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RESEARCH ARTICLES

Cholesterol Solubility in Model Bile Systems: Implications in Cholelithiasis

DANIEL MUFSON[x], KRISNA TRIYANOND, JOHN E. ZAREMBO, and LOUIS J. RAVIN

Abstract □ Studies of cholesterol solubility in a physiologically realistic model bile system as a function of its (crystalline) form revealed significant behaviorial differences in the rate of solution and the approach to equilibrium. The solubility of the equilibrium species, hydrated cholesterol, and of fresh human gallstones was found to be approximately 5 mole %. Anhydrous and coprecipitated cholesterol samples attained metastable solubility values. The lag time for nucleation and growth to occur in these systems could account for lab-to-lab variations in previous studies and could be of extreme importance *in vivo* when lithogenic bile is present. These studies were extended to measurement of the solubility as a function of the bile salt–lecithin ratio. Evaluation of these data with respect to available clinical data reveals that many normal individuals have bile that is supersaturated with respect to cholesterol. The principles governing the maintenance of supersaturation in these persons are discussed and suggestions for future studies are offered.

Keyphrases □ Cholesterol solubility in model bile systems—relationship to solid-state (crystalline) properties and bile salt–lecithin ratio, implications in cholelithiasis □ Gallstones and cholesterol-supersaturated bile in man—implications of cholesterol solubility in model bile systems, solution rate and equilibrium considerations □ Bile salt–lecithin ratio—relationship to cholesterol solubility, model

Cholesterol is the major component of gallstones found in western man. This water-insoluble lipid is primarily excreted in the bile. The nature of the interactions between cholesterol and the other biliary lipids, namely bile salts and phospholipids, has been the subject of many investigations. Transport of the cholesterol has been shown to be mediated by a lecithin–bile salt micelle system. Overloading this system can result in cholelithiasis, which is the deposition of cholesterol as gallstones, a disease state that has been estimated to affect some 15 million Americans. "More than 300,000 gallbladders are removed annually because of cholelithiasis while medical, surgical, and hospitalization expenses ascribed to gallstone disease total almost 1 billion dollars a year" (1).

The functions of bile almost seem paradoxical; on the one hand it is involved with intestinal digestion and absorption of lipids, and on the other it is responsible for the excretion of the otherwise water-

insoluble cholesterol. Bile is a complex mixture, secreted by the liver, consisting of bile salts, phospholipids, cholesterol, pigment, proteins, and mucus. During nonmeal periods, the bile is held in a reservoir, the gallbladder. Upon hormonal stimulus due to a meal, the gallbladder slowly contracts, releasing the bile into the duodenum. Here the bile salts facilitate the digestion and absorption of lipids. The majority of these bile salts are actively reabsorbed in the ileum and then are rapidly picked up by the liver for resecretion into the bile. Because of its many components, numerous theories as to bile's mode of biliary cholesterol transport were proposed.

DISCUSSION

Isaksson's (2) *in vitro* studies served to establish the importance of both bile salts and lecithin in the solubilization of cholesterol. Above a critical concentration, bile salts in aqueous media spontaneously form micelles. These aggregates have little capacity to solubilize cholesterol. However, the addition of lecithin to micellar bile salt results in micelles with the capacity to solubilize substantial quantities of cholesterol. Normal human bile contains approximately 10% by weight of bile salts and lecithin.

Several different disease processes, such as inproportionate secretion of cholesterol by the liver, infection, or absorption of bile acids through the gallbladder wall, may act to overwhelm the ability of bile to carry cholesterol, resulting in the production of bile supersaturated with cholesterol. Relief of the supersaturation by cholesterol precipitation can proceed to the point of filling the gallbladder and/or blocking the bile ducts. Such blockage generally necessitates surgical removal of the deposit, a gallstone. Autopsy data (3) indicate that 10% of the world's population has gallstones. To study rationally the development of such stones, the solubility of cholesterol in bile must be well defined.

With advances in analytical and preparative methods, it has been possible to prepare model systems for systematic studies on the pathogenesis and therapy of cholelithiasis. Through the use of such systems the mechanism of cholesterol's solubilization has been investigated. While the chemical identity and purity of these lipids are generally well documented, the same cannot be said for their physical properties. Although there have been many investigations on this subject, the literature is confusing, no one value has been universally accepted. "Obviously, establishment of a solubility limit to which everybody can agree is called for" (4).

Table I is a compilation of the solubility methods and equilibrium times employed in representative studies. These studies generally suffer from a lack of definition of the physical state of the cholesterol employed and/or from ill-defined equilibrium states. The most widely utilized procedure of sample preparation was that of simultaneous (evaporation) precipitation or coprecipitation of the lecithin, bile salt, and cholesterol from an organic solvent system. Upon evaporation, a suitable aqueous phase is added. It is a very convenient method to use for ease in quantifying addition of each component and to ensure mixing. However, while this coprecipitate system offers much in the way of facilitating laboratory manipulation[1], it can alter the physical properties of the lipids. This is not implicitly bad; it is simply a fact that must be recognized in the interpretation of the data. Where the use of crystalline cholesterol was reported, its exact nature was generally not specified; different methods and solvents of crystallization were used. Hydrated cholesterol has been reported as the physiologically stable form of cholesterol (15–17). Upon storage at low humidities, the hydrate becomes anhydrous (16, 18).

The solubility of cholesterol in these studies was judged by methods varying from gross determination of a vial for the presence of cholesterol to actual assay of micellar cholesterol. The temperature of these studies was not always physiological. Furthermore, the solubility data were reported in various ways, *i.e.*, milligrams per milliliter or molar ratio of bile salts to cholesterol, which makes an overall assessment difficult. The presentation of such data was put on a rational basis by application of phase diagrams (6). These diagrams emphasize the molecular interactions between the four species involved in the solubilization: cholesterol, bile salt, lecithin, and water. Additional information regarding phase behavior may be gleaned from such diagrams; systematic analysis of the effects of composition on the physical-chemical properties of the system revealed the presence of regions containing crystalline cholesterol, liquid crystalline material, or both.

Later papers attempted to correlate these model data with *in vivo* findings. Admirand and Small (19) were able to show that the bile composition of patients with cholelithiasis fell outside the micellar region while normal patients were generally within the micellar boundaries. However, while the use of the quaternary phase diagram has greatly increased comprehension of the interactions of these lipid materials, it does not seem to be accurately distinguishing between the biles of normal or stone-forming human (20–24) or animal (25) subjects as originally reported. In one study, the control population was found to possess a greater mean cholesterol level than the gallstone patients (24). The poor correlation in these studies might be attributed to errors in bile sample collection and manipulation (26) or to deficiencies in the data used to establish the micellar region (27). A discussion of this latter point is the subject of this report.

Studies in these laboratories indicated that the experimental methods employed to produce these diagrams may have acted to bias the results in favor of supersaturated cholesterol levels. This bias is due in part to the slow rates at which these systems move to equilibrium. Experiments were designed to monitor the solubility of cholesterol in model bile systems as a function of the solid-state properties of cholesterol and of the bile salt–lecithin ratio.

EXPERIMENTAL

Cholesterol—Cholesterol[2] was recrystallized from 95% ethanol. TLC in systems designed to reveal autoxidative products and other common impurities showed the sample to be equivalent to National Bureau of Standards reference cholesterol. The cholesterol was stored at 4° under nitrogen.

Cholesterol-4-^{14}C[3] was tested for radiopurity upon receipt and found to be >99%.

Prior to the production of different physical forms of cholesterol, the radioactive ^{14}C-cholesterol was mixed with chemically pure cholesterol. The benzene solution of ^{14}C-cholesterol as received from the manufacturer was carefully added to a hot solution of cholesterol in 95% ethanol. The solution was brought to boiling and then allowed to cool, and the solvent was removed by vacuum. The specific activity was then determined. This cholesterol was used to prepare the following samples.

Hydrated Crystal—Hydrated cholesterol has been reported to exist as a monohydrated species. Some difficulty was experienced in producing and maintaining this species. Samples of cholesterol gain and lose water readily at room temperature. Hydrated cholesterol was prepared by crystallization from 95% ethanol (17). However, it is difficult to estimate its water composition by thermal methods because large quantities of residual ethanol remain in the sample. Attempts to remove the ethanol selectively did not meet with success. Although titrimetric analysis for water could be employed, it was not considered desirable to utilize crystals containing ethanol in these studies. In the method finally selected, the hydrate is precipitated from 95% ethanol, dried under vacuum, and then allowed to equilibrate in an atmosphere saturated with water vapor.

Anhydrous Crystal—Anhydrous cholesterol was prepared by dissolving cholesterol in an excess of dry ether. The solvent was then evaporated *in vacuo*, and the resulting dry powder was ground and stored at 4°.

Coprecipitated—Appropriate quantities of stock solutions of the lipids [10% bile salts in methanol, 10% lecithin in methanol, and 10% cholesterol in methanol–benzene(1:1)] were accurately mixed in a round-bottom flask. The solvent was removed under vacuum.

[1] Woodford reported that upon adoption of coprecipitation he was able to solubilize 10 times more cholesterol in his taurocholate micelles (9).

[2] Eastman Organic Chemicals, Rochester, N.Y.

[3] New England Nuclear, Boston, Mass.

Table I—Summary of Literature Data Pertaining to the Solubility of Cholesterol

Reference	Method	Equilibration Time
2	Cholesterol, lecithin, and bile salts coprecipitated	1 hr
5	Cholesterol adsorbed onto Hyflo Super-Cel added to human or dog bile	4 hr
6	Cholesterol, lecithin, and bile salts coprecipitated	2–7 days
7	Crystalline cholesterol added to lecithin–bile salt solution	6 days
8	Cholesterol and phospholipid coprecipitated, added to aqueous bile salt solution	4 hr
9	(*a*) Aqueous taurocholate and crystalline cholesterol; (*b*) coprecipitated taurocholate and cholesterol	1–3 days? Immediate?
10	Cholesterol, lecithin, and bile salts coprecipitated	3 days
11	Crystalline cholesterol added to lecithin–bile salt solution	6 days
12	Cholesterol, lecithin, and bile salts coprecipitated	3 days
13	Crystalline cholesterol added to canine bile	5-min sonication or longer unspecified times
14	Cholesterol, lecithin, and bile salts coprecipitated	3 days

Human Gallstones—Upon their surgical removal, the gallstones[4] were placed in saline for storage. The stones used for this study were all from a single patient who yielded 127 stones totaling 12.6 g. Aliquots were ground and sized immediately prior to their use. TLC was used to show the presence of cholesterol in these stones. Sectioning revealed the presence of bile pigments.

Diffractograms were obtained on the powdered cholesterol samples using a diffractometer[5] (CuKα radiation).

Bile Salts—Conjugated bovine bile salts[6] were purified following the method of Small *et al.* (28). TLC revealed the ratio of trihydroxy to dihydroxy bile salts to be about 1.5:1 with about equal quantities of taurine and glycine conjugates.

Lecithin—Lecithin was prepared from fresh chicken egg yolks by the procedure of Small *et al.* (28) with the following additions. Following alumina chromatography, the lecithin-containing fractions were dried under vacuum. The residue was dissolved in petroleum ether and then slowly added to acetone, thereby precipitating white, waxy lecithin. The precipitate was collected and dried under vacuum, and the dry lecithin was stored at 4° under nitrogen.

The fatty acid composition of the lecithin determined by GLC compared favorably to literature values (11, 28). The phosphorous–nitrogen ratio was 1.04. Thin-layer silica gel chromatography revealed no nonphospholipid impurities.

Lecithin–Bile Salt Solution—Literature values for the composition of normal human bile show wide variations. The values presented (135.2 ± 64.6 m*M* bile salt and 38.0 ± 19.6 m*M* lecithin) by Admirand and Small (19) for normal human gallbladder bile were chosen for the preparation of the model dissolution system because they appeared to be representative. It is necessary to know the relative composition of the biliary lipids for interpretation of the phase behavior. It is also important to know the actual quantity of such lipids. These are usually expressed in terms of percent solids. Unfortunately, this term has been used in the cholelithiasis literature to express two different groups of solids: the total solids remaining after evaporation of water from whole bile or the quantity of lecithin, bile salt, and cholesterol. From a solubilization point of view, the most meaningful description would be that of the quantity of solids present in bile capable of solubilizing cholesterol: lecithin and bile salt expressed as a weight/volume percentage of the bile (percent solids as lecithin–bile salt). One might then assume that the greater the percentage of such solids the greater the actual cholesterol-holding capability of bile. However, there may not be a strictly linear function between the two. Ekwall *et al.* (29) presented data showing changes in bile salt micelles as a function of concentration and defined three limits of solubility. It is possible that such discontinuities could occur in the more complex lecithin–bile salt–cholesterol system. The experience of the authors and of others (30, 31) has been that changes in micellar behavior are observed as the percentage of solids and the intramicellar solution are altered by dilution. Similar alterations in electrolyte composition could be expected during storage of hepatic bile in the gallbladder. It is also possible that variations in hormonal levels due to the menstrual cycle, oral contraceptive agents, or pregnancy and their subsequent effects on electrolyte balance (32, 33), biliary components (34, 35), and biliary function (36, 37) could play a role in cholelithiasis by modifying the cholesterol carrying capacity of bile.

Phase work to date (19) has generally been done at 10% solids (calculated as lecithin, bile salt, and cholesterol), the approximate level of biliary solids. Extrapolations of phase boundaries from one well-defined system to a system of differing percentage solids might not be correct. For instance, the phase diagram approximating hepatic bile may not accurately serve to describe the phase behavior of the more concentrated gallbladder bile. Ideally, authors would state molar (or weight) biliary compositions—from this, anyone could calculate mole percent if so desired. The percent solids, weight-to-volume (as lecithin–bile salt), employed in these studies was 10.3 ± 0.7. The formula used to prepare the lecithin–bile salt model system for dissolution studies was 135 m*M* conjugated bovine bile salts and 38 m*M* egg lecithin in a 0.05 *M* phosphate buffer, pH 7.4.

Preparation of Lecithin–Bile Salt Solutions—Stock solutions of lecithin and bile salt were mixed to the required ratio in a round-bottom flask. The solvent was evaporated under vacuum, and buffer solution was added to the dried powder to form the micellar system. Initial solubility experiments were adversely influenced by the presence of microorganisms, necessitating the application and design of aseptic techniques and equipment; the reconstituted lecithin–bile salt was cold sterilized and stored in a sterile incubator-stirrer (38).

Determination of Cholesterol Solubility—*Crystalline Cholesterols*—An excess (20 mg/ml) of the crystalline cholesterol was added to the lecithin–bile salt system contained in glass vials. The vials were sealed and placed in a ferris-wheel-type rotating thermostated apparatus. Equisized fractions of all cholesterol samples were utilized for dissolution experiments.

Coprecipitated Cholesterol—The coprecipitate consisting of cholesterol (to supply 20 mg/ml of reconstituted system), lecithin, and bile salts was carefully weighed into glass vials and warmed to 37°. At time zero, appropriate quantities of prewarmed buffer were added and the vials were placed in the rotating apparatus.

Assay Method—The solubility of the ^{14}C-cholesterol in the lecithin–bile salt system was determined by liquid scintillation counting of an aliquot of the filtrate obtained by passage of the system through a glass-fiber prefilter and a cellulose ester membrane filter[7]. Preliminary experiments showed no significant loss of cholesterol by this filtration technique. The membrane, its stainless steel "Swinney" holder, sampling syringe, and cannula were all prewarmed to 37° to prevent precipitation of cholesterol during sampling. A scintillation counting solution was developed, following Bray (39), to accept both aqueous and organic additives as follows: naphthalene, 100 g; 2,5-diphenyloxazole, 7 g; 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, 0.3 g; toluene, 200 ml;

[4] Obtained through the courtesy of Dr. R. T. Holzbach, St. Luke's Hospital, Cleveland, Ohio.
[5] General Electric XRD-5.
[6] General Biochemicals, Chagrin Falls, Ohio.
[7] Type AP20 and HA, Millipore Corp., Bedford, Mass.

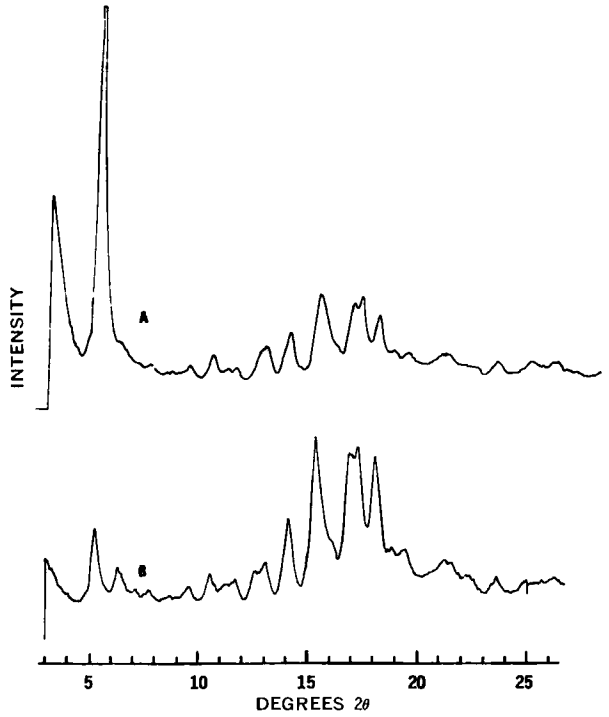

Figure 1—*Diffractogram of anhydrous (A) cholesterol and National Bureau of Standards anhydrous (B) cholesterol.*

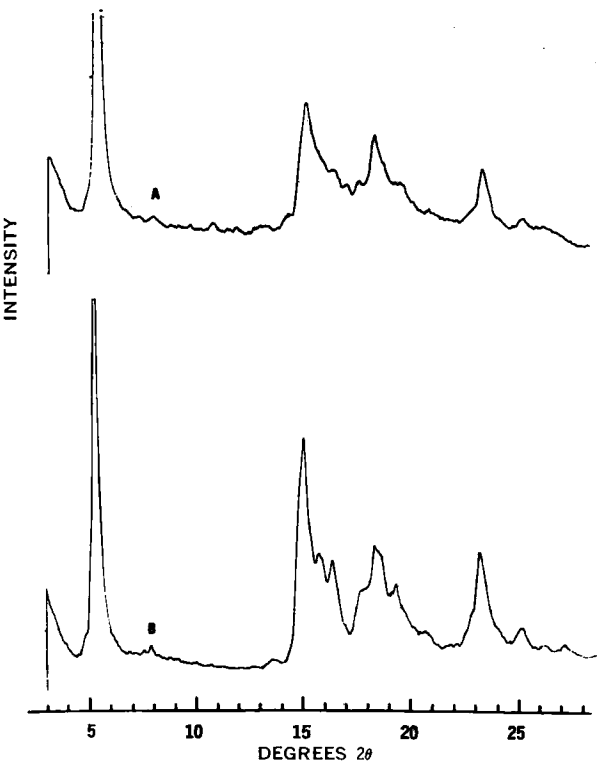

Figure 2—*Diffractogram of human gallstone (A) and of hydrous (B) cholesterol.*

methanol, 100 ml; and *p*-dioxane, *qs* to 1.0 liter. The sample was counted[8] employing an automatic external standardization procedure to account for quenching. The total count collected was $>10^4$.

RESULTS

X-Ray Diffractometry—X-ray diffractometry was utilized to provide information about the crystallographic nature of the cholesterol samples. In Fig. 1 the diffractogram of the anhydrous sample is plotted along with that of National Bureau of Standards reference cholesterol. Good agreement between these and reported data (16) for anhydrous cholesterol was obtained. The hydrated cholesterol and the human gallstone samples yielded similar diffractograms (Fig. 2), which compare favorably with literature values (16). When care is taken to prevent water loss, the hydrated form of cholesterol is observed in pathological deposits. The diffractogram of the coprecipitated sample (Fig. 3) is devoid of well-defined peaks, indicating that the cholesterol in this sample exists as extremely small crystallites or that it is molecularly dispersed with the lecithin and bile salts in a noncrystalline, amorphous matrix.

Dissolution Behavior—The dissolution behavior of cholesterol as a function of its physical form in a model bile system is shown in Fig. 4, where the amount of cholesterol in solution (in mg/ml) is plotted as a function of time[9]. It is useful to express the solubility of cholesterol in moles as a percentage of the molar quantities of cholesterol, lecithin, and bile salt present (in units of mole percent). The solubility expressed in this manner can be thought of as defining the boundary between the cholesterol–lecithin–bile salt micellar phase and the heterogeneous phases possible when excess cholesterol and/or phospholipid are present. The solubility of hydrated cholesterol in this model bile system can be expressed as 5 mole %. The rate of dissolution of the hydrate is in marked contrast to the rapidity of dissolution observed with the anhydrous and coprecipitated forms and even the powdered human gallstone sample. The anhydrous species dissolved quickly and appeared to reach an "equilibrium" solubility of 7 mole % within several hours. The driving force for the dissolution of the anhydrous species must therefore be greater than that of the hydrate, since all other factors relevant to dissolution were maintained constant, *i.e.*, rate of agitation and particle size. The quantity of cholesterol in solution in the "anhydrous system" was monitored over many days and found to remain constant. After a week on this plateau, however, the apparent cholesterol solubility slowly began to fall, eventually converging with that of the hydrated species. Samples of the excess cholesterol removed from the system at this time were found to be hydrated.

The amount of cholesterol in solution following addition of the buffer to the coprecipitate sample reached a plateau within 20 min. This value, 7.5 mole %, higher than that obtained with either of the two crystalline forms of cholesterol, was undoubtedly a result of the ease with which the solvent was able to effect solution of the amorphous cholesterol. The solvent (aqueous buffer) must first act to dissolve the bile salts and lecithin. These materials dissolve instantaneously (observed microscopically) and form micelles. The cholesterol, being essentially molecularly dispersed with the bile salts and lecithin in the coprecipitate, can be rapidly incorporated within the micelles as they form. The coprecipitation process has been employed to induce rapid dissolution and the attainment of high solubility values as an aid to the bioavailability of pharmaceuticals.

A human gallstone sample dissolved more rapidly than the hydrate sample; but unlike the anhydrous or coprecipitated sample, it directly equilibrated at the solubility value of the hydrate. This would seem to imply that its crystalline properties as related to solubility were identical to those of the hydrated system. The more rapid rate of dissolution may be ascribed to particle-size phenomena. Although all samples were screened to provide similar size distributions, it is possible that the gallstone particles containing a mixture of cholesterol and stone constituents actually provided cholesterol with a greater degree of subdivision to the dissolution media.

As with the anhydrous form, the amount of cholesterol in solution following dissolution of the coprecipitate was monitored *versus* time. The time lag for the relief of the supersaturation was not as long for the coprecipitate as for the anhydrous material. This could be due to the greater degree of supersaturation (as compared to the equilibrium hydrated state) in the coprecipitate system. The major driving force in crystal nucleation and growth processes is the degree of supersaturation.

The basic relationships pertaining to the transformation of a

[8] Packard Tri-Carb.
[9] A portion of these data appeared in a preliminary report (27).

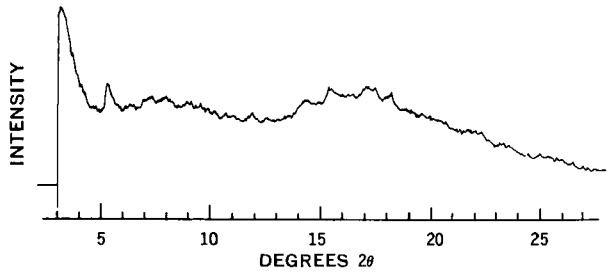

Figure 3—*Diffractogram of coprecipitated cholesterol.*

high activity, supersaturated state to an equilibrium state can be explained by use of Fig. 5. In the upper curve, the quantity of solute is steadily increased above the saturation solubility. A point is eventually reached, where the number of solute molecules is great enough to aggregate spontaneously into a stable cluster, the nucleus, on which crystal growth can proceed. At this point, the critical supersaturation, spontaneous homogeneous nucleation is said to begin, with the system rapidly moving toward equilibrium. Another case can be imagined where the quantity of solute present in a system exceeds the saturation solubility but is below the critical saturation level. Although thermodynamic principles predict that such a system should adjust to the lower energy state, during long periods of observation no such changes may be found; kinetic barriers may be operative which retard the transformation. Such a system is termed metastable. Upon the addition of a suitable catalyst, however, the barriers are lowered and the chemical potential of the system decreases as nucleation and growth commence. Nucleation in this case is termed heterogeneous; in a complex system like bile, nucleation is undoubtedly of this type. A specialized case of heterogeneous nucleation known as epitaxy has been proposed as a mechanism for cholesterol gallstone development (40).

Considering the cholesterol–lecithin–bile salt system, it can be seen that a critical supersaturation must exist above which almost instantaneous precipitation will occur, with lesser supersaturation ratios being potentially lithogenic. Metzger *et al.* (41) suggested the use of a "lithogenic index" as a measure of the relative supersaturation of biles. Metastable systems will be strongly influenced by the presence of "foreign" materials which could act as nucleating sites or conversely as crystal growth inhibitors (42, 43). It is intriguing that in the present model system, where thermodynamic considerations forecast precipitation of cholesterol levels greater than 5 mole %, a supersaturated state could exist for days. It is interesting to speculate that perhaps this time lag to deposition is beneficial in that it gives the body a chance to rid itself of any lithogenic bile during the normal emptying cycles of the gallbladder. However, extended bile residency times such as are present during stasis due to fasting, pregnancy, or vagotomy or as induced physically by viscosity-producing agents in microareas of the gallbladder (44–46) may allow sufficient residence time for the pathogenic bile to deposit cholesterol. During this time, other components can further alter bile composition, increasing the possibilities of deposition (47).

Solubility as Function of Bile Salt–Lecithin Ratio—The finding that physical differences in cholesterol could explain solubility variations between laboratories prompted a more complete study of cholesterol solubility as a function of the bile salt–lecithin ratio. The data are plotted in Fig. 6 on a mole percentage basis. The cross-hatched area within the triangular diagram serves as a guide to the position of the system under study; the rectangular diagram represents the area of primary physiological concern. The tie-line connecting the data points describes the boundary between the clear micellar phase and a multiphase (micellar plus crystalline and/or liquid crystalline) system. The micellar limit in the human physiological range of the bile salt and lecithin can be seen to be about 5 mole %. The significance of this value lies in the fact that many persons considered normal because no crystals of cholesterol or gallstones can be demonstrated in their bile have been found to possess far greater quantities (>5 mole %) of cholesterol in their bile (14, 20, 22–24, 48, 49). In a recent paper (25), the gallbladder bile composition of normal subjects was compared to that of patients with cholesterol gallstones. The control population had no (clinical or radiological)

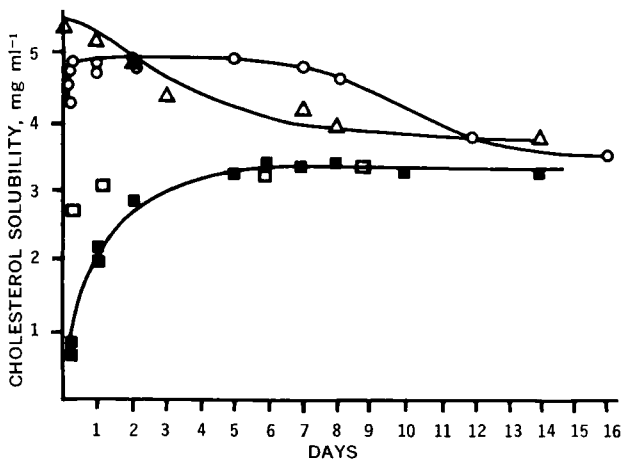

Figure 4—*Dissolution behavior of coprecipitated* (△), *anhydrous* (○), *and hydrated* (■) *cholesterol and of human gallstone* (□) *in model bile system.*

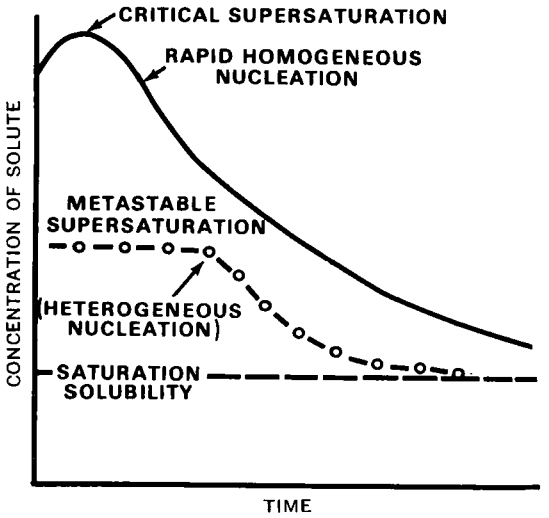

Figure 5—*Plot describing the relationship between degree of supersaturation and precipitation behavior.*

evidence of gallbladder disease. The bile composition of two out of the 12 (16%) control patients was found to be out of the micellar region (>10 mole % cholesterol) as defined by Admirand and Small (19). If the micellar boundary defined in Fig. 6 is used, 75% of these normal patients are found to possess bile supersaturated with respect to cholesterol. Of the patients with cholesterol gallstones, nine out of the 16 (56%) had a bile composition within the micellar zone of Admirand and Small (19) while only one of the 16 (6%) was below the boundary of Fig. 6. Recently, it was reported that normal persons began to secret a lithogenic bile during fasting (50). Thus, lithogenic or metastable bile in man would seem to be the rule rather than the exception. The definition of this lower value for cholesterol solubility prompts some thought into what factors might be responsible for the actual precipitation of cholesterol. The phase diagram serves to inform us as to the value of cholesterol's solubility in a given milieu at equilibrium. It does not take into account the role of other (nonsolubilizing) biliary components which can act to modify the rate of attainment of equilibrium. The importance of such materials on the precipitation behavior of cholesterol awaits further study.

During these studies, the authors became aware of other investigators who were employing similar methodologies (use of crystalline anhydrous cholesterol in model or whole bile systems) and arriving at similar values for the cholesterol-holding capacity of bile. Unlike earlier workers who also employed anhydrous cholesterol, Hegardt and Dam (11) and Holzbach *et al.* (51) presented data that essentially coincide with this study's data. Discrepancies with earlier literature values are undoubtedly due to labora-

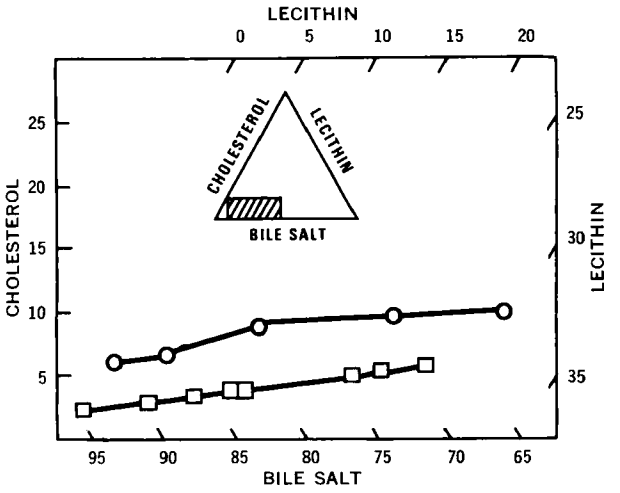

Figure 6—*Equilibrium solubility of cholesterol as a function of lecithin–bile salt ratio* (□) *and the data from Admirand and Small* (*19*) (○).

tory variation in those factors responsible for rate of attainment of equilibrium, *e.g.*, intensity and extent of agitation. It is of importance that three laboratories have independently arrived at similar values.

The application of physical-chemical concepts and methodology to the study of this disease state has been most rewarding. The mechanisms by which gallstones may dissolve was recently reported (52). Chemotherapy of cholelithiasis is becoming a reality (24, 53). Agreement on a value for the equilibrium solubility of cholesterol in bile coupled with the observation that many (most?) nonstone-bearing individuals have a bile that is supersaturated with respect to cholesterol, at least part of the time, points the way for further application of physical-chemical (kinetic) methods to clarify the factors involved in the maintenance of this metastable condition or, additionally, the factors influencing the nucleation and growth of cholesterol crystallites and their aggregation into stones.

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