variation of solute concentration near the gallbladder wall. This variation most likely arises from the rapid concentration of hepatic bile in the gallbladder. Consequently, the concentration of cholesterol very near the wall of the gallbladder may be above saturation in many instances. Precipitation of microcrystals in this region can occur irrespective of bulk concentration. The question then becomes not why some individuals have gallstones, but why everyone does not.

Theory. The gallbladder operates in a cyclic fashion: filling with hepatic bile; simultaneously concentrating the bile constituents and increasing in volume; and finally, periodically emptying the contents into the intestine. The bile constituents are predominantly cholesterol, lecithin, and bile acid salts. During this cycle, the constituents are concentrated by the active transport of water and small electrolytes^{9,10} through the gallbladder wall. This flow may be assumed to form a layer of bile constituents near the wall, as if filtration were occurring at the surface. This layer will not disappear, since the constituents in the liquid diffuse only slowly away from the wall. The layer will get thicker as abstraction of water continues, decreasing the rate of water removal per unit area.¹¹ However, since the volume of the gallbladder, and hence its wall area, increases with time, the total rate of water removal need not decrease.

If these assumptions are made it can be demonstrated that the cholesterol concentration near the bladder wall may be above saturation and cholesterol can precipitate. This precipitation might take two extreme forms: microcrystals that are rapidly formed but expelled before they coagulate, and larger particles, 1-2 mm in diameter, near the wall⁷ that are not expelled but slowly generated over many cycles. Regardless of the form of this precipitation, there may be a tendency for precipitated material to be held against the wall by the large water flow during the period of concentration of bile. The amount of precipitated cholesterol will be small if the rate of water flow through the wall is large, since the thickness of the layer can be shown to vary inversely with this flow. Between these two extremes, a moderate flow of water may give a layer thick enough to precipitate significant amounts of cholesterol. Thus, flux through the gallbladder wall, and gallbladder filling and emptying, may be even more important than the average concentration of bile constituents in the gallbladder.

To show that these factors are central to an understanding of both gallbladder

function and gallstone formation, we now examine them quantitatively in the three following sections. First, we derive expressions for the variation of bile constituent concentration near the gallbladder wall. Second, we determine the variation of gallbladder volume with time. Finally, we show that these results predict significant cholesterol precipitation at varying concentrations of cholesterol relative to bile acid and lecithin concentration.

Concentration profiles: To quantitatively calculate the variation of concentration of constituents as a function of distance from the wall, we assume that the gallbladder may be approximately modeled as a sphere of varying volume. The common bile duct is assumed to end at the center of the sphere and to deliver hepatic bile at a constant flow rate. The approximation of the gallbladder as spherical is reasonable, since it is found that concentration varies significantly only near the gallbladder wall. The assumption that the flow is spherically symmetric is more limiting, since it implies that the bile will never be layered. This layering may contribute significantly to cholesterol precipitation^{12,13} but is neglected here; it can be included in a more sophisticated model by allowing for unsteady flow of hepatic bile, a nonspherical pear-shaped gallbladder, and the effects of changes in position with bile of differing density.

In this simple model, bile is assumed to be a binary system of constant density. The solvent of this binary system is taken as all species which freely pass through the cell wall, such as water and small electrolytes. The solute includes cholesterol, lecithin, bile acid salts, and bile pigments. The constant density assumption, common in liquid diffusion theory, has been justified in a wide variety of experimental situations.¹⁴ Symbolically, this assumption is:

$$\rho = c_1 + c_2 \quad (1)$$

where ρ is the density of bile, in g/cm³, and c_1 and c_2 are the concentrations of solvent and solute in g/cm.³

As a result of these assumptions, bile can be considered to flow only in the radial direction. The velocity v_r of this flow is easily shown to be:⁹

$$v_r = Q/4\pi r^2 \quad (2)$$

where Q is the flow into the gallbladder in cm³/sec and r is the radial coordinate in the gallbladder. The continuity equation¹⁵ for the water in the bladder is

$$(\partial c_1/\partial t) + v_r (\partial c_1/\partial r) = (D/r^2) [\partial(r^2 \partial c_1/\partial r)/\partial r], \quad (3)$$

where D is the diffusion coefficient in cm²/sec, and t is the time. The gallbladder is assumed initially to be filled with bile at some known concentration. In addition, the concentrations at the wall of the gallbladder are taken to be constant: when $r = 0$, $c_1 = c_1^0$ and when $r = R_w$; $c_1 = c_1^w$, where R_w is the radius of the wall. The solution of Eq. (3) depends on the value for R_w , which in turn depends on the equation for the gallbladder's volume, V :

$$\partial V/\partial t = \partial \left(\frac{4}{3} \pi R_w^3 \right) / \partial t = Q - J_1/\rho, \quad (4)$$

where J_1 is the total solvent flow in g/sec through the bladder wall, relative to

the velocity of the bladder wall. But the calculation of J_1 in turn requires a solution to Eq. (3). Hence, Eqs. (3-4) must be solved simultaneously.

To obtain a simultaneous solution of these equations is only possible numerically, and does not seem justified in view of the variety of assumptions made above. However, an approximate solution can be obtained because the gallbladder concentrates liver bile at least 5 or 10 times. This means that Q and v_r in the above equations will be quite large, and will dominate the concentration profile. Hence, $v_r (\partial c_1 / \partial r) \gg (\partial c_1 / \partial t)$. Equation (3) then becomes

$$v_r = (D/r^2) [\partial(r^2 \partial c_1 / \partial r) / \partial r] \quad (5)$$

Appropriate boundary conditions for this equation assume that the center region of the gallbladder is well mixed by free convection. As a consequence, the concentration in this region is uniform. The concentration of constituents at the wall of the gallbladder is assumed to be fixed by the intense solvent flux across the wall. The physical analogue of this behavior occurs during the process of filtration. A formulation of the boundary conditions is as follows: when $r = R_c$, $c_1 = c_1^c$ and $r = R_w$, $c_1 = c_1^w$ where R_c is a small radius characteristic of the well-mixed region, and c_1^c is the solvent concentration in this center region. The wall concentration remains fixed, but the concentration in the well-mixed center can change with time. Eq. (5) is easily solved for the conditions given:

$$(c_1 - c_1^c) / (c_1^w - c_1^c) = (e^{-PéR_w/r} - e^{-PéR_w/R_c}) / (e^{-PéR_w/R_c}) = e^{Pé(1 - R_w/r)} \quad (6)$$

where $Pé (= Q/4\pi DR_w)$ is the Péclet number, a dimensionless quantity giving the relative effect of flow and diffusion. The approximation holds because $R_w \gg R_c$. In gases, where the Péclet number is often small, the effects of flow and diffusion are of similar magnitude. But in liquids such as bile, the Péclet number is large, and flow processes control diffusion effects. With the assumption of constant density (Eq. 1),

$$(c_2 - c_2^c) / (c_2^w - c_2^c) = e^{Pé(1 - R_w/r)} \quad (7)$$

The solvent flux n_1^w at the gallbladder wall relative to fixed coordinates is

$$n_1^w = -D[\partial c_1 / \partial r]_{r=R_w} + [c_1^w v_r]_{r=R_w} = (c_1^c Q) / (4\pi R_w^2) \quad (8)$$

A similar relation exists for the solute:

$$n_2^w = (c_2^c Q) / (4\pi R_w^2) \quad (9)$$

This completes the development of the equations for the concentration profiles (Eqs. 6-9), the first of the three steps in the quantitative theory. However, two quantities in these equations, R_w and c_2^c , are unspecified.

Gallbladder volume: To determine c_2^c and R_w (or V), we must solve for the variation of gallbladder volume with time, using the overall conservation equations. The overall equation for the solute is

$$\partial(c_2^c V) / \partial t = Q c_2^0, \quad (10)$$

where c_2^0 is the inlet concentration of solute from the common bile duct. This is easily integrated:

$$c_2^c V = Q c_2^0 t \quad (11)$$

The overall equation for the volume (Eq. 4) requires determination of the flow J_1 relative to the wall

$$J_1 = 4\pi R_w^2 [n_1^w - c_1^w v_w] \quad (12)$$

where v_w is the velocity of the wall. But because no solute passes through this wall, we have an additional boundary condition:

$$v_w = v_2|_{r=R_w} = n_2^w/c_2^w \quad (13)$$

where $v_2|_{r=R_w}$ is the solute velocity at the wall. Eqs. (1, 5, 8, 9, 12, and 13) give

$$\partial V/\partial t = c_2^0 Q/c_2^w \quad (14)$$

When this result is combined with Eq. (15) and integrated, we obtain

$$c_2^c = [c_2^0 c_2^w]^{1/2} \quad (15)$$

$$V = [c_2^0/c_2^w]^{1/2} Q t = (c_2^0/c_2^c) Q t \quad (16)$$

These equations show that the volume V increases linearly with time t until the gallbladder empties as part of its cyclic operation, and that the solute concentration in the center region, c_2^c , is independent of time. This information is sufficient to specify the concentration profile as a function of distance from the gallbladder wall and time.

Cholesterol precipitation: The final step in this theory is the calculation of the amount of cholesterol precipitated. The total amount of precipitated cholesterol is calculated from the total solute-concentration profile, implicitly assuming that cholesterol is a small fraction of the total solute. As the cholesterol concentration near the wall becomes greater than the saturation value, cholesterol begins to precipitate. Nucleation and precipitation are assumed rapid and complete; this assumption is discussed further below. Since the concentration of cholesterol varies only near the wall, the total fraction of cholesterol precipitated is:

$$F = \left[\int_{R^*}^{R_w} (c_3 - c_3^*) 4\pi r^2 dr \right] / \left[\frac{4}{3} \pi R_w^3 c_3^c \right] \quad (17)$$

where c_3 is the total cholesterol concentration, c_3^c is the cholesterol concentration in the center, c_3^* is the concentration at saturation, and R^* is the radius where precipitation first starts to occur. Both R_w and R^* vary with time. However, if this integral is evaluated just before the gallbladder empties, i.e., when the volume is a maximum, F is the maximum fraction precipitated in that cycle.

Since c_3 is linearly proportional to c_2 , we see from Eq. (7) that

$$dc_3 = P\dot{\epsilon}(R_w/r^2) (c_3 - c_3^c) dr \quad (18)$$

When we combine this result with Eq. (17) and remember that the concentration varies significantly only near the wall, we obtain

$$F = (3/c_3^c P\dot{\epsilon}) [c_3^w - c_3^* - (c_3^* - c_3^c) \ln [(c_3^w - c_3^c)/(c_3^* - c_3^c)]] \quad (19)$$

a result restricted to cases where the concentration gradient varies only near the wall. This result completes the theoretical development of this model for the gallbladder.

Numerical Examples. To illustrate the significance of the above equations, we now give numerical examples for the concentration profiles and for resulting cholesterol precipitation. The numerical values used in these calculations are representative but by no means exhaustive. One may choose different values and obtain the same qualitative behavior.

Solute concentration profiles: Typical solute concentration profiles calculated from Eqs. (6-7) are shown in Fig. 1. The concentration is seen to vary rapidly

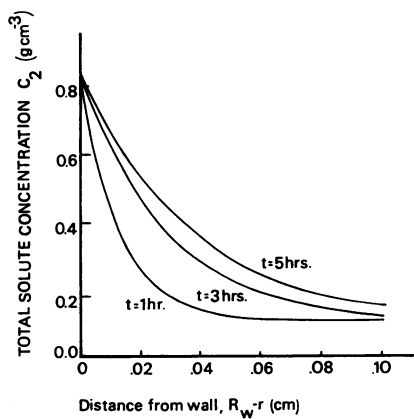


FIG. 1. Solute concentration profiles near the gallbladder wall.

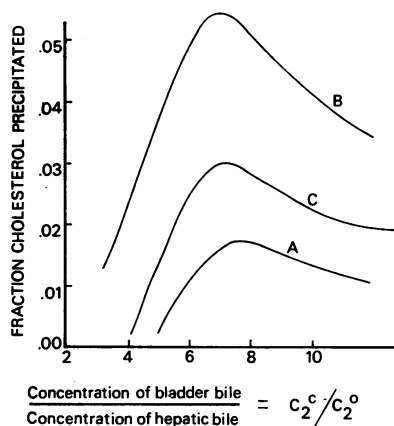


FIG. 2. Cholesterol precipitation below saturation in the gallbladder.

near the wall, reaching within 0.01 g/cm^3 of the bulk value only 0.05 cm from the gallbladder wall. Moreover, the concentration does not vary rapidly with time, consistent with the steady state approximation made in going from Eq. (3) to Eq. (5). In these calculations, the following values are assumed as typical: the bile is concentrated six times, so that $c_2^c/c_2^0 = 6$; the solute concentration c_2^c is 0.14 g/cm^3 ; the diffusion coefficient is $5 \times 10^{-6} \text{ cm}^2/\text{sec}$; and the gallbladder reaches a volume of 60 cm^3 in 5 hr, then empties. This constant value for the diffusion coefficient is consistent with our preliminary experiments.¹⁶

To make these calculations, we first find the flow rate Q from Eq. (13) ($Q = 0.02 \text{ cm}^3/\text{sec}$) and use this to calculate the total flow J_1 out of the gallbladder ($J_1 = (360-60)\text{cm}^3/5 \text{ hr} = 0.017 \text{ cm}^3/\text{sec}$). Now from Eqs. (1) and (12-13), we find $c_2^w = 0.84 \text{ g/cm}^3$. This very high solute concentration at the wall is purely a result of the concentrating ability of the gallbladder. From Eq. (7) we may calculate the results shown in Fig. 1, remembering that R_w is a function of the volume, and hence of time (Eqs. 4, 16).

Cholesterol precipitation: The precipitation of cholesterol depends strongly on the concentration of bile constituents and on the ability of the gallbladder to concentrate hepatic bile. To demonstrate this, we have calculated the fraction

of cholesterol precipitated for three typical solutions of bile constituents, such as those given in Table 1. Each solution is close to values found in *centrifuged* samples of human bile containing 14 g/100 ml solutes. It should be emphasized that these are true, stable solutions, containing no suspended precipitates, microcrystals, or supersaturated solutes.

TABLE 1. *Sample bile concentrations.**

	A	B	C
Bile salts	0.105	0.090	0.075
Lecithin	0.030	0.040	0.055
Cholesterol	0.005	0.010	0.010
Cholesterol at saturation	0.020	0.022	0.032

* All values are in g/cm.³

The fraction of cholesterol precipitated, F , for each of these solutions is plotted in Fig. 2 vs. c_2^c/c_2^0 , a measure of the ability of the gallbladder to concentrate hepatic bile. In the calculations for Fig. 2, we have again assumed that the diffusion coefficient is 5×10^{-6} cm²/sec and that the gallbladder expands to a volume 60 cm³ in 5 hr, and then empties.

Discussion. It can be seen that when the bile-constituent concentrations vary with position within the gallbladder, significant cholesterol precipitation can take place. Because of the nature of the concentration profiles, as shown in Fig. 1, this precipitation occurs near the gallbladder wall. The amount precipitated depends primarily on the concentration of constituents and on the ability of the gallbladder to concentrate bile. This is shown in Fig. 2 and Table 1. Comparison of curves *A* and *B* shows that increased cholesterol concentration leads to increased precipitation. Comparison of curves *B* and *C* shows that increased lecithin concentration leads to decreased cholesterol precipitation. Depending upon the flux of water across the gallbladder, the amount of cholesterol precipitated may be increased or decreased by a factor of two. It should be remembered that saturation considerations alone would have predicted no precipitation in the three cases considered in the calculations of Fig. 2.

The model developed here has two major deficiencies: it makes no predictions about the form of the precipitated cholesterol nor the extent to which the gallbladder empties *in vivo*. The nature of precipitated cholesterol will depend upon its nucleation.¹¹ In the absence of nucleation sites, the cholesterol will supersaturate and no precipitation will occur. If large numbers of nucleation sites are present, cholesterol will be precipitated as very small particles. The agglomeration of these particles will be very slow, limited by their diffusion through viscous, concentrated bile. At intermediate concentrations of nucleation sites, the growth of particles is controlled by the diffusion of individual cholesterol molecules which is not significantly changed in the highly viscous solution. This can lead to the formation of stones of significant size.

The second deficiency, that nothing is said about the emptying of the gallbladder, means that the consequences of incomplete emptying are ignored. Since some cholesterol precipitation may frequently occur in human bile, this precipitated material must be expelled at the end of each cycle. Otherwise, the microcrystals which are retained on the wall can grow over many cycles.

When the form of the precipitation and the extent of emptying are considered, the model developed here will describe the four facts given in the introduction:

(1) Gallstone formation does not correlate with concentration of cholesterol, lecithin, and bile salts because not enough variables have been measured. Specifically, concentration measurements must be supplemented with studies of the amount of hepatic bile concentrated, of the nature of the precipitated cholesterol, and of the way in which the gallbladder empties.

(2) When the gallbladder is flaccid, the probability of the formation of stones is increased because the ability to concentrate bile is impaired and because incomplete emptying allows microcrystal growth over many cycles.

(3) Gallstones occur commonly at the wall because the concentration of cholesterol is predicted to exceed saturation in this region.

(4) The formation of gallstones in only one limb of double gallbladders supports the concept that flow, and the kinetics of gallbladder filling and emptying, are more critical than the relative concentrations of biliary constituents presented to the gallbladder in hepatic bile.

In conclusion, the fallacy of interpretations based solely on solubility measurements alone is obvious. The optimal functioning of the gallbladder depends on its cyclic operation, particularly on the ability to concentrate bile, and on its degree of emptying. The nature of the nucleation of cholesterol in the gallbladder must also be a critical factor. The assumptions made in the development of this theory require experimental verification. The predictions are compatible with qualitative clinical observations and point out the necessity for obtaining more precise quantitative information about the dynamic features of gallbladder operation. Data of this sort are urgently needed to improve our understanding of cholesterol-gallstone formation.

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¹⁵ For a derivation of this equation see ref. 14, p. 559.

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