A definition of lithogenic bile

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In recent years, the descriptive term *lithogenic* has come into common use when applied to bile obtained from a patient with cholesterol cholelithiasis. It is assumed that in the presence of cholesterol gallstones, conditions in the surrounding bile solution are physicochemically suitable for precipitation of cholesterol and thus ultimately for the beginning of stone formation. The difficulty is that despite extensive use of the word *lithogenic,* a rational definition in terms of process based on direct evidence remains unavailable.

In the mid- to late 1960s work was done which for the first time attempted to provide a definition of saturation in terms of maximum micellar capacity to dissolve cholesterol in bile.[1] By inference, when the content of cholesterol in bile exceeded this limit, the system was said to be supersaturated. The latter indicated an unstable state in which cholesterol precipitation was likely to occur almost immediately so that supersaturation, thusly defined, was regarded as equivalent to *lithogenic.*[2] Existence of a more prolonged state of metastable supersaturation in solubility systems was well known to physical chemists and

biochemists who were familiar with such solutions, but applicability of this concept to biological systems had not been widely considered.

Work done within the past 5 years on precipitate formation in urolithiasis and cholelithiasis has indicated that both of these solubility systems are capable of and commonly exemplify the state of metastable supersaturation as applied to biological fluids,[3] as indicated for normal human biles in *Figure 1.* This new concept has had some important implications. Among them is that a definition of saturation in these systems, although heuristically useful, will not alone explain the phenomena of nucleation and precipitation as a prelude to stone formation. To date, the work defining the initial nidus and precipitate for renal stones is further developed than that applicable to human bile. In part, this is true because an ionic activity solubility system is simpler to define, especially when one is working primarily with a single cation, calcium, and a single anion, phosphate.[4] In contrast, the micellar lipid solubility system for cholesterol in bile involves at least two other molecularly dissimilar compounds, lecithin and bile salts. Nevertheless, an accurate definition of saturation for bile has been recently achieved. Direct applicability has been shown to both human bile and to artificial model solutions patterned after bile.[5] Work on solubility definitions for urine is relatively more advanced in that the definitions both of saturation and more importantly that of metastability limit have been determined. A metastability limit denotes the relative concentration of those chemicals in solution which, when exceeded, will be accompanied by immediate precipitation of an insoluble material. According to recent work, in urine this precipitate is a dihydrated acidic salt of calcium and phosphate, termed brushite ($CaHPO_4 \cdot 2H_2O$). This metastability limit in urine ranges between 2 and 3.5 times the saturation limit, the variability influenced largely by the concentration of inhibitors of calcification, e.g., pyrophosphate.[6] Therefore, such a metastability limit cannot be predicted, a priori, for any given urine specimen, but must be measured in each case to determine operationally the actual metastability limit as affected by the given concentrations of appropriate ionized substances.

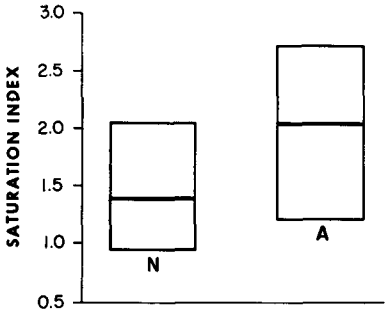

Fig. 1. Comparison of degree of saturation in normal (N) (n = 205) and abnormal (A) (gallstone-associated) (n = 283) bile specimens; mean and range; 1.0 represents 100% saturation or the equilibrium saturation limit.

By analogy, it seems probable that similar determinants are applicable to cholesterol solubilization in bile. Such a metastability limit has recently been defined for artificial solution systems resembling bile. As a general statement the metastability limit for the precipitant, in this case, cholesterol, was approximately twice the saturation value.[7] In a comparatively simple and controllable *in vitro* system, such a metastability limit can be precisely and reproducibly defined as a bound-

ary. When the cholesterol content in the system exceeds this, nearly immediate spontaneous nucleation and precipitation occur.

Observations have already been made, however, which indicate that this precise and reproducible metastability limit, so important for understanding the initiating process in gallstone formation from lithogenic bile, does not apply *in vivo* to control human bile samples from cases not associated with gallstones.[8] This can only indicate that the *in vitro* metastability limit cannot be validly applied to the more complex solution of lipids and other ingredients contained in bile. Determination by direct measurement of the *metastability limit* for normal human bile is therefore the first requirement toward improved understanding of cholelithiasis. Based on analogy derived from these phenomena in urolithiasis, it may reasonably be predicted that the observed *in vivo* metastability limit will be a ratio or multiple in the order of two to three times the saturation limit. Additional hypotheses stem from these considerations: (1) The metastability limit will not be a fixed boundary equally applicable to all bile solutions but rather a range with multiples of the saturation limit determined by observed values obtained from the samples. (2) Further study of nonlipid constituents normally present in bile, such as calcium, protein, and bilirubin, will reveal those which have as a net effect the property of either raising or lowering the metastability limit. (3) Abnormal or truly lithogenic bile might be expected to demonstrate the property of a reduced range for metastability limit; therefore, cholesterol precipitation would be expected to occur in the presence of significantly lower relative cholesterol concentrations in these systems than would be the case in normals. Those factors which operate to lower the metastability limit abnormally and thus to promote cholesterol precipitation from a previously metastably supersaturated solution await further delineation. They perhaps ultimately hold the key to a better understanding of the process of the human gallstone formation.

From these considerations, new strategies aimed at prevention or chemotherapy for cholelithiasis may emerge. Present chemotherapeutic strategies, which in the case of chenic acid (chenodeoxycholic acid) are already nearing clinical trials,[9] appear to alter biliary lipid composition, so that it either approaches or falls below the cholesterol saturation limit.[10] The effect reduces the likelihood of cholesterol precipitation and favors redissolution of existing precipitates.

But responses recently reported to oral administration of chenic acid for gallstone chemotherapy[11] indicate that although the drug is often specifically effective, it is also quite slow and subject to failure (*Table*). The millennium is obviously not yet here. To quote Samuel Johnson, the situation is "like a dog's walking on his

Table. Clinical experience with chenic acid therapy for dissolving gallstones[10]

Duration	Response of radiolucent stones	No. of patients
6 mo	Decreased size or number	11/18
12 mo	Dissolved	2/5
24–36 mo	Dissolved, others decreased size	3/7

Radiopaque stone response (duration not known) by contrast = 2/13 patients.

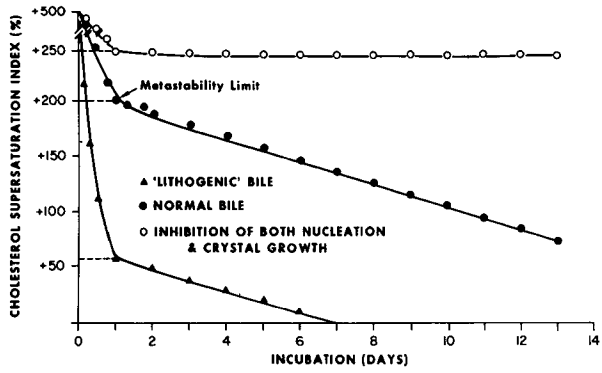

Fig. 2. Hypothetical depiction of (a) lower state of saturation associated with initiation of crystal nucleation in lithogenic biles [▲] and more rapid crystal growth rate when compared with normals [●], and (b) inhibition of both functions in bile specimen [○] by chemotherapeutic agent.

hind legs. It is not done well, but the wonder is that it is done at all." [12] With respect to gallstone chemotherapy, at present we are probably in a state of development similar to that in 1932, when 2,4-diaminoazobenzene-4-sulfonamide hydrochloride (Prontosil) was first introduced as the prototype antimicrobial sulfa drug by Domagk[13] of I. G. Farbenindustrie. For discovery of its chemotherapeutic value, he was later awarded the Nobel Prize for Physiology and Medicine in 1939. From this beginning, advances in the field of antibiotic therapy are a matter of historical record.

All this underscores the importance of greater basic understanding of the process of nucleation and precipitation of cholesterol in bile and developing optional or alternative chemotherapeutic approaches. A second entirely new strategy suggested here would entail manipulation of the metastability limit of a given bile solution. A hypothetical representation of this mechanism is shown in *Figure 2.* This latter approach would not alter the cholesterol concentration in bile with reference to the saturation limit, but instead would inhibit its nucleation and precipitation at existing concentration levels as exemplified by the recently published inhibiting effect of diphosphonate in urolithiasis.[14] Although much work still needs to be done to confirm or refute the premises and hypotheses outlined here, the described therapeutic strategies recently have been successfully deployed in the case of urolithiasis. Major scientific advances in the cause, prevention, and treatment of human cholesterol cholelithiasis should be attainable by attacking these lines of investigation. Despite the high speculation to fact ratio in this paper, an important aim has been to provide a broad and practical view of the impact of basic chemistry on common clinical problems.

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