JCI The Journal of Clinical Investigation

Pathogenesis of calcium-containing gallstones. Canine ductular bile, but not gallbladder bile, is supersaturated with calcium carbonate.

R V Rege, E W Moore

J Clin Invest. 1986;77(1):21-26. https://doi.org/10.1172/JCI112278.

Research Article

Calcium precipitation in bile is a requisite event in the initiation and growth of all pigment gallstones. Calcium solubility in bile is thus of great importance. This is the first attempt to define the ion-product of CaCO3 in bile in any species. If the ion-product: [Ca++] X [CO = 3] exceeds solubility product (K'sp), the sample is supersaturated and CaCO3 precipitation is thermodynamically possible. We have recently determined K'sp of calcite to be 3.76 X 10(-8) mol/liter at 37 degrees C and total ionic strength = 0.16 M. Gallbladder (GB) bile was obtained from 15 anesthetized dogs after 12-24-h fasts. Duct bile was obtained from three dogs (n = 12) during variable taurocholate infusion. Samples were assayed for pH, partial pressure of carbon dioxide (PCO2), total bile salt concentration ([TBS]), total calcium concentration ([Ca]), and free calcium ion concentration ([Ca++]). With increasing [TBS] in both GB and duct bile, there was a linear decline in pH, a curvilinear decline in [HCO-3] and [CO = 3], and linear increase in [Ca++] and [Ca]. All ductular samples were supersaturated with CaCO3, with saturation indices (SI) as high as 17.5 and a mean of 8.36 +/- 1.43 (SE). In sharp contrast, none of the GB samples were supersaturated, due to the marked decline in [CO = 3] upon concentration and acidification of bile. […]

Pathogenesis of Calcium-containing Gallstones

Canine Ductular Bile, but Not Gallbladder Bile, Is Supersaturated with Calcium Carbonate

Robert V. Rege and Edward W. Moore

Department of Surgery, Northwestern University Medical School and Veterans Administration Lakeside Medical Center, Chicago, Illinois 60611, and Departments of Medicine and Physiology, Medical College of Virginia, Health Sciences Division of Virginia Commonwealth University, Richmond, Virginia 23298

Abstract

Calcium precipitation in bile is a requisite event in the initiation and growth of all pigment gallstones. Calcium solubility in bile is thus of great importance. This is the first attempt to define the ion-product of $CaCO_3$ in bile in any species. If the ion-product: $[Ca^{++}] \cdot [CO_3^{=}]$ exceeds solubility product (K'_{sp}), the sample is supersaturated and $CaCO_3$ precipitation is thermodynamically possible. We have recently determined K'_{sp} of calcite to be 3.76 $\times 10^{-8}$ mol/liter at 37°C and total ionic strength = 0.16 M. Gallbladder (GB) bile was obtained from 15 anesthetized dogs after 12–24-h fasts. Duct bile was obtained from three dogs (n = 12) during variable taurocholate infusion. Samples were assayed for pH, partial pressure of carbon dioxide (P_{CO_2}), total bile salt concentration ([TBS]), total calcium concentration ([Ca]), and free calcium ion concentration ($[Ca^{++}]$). With increasing [TBS] in both GB and duct bile, there was a linear decline in pH, a curvilinear decline in $[HCO_3^-]$ and $[CO_3^{=}]$, and linear increase in $[Ca^{++}]$ and [Ca]. All ductular samples were supersaturated with $CaCO_3$, with saturation indices (SI) as high as 17.5 and a mean of 8.36±1.43 (SE). In sharp contrast, none of the GB samples were supersaturated, due to the marked decline in $[CO_3^{=}]$ upon concentration and acidification of bile. In GB bile, the SI ranged from 0.006 to 0.126, with a mean of 0.039±0.011. The gallbladder thus produced a change in the SI from a value as high as 17.5 to a value as low as 0.006 in concentrated GB bile, which is a nearly 3,000-fold change. The average change in the SI was ~215-fold. Since all duct samples were supersaturated, and since the dog does not normally form gallstones, the data support our previous hypotheses that: (*a*) in canine bile, as in canine pancreatic juice, a nucleating factor is necessary for $CaCO_3$ precipitation; (*b*) bile salts are important buffers for Ca^{++} in bile; and (*c*) normal GB mucosal function (concentration and acidification of bile) plays an important role in reducing $CaCO_3$ lithogenicity in GB bile.

Introduction

Calcium precipitation in bile is a requisite physicochemical event in the initiation and growth of all pigment gallstones. Such stones contain calcium salts of one or more readily precipitable anions in bile, which we (1) have recently termed "calcium-sensitive": carbonate, bilirubinate, phosphate, and long-chain fatty acids (2). Calcium solubility in bile is thus of critical importance in the formation of pigment stones.

For cholesterol stones, the importance of cholesterol supersaturation in bile was clearly demonstrated by the studies of Admirand and Small (3). More recent work has highlighted the importance of nucleation factors in the formation of cholesterol stones, since supersaturated bile occurs in individuals who do not have gallstones (4–6), and since bile from cholesterol gallstone patients nucleates more rapidly in vitro than bile samples from subjects without gallstones for a given degree of cholesterol supersaturation (7–9).

Precipitated calcium salts in bile are believed to serve as a nucleus for cholesterol precipitation since cholesterol stones have been found to contain pigment stone centers (10, 11). Therefore, we (1, 12) have recently advanced the hypothesis that calcium precipitation is important in the initiation of cholesterol gallstones by forming a nucleus on which cholesterol can precipitate from its supersaturated state. A similar view has been expressed by Williamson and Percy-Robb (13).

In addition to possibly having a role in the initiation of cholesterol stones, calcium precipitation may occur on the surface of such stones either spontaneously (14), during chenodeoxycholic acid treatment (15), or during ursodeoxycholic acid treatment (14). Such precipitation is clinically important, as it usually precludes successful gallstone dissolution.

In any given bile sample, the limiting solubility of a calcium salt is determined by the ion-product: $[Ca^{++}] \cdot [anion^-]$ for that salt. If this product exceeds the solubility product constant (K'_{sp})[1] of that salt, the sample is supersaturated (metastable) and calcium precipitation is thermodynamically possible. Of the four anions listed above that precipitate with calcium in bile, only the K'_{sp} of $CaCO_3$ (calcite) has been determined under physiologic conditions. Recently, using the Ca^{++} electrode, Moore and Verine (16) have reported K'_{sp} for calcite to be 3.76 $\times 10^{-8}$ mol/liter at 37°C. Comparison of the ion-product: $[Ca^{++}] \cdot [CO_3^{=}]$ in a given bile sample at 37°C with the value of K'_{sp} allows a quantitative statement as to whether that sample is supersaturated with respect to calcite.

This study is the first attempt to determine the ion-product of a calcium salt in bile in any species. We will show that canine ductular bile, but not gallbladder bile, is supersaturated with calcium carbonate. The results suggest that: (*a*) nucleating factor(s) are necessary for calcium precipitation in bile, and (*b*) normal gallbladder mucosal function (absorption and acidifi-

Portions of this work have been published in abstract form, 1985. *Gastroenterology.* 88:1079*a*. This work was presented in part at the meeting of the American Association for the Study of Liver Diseases, New York, May 1985.

Address correspondence to Dr. Moore, Box 711, MCV Station, Medical College of Virginia, Richmond, VA 23298.

Received for publication 17 April 1985 and in revised form 30 July 1985.

1. *Abbreviations used in this paper:* [Ca], total calcium concentration; $[Ca^{++}]$, free calcium ion concentration; $[HCO_3^-]$, bicarbonate ion concentration; K'_{sp}, solubility product constant; P_{CO_2}, partial pressure of carbon dioxide; [TBS], total bile salt concentration.

J. Clin. Invest.

0021-9738/86/01/0021/06 $1.00
Volume 77, January 1986, 21–26

cation) greatly reduces the $[Ca^{++}] \cdot [CO_3^=]$ ion product, so that gallbladder bile is normally unsaturated with calcite.

Methods

Gallbladder bile was obtained from 15 mongrel dogs anesthetized with sodium pentobarbitol (25 mg/kg) after 12–24-h fasts. The period of fasting was intentionally varied to achieve variable degrees of bile concentration and acidification. Laparotomy was performed and gallbladder bile aspirated anaerobically into a syringe using a 20-gauge needle. Care was taken to empty the gallbladder completely to minimize stratification effects on bile composition (17).

12 samples of common duct bile were also obtained from three dogs during variable infusion of sodium taurocholate (0–100 μmol/min), obtained from Calbiochem-Behring Corp., La Jolla, CA (purity > 98%). At laparotomy, the cystic duct was ligated, and the common duct was intubated with PE-190 tubing, and ligated distally. For each rate of infusion, bile flow was allowed to stabilize until it was relatively constant. At this time, a sample of duct bile was slowly withdrawn anaerobically into a syringe. A second higher rate of taurocholate infusion was then begun and the procedure repeated.

All samples were immediately analyzed at 37°C for pH and partial pressure of carbon dioxide (P_{CO_2}) using a blood gas analyzer (model 513; Instrumentation Laboratory, Inc., Lexington, MA). Total CO_2 content ($[TCO_2]$) was determined in triplicate with a S/Pecial Chem Micro CO_2 System (American Scientific Products, McGaw Park, IL). Total bile salt concentration was assessed by the 3-hydroxysteroid dehydrogenase method (18). Total calcium concentration ([Ca]) was determined by atomic absorption spectroscopy using a spectrometer (model 305B; Perkin-Elmer Corp., Instrument Div., Norwalk, CT). Free ionized calcium concentration ($[Ca^{++}]$) was determined anaerobically at 37°C with a Ca^{++} electrode (Model SS-20 Ionalyzer; Orion Research, Inc., Cambridge, MA).

In each sample, the bicarbonate ion concentration ($[HCO_3^-]$) was calculated from the Henderson-Hasselbalch equation:

$$pH = pK'_1 + \log \frac{[HCO_3^-]}{P_{CO_2} \cdot Q'} \quad \text{(Eq. 1a)}$$

$$[HCO_3^-] = (pCO_2 \cdot Q') \times (10^{pH - pK'_2}) \quad \text{(Eq. 1b)}$$

where $pK'_1 = 6.1$ at 37°C (19), and Q' is the CO_2 solubility coefficient. The product: $P_{CO_2} \cdot Q'$ is the concentration of dissolved CO_2. We have recently reported Q' to be 0.0288 mol/liter per mmHg in canine bile at 37°C (20); this value was used in the calculations. In each sample, the carbonate ion concentration ($[CO_3^=]$) was similarly calculated:

$$pH = pK'_2 + \log \frac{[CO_3^=]}{[HCO_3^-]} \quad \text{(Eq. 2a)}$$

$$[CO_3^=] = [HCO_3^-] \times (10^{pH - pK'_2}) \quad \text{(Eq. 2b)}$$

where $pK'_2 = 9.8$ at 37°C (19). The ion-product: $[Ca^{++}] \cdot [CO_3]$ was then determined for each sample from observed electrode $[Ca^{++}]$ and calculated $[CO_3^=]$ concentrations. All calculations were made with a computer (PC-XT; IBM Instruments, Inc., Danbury, CT); graphs were made using Lotus 1-2-3 software and Hewlett-Packard Model 7470A plotter.

Results

pH vs. total bile salt concentration ([TBS]). As shown in Fig. 1, there was an apparently linear decline in pH with increasing [TBS]. The slopes of the regressions for duct and gallbladder bile samples were not significantly different from one another (variance analysis), so the two sets of data were combined to yield the tentative regression: pH = 8.03 − 0.008 [TBS], (r = 0.97). In duct samples, with increasing taurocholate infusion and increasing [TBS], pH declined from 7.85 to 7.37. The linear decline in pH continued in gallbladder samples as bile salt concentration rose during sodium and water reabsorption. Thus, in the most concentrated gallbladder bile sample, [TBS] was 305 mM and pH was 5.86; the lowest observed pH was 5.81 at a [TBS] of 298 mM.

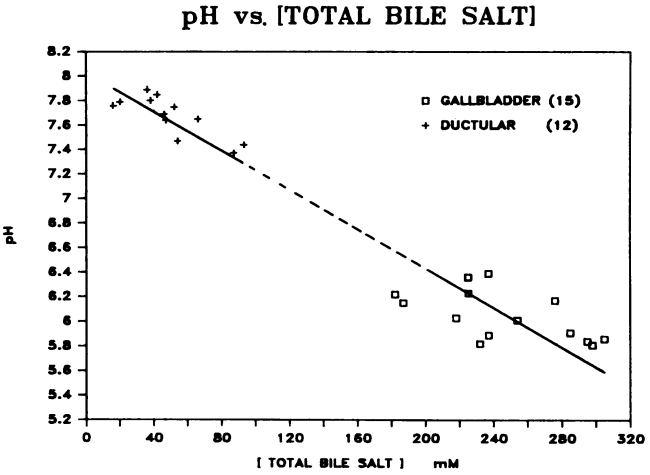

Figure 1. Tentative linear relation between pH and total bile salt concentration in 12 ductular and 15 gallbladder bile samples: pH = 8.03–0.008 [TBS], r = 0.97; y = 8.03 − 0.008 x.

$[HCO_3^-]$ and $[CO_3^=]$ vs. [TBS]. The above-mentioned decline in pH was accompanied by a curvilinear decrease in calculated bicarbonate and carbonate ion concentrations, as shown in Fig. 2. In ductular bile, maximal observed $[HCO_3^-]$ was 38.6 mM, with a decline to <1 mM in concentrated gallbladder bile. Similarly, $[CO_3^=]$ declined from a maximal value of 0.34 mM in ductular bile to $< 7 \times 10^{-8}$ M in concentrated and acidified gallbladder bile. This decline was ~5,000-fold. Thus, the normal concentration and acidification of bile in the canine gallbladder resulted in a striking decrease in the concentration of one component of the ion-product: $[Ca^{++}] \cdot [CO_3^=]$. This decline in $[CO_3^=]$ would correspondingly increase the maximal allowable $[Ca^{++}]$ concentration within the limits of calcium carbonate solubility, as dictated by K'_{sp}.

[Ca] and free $[Ca^{++}]$ vs. [TBS]. In sharp contrast to the above decrease in $[CO_3^=]$, the concentrations of total calcium and free ionized Ca^{++} rose linearly with increasing bile salt concentration in both ductular and gallbladder bile samples. For total calcium (Fig. 3), the combined tentative regression was: [Ca] = 2.73 + 0.026 [TBS], (r = 0.96), while for free Ca^{++} (Fig. 4) it was: $[Ca^{++}]$ = 1.16 + 0.0063 [TBS], (r = 0.90). The $[Ca^{++}]$-intercept of the latter regression, 1.16 mM, is the approximate normal canine ionized calcium concentration in plasma. This suggests that free Ca^{++} ion in the proximal biliary tract is at or near electrochemical equilibrium with plasma.

The slope of 0.0063 (Fig. 4) for the rise in $[Ca^{++}]$ was considerably lower than the slope of 0.026 (Fig. 5) observed for total calcium, which indicates that free $[Ca^{++}]$ rises more slowly than [Ca] during bile concentration. Thus, in ductular samples, mean [Ca] was 3.98±0.18 mM (SE), whereas in gallbladder bile it was 9.30±0.28 mM. In contrast, mean $[Ca^{++}]$ rose from 1.47±0.08 mM in ductular bile to a mean of only 2.71±0.11 mM in gallbladder bile.

This difference between total and free Ca^{++} concentrations largely reflects binding of calcium to bile salts. Thus, the ratio of mean $[Ca^{++}]$ to mean [Ca]: 2.71/9.30 = 0.291 in gallbladder bile indicates that only about 29.1% of total calcium was present as the free ion, while 70.9% was present as bound and complexed

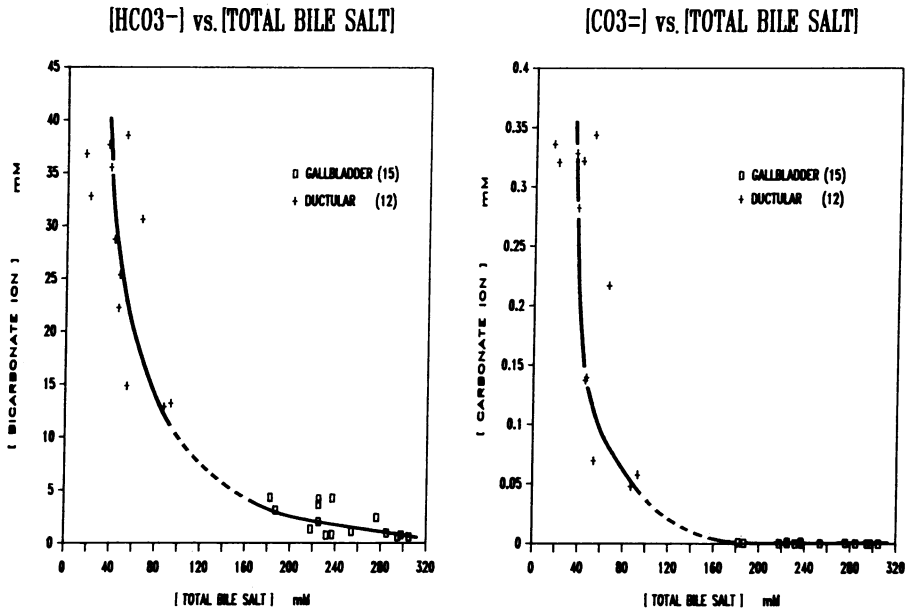

Figure 2. Left, curvilinear decline in $[HCO_3^-]$, with increasing bile salt concentration in 12 ductular and 15 gallbladder bile samples. *Right,* curvilinear decline in $[CO_3^=]$, with increasing bile salt concentration. Note the approximate 100-fold difference in scales between the two panels.

species. Significant calcium-binding would be expected, since taurocholate is the major bile acid in canine bile, and since we have recently found affinity constants of ~151 liters/mol and 7.1 liters/mol for the binding of calcium to free and micellar taurocholate species, respectively (1, 12).

Ion product: $[Ca^{++}] \cdot [CO_3^=]$. The above-mentioned decline in $[CO_3^=]$ was much greater than the rise in free $[Ca^{++}]$, so that the ion-product: $[Ca^{++}] \cdot [CO_3^=]$ sharply declined with increasing bile salt concentration (Fig. 5). The dashed line in the figure at 3.76×10^{-8} mol/liter is the value of K'_{sp} for calcite at 37°C and total ionic strength 0.16 M (17). It can be seen that all duct bile samples were supersaturated, with ion-products that exceeded K'_{sp}. The most marked degrees of supersaturation were at the lowest bile salt concentrations. These data indicate that canine ductular bile is metastable with respect to calcium carbonate (calcite). In sharp contrast, all gallbladder bile samples were far below K'_{sp}, which indicates that canine gallbladder bile is normally unsaturated with respect to calcite.

Saturation index. Another way of considering calcium solubility in bile is the saturation index, which we will define as the ratio of the observed ion-product to K'_{sp}:

$$\text{Saturation index} = \frac{[Ca^{++}] \cdot [CO_3^=]}{K'_{sp}} \qquad \text{(Eq. 3)}$$

A saturation index of 1.0 is the limit of a stable thermodynamic state. An index < 1.0 indicates that the sample is unsaturated, while a value > 1.0 indicates that the sample is supersaturated (metastable) and $CaCO_3$ precipitation is thermodynamically possible.

Results are shown in Fig. 6. In ductular bile, the maximum observed saturation index was 17.5, decreasing to a minimum of 2.1 with increasing bile salt concentration. The mean saturation index for the 12 ductular samples was 8.36±1.43. On the average, therefore, ductular samples were supersaturated by a factor of 8.36. In gallbladder bile, the saturation index ranged

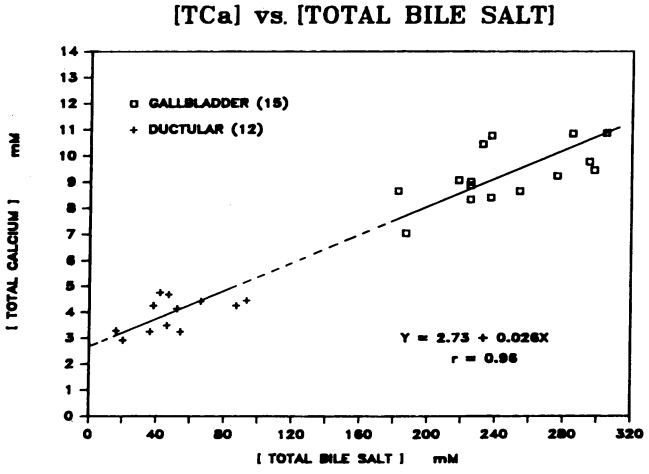

Figure 3. Tentative linear relation between [Ca] and [TBS], in 12 ductular and 15 gallbladder bile samples: [Ca] = 2.73 + 0.026 [TBS], r = 0.96; y = 2.73 + 0.026 x.

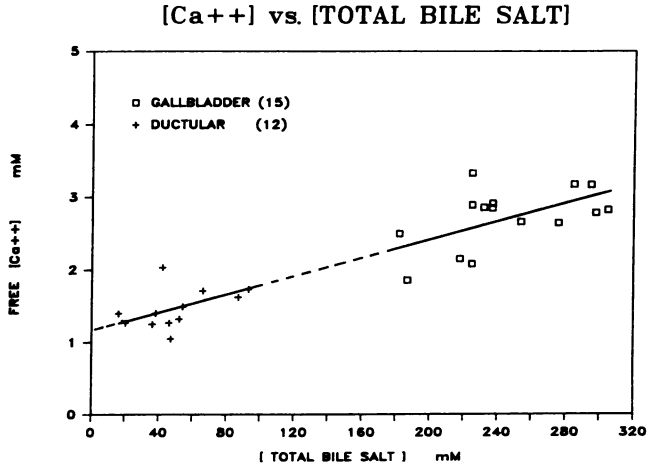

Figure 4. Tentative linear relation between free ionized calcium and total bile salt concentrations in 12 ductular and 15 gallbladder bile samples. Free $[Ca^{++}]$ was related to [TBS] by the function: $[Ca^{++}]$ = 1.16 + 0.0063 [TBS], r = 0.91. Note the $[Ca^{++}]$ intercept of 1.16 mM at zero [TBS]; this is the approximate $[Ca^{++}]$ in canine plasma. y = 1.16 + 0.0063 x.

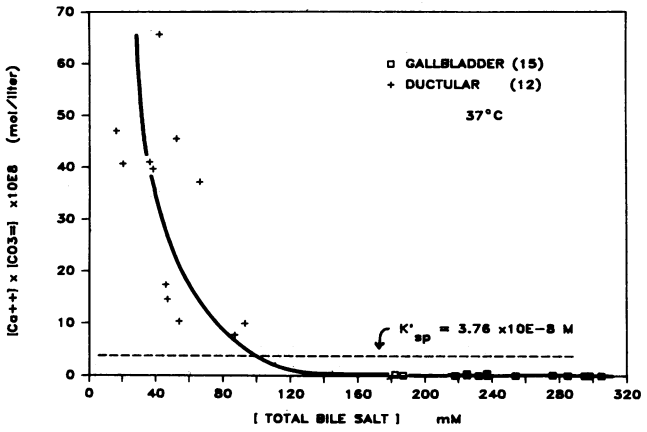

Figure 5. Observed ion-products: $[Ca^{++}] \cdot [CO_3^{=}]$, in 12 ductular and 15 gallbladder bile samples at 37°C as a function of total bile salt concentration. The dashed line is the value of the solubility product constant, K'_{sp}, for calcite at this temperature. Note that all ductular samples were supersaturated (metastable), whereas all gallbladder bile samples were unsaturated.

from 0.006 to 0.126, with a mean of only 0.039±0.011. Thus, the mean ion-product in gallbladder bile was only 3.9% of K'_{sp} so that, on the average, gallbladder bile was unsaturated by a factor of ~25.

These data show that normal gallbladder mucosal function, i.e., concentration and acidification of bile, produced a change in the saturation index from a value as high as 17.5 in ductular bile to a value as low as 0.006 in gallbladder bile, a nearly 3,000-fold change. The average change in the saturation index from ductular (8.34) to gallbladder (0.039) bile was ~215-fold.

Discussion

These studies represent the first attempt in any species to determine whether bile is supersaturated with respect to calcium carbonate. Using the Ca^{++} ion electrode for measurement of free ionized calcium, and established relationships and constants for calculation of free carbonate ion concentration, the ion-product: $[Ca^{++}] \cdot [CO_3^{=}]$ has been assessed in 12 samples of canine ductular bile and in 15 samples of gallbladder bile. The results show striking differences between ductular and gallbladder bile. Thus, all ductular samples, but none of the gallbladder samples, were supersaturated with calcium carbonate. We believe these findings have important implications regarding the pathogenesis of calcium-containing gallstones.

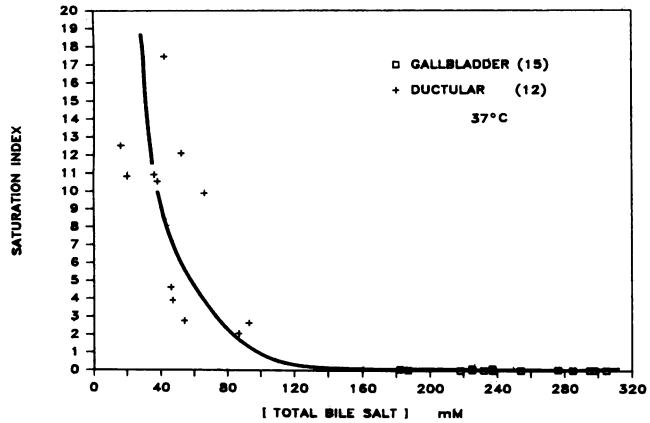

Figure 6. Saturation indices (SI), calculated from the data in Fig. 5. Note that all ductular bile samples showed indices well above 1.0 (supersaturated), whereas all gallbladder bile samples showed indices well below 1.0 (unsaturated). The average change in the saturation index between duct and gallbladder bile was ~215-fold.

First, a metastable state has been shown to exist in ductular bile samples of the dog, a species that does not normally form gallstones. This strongly suggests that a nucleating factor is necessary for $CaCO_3$ precipitation to occur in bile. We have previously reported a very analogous situation in canine pancreatic juice (21), in which both basal and stimulated samples were consistently supersaturated with respect to calcite, yet the dog is not known to form pancreatic stones. Pancreatic stones have been reported only in humans and cattle (22, 23), and are composed largely or entirely of calcite in both species (24–26). The presence of supersaturated juice, but no stones, strongly suggests that for a pancreatic stone to develop, a nidus for $CaCO_3$ precipitation is necessary. Verine and Lafont (27) have demonstrated the presence of protein in pancreatic stones from humans and cattle, and Sarles (22) has suggested that such a protein "plug" forms a nidus for $CaCO_3$ precipitation in human pancreatic calcific disease.

The present studies thus indicate that there are strong analogies between bile and pancreatic juice in the dog; both fluids are supersaturated with $CaCO_3$, yet neither gallstones nor pancreatic stones have been reported in this species. These findings suggest that a nucleating factor, presumably mucus and/or protein, is required for $CaCO_3$ precipitation in bile, similar to the "protein plug" theory of Sarles for $CaCO_3$ precipitation in pancreatic juice. Any measure that reduces the free Ca^{++} concentration (activity) in bile will reduce the likelihood of pigment gallstone formation.

The second implication of these studies is that bile salts are the major buffers for free Ca^{++} in bile, in conformity with our previous hypothesis in studies of calcium-binding to taurocholate (12). This is indicated by the finding that, on the average, only about 29% of the total calcium in gallbladder bile was present as free Ca^{++} ion; the remainder was present as bound or complexed species. At given total calcium, this buffer action would tend to reduce free Ca^{++}, and thus serve to reduce the $[Ca^{++}] \cdot [CO_3^{=}]$ ion product, thereby reducing the likelihood of $CaCO_3$ precipitation in bile.

The third implication of the present study relates to gallbladder mucosal function, and the protection that this provides against calcium precipitation in bile. Thus, in Fig. 5 it was seen that all ductular samples were supersaturated with $CaCO_3$, whereas none of the gallbladder bile samples were supersaturated. This reduction in lithogenicity of gallbladder bile occurred in the face of increasing $[Ca^{++}]$, and was due to a marked reduction in free carbonate ion concentration. We now will consider the mechanisms by which $[CO_3^{=}]$ may be so dramatically reduced in gallbladder bile.

The reduction in free $[CO_3^{=}]$ is due primarily to two factors: (*a*) reduction in total carbonate (total CO_2 in all forms) in gallbladder bile, and (*b*) reduction in the relative proportion of $[CO_3^{=}]$ as pH declines. In the past, it has been generally held that the reduction in total carbonate in gallbladder bile that occurs during the concentrating process is due to bicarbonate ion reabsorption. Recently, however, in studies reported elsewhere (Rege, R. V., and E. W. Moore, manuscript submitted for publication), we have shown that in the dog, there is substantial H^+ ion secretion by gallbladder mucosa. This presumably represents a

Na^+/H^+ exchange in the epithelial cell, similar to that recently described in *Necturus* gallbladder in vitro by Weinman and Reuss (28). Acidification by H^+ secretion would result in a greater pH decline, at given luminal bicarbonate, than would occur by simple bicarbonate absorption alone, due to an increase in the denominator of the Henderson-Hasselbalch equation (increase in CO_2 from luminal bicarbonate neutralization). As a result of this pH decline, the proportion of total CO_2 present as carbonate ion would be substantially lower.

These considerations regarding gallbladder mucosal function may be summarized as follows: upon delivery of ductular bile to the gallbladder, the mucosa absorbs Na^+ and water, while secreting H^+ ion. The latter reacts with luminal bicarbonate to produce CO_2 and water. As a result of bicarbonate neutralization and CO_2 production, there is a decline in pH and total CO_2, with dramatic decline in the concentration of free $[CO_3^=]$ ion. The decrease in $[CO_3^=]$ is so substantial that gallbladder bile remains very unsaturated with respect to $CaCO_3$, despite a rise in free $[Ca^{++}]$ during the concentrating process. In addition, upon removal of water by mucosal function (Na^+ absorption), the total bile salt concentration increases, with a corresponding increase in calcium-binding capacity, so that free $[Ca^{++}]$ remains relatively low in the face of increasing total calcium. Resulting $[CO_3^=]$ and $[Ca^{++}]$ are thus sufficiently low to produce an ion-product that is well below K'_{sp} for calcite.

Note that assessment of $CaCO_3$ solubility is strongly dependent on the constants used in the calculations: pK'_1, pK'_2, and K'_{sp}. For the first two of these, we have used values given by Edsall and Wyman (19), who have noted that these are probably accurate to within 0.1 pH unit (29). For K'_{sp}, we have used the value 3.76×10^{-8} mol/liter, recently obtained with the Ca^{++} electrode (16); this value is similar to that of Hastings, Murray, and Sendroy (30) who, in 1926, obtained a K'_{sp} of 3.98×10^{-8} mol/liter (37°C, total ionic strength [μ] = 0.15 M), and is almost identical to the results of Broman and Hastings (31) who, in 1937, obtained a calcite K'_{sp} value of 3.72×10^{-8} mol/liter.

All of the above K'_{sp} values were obtained at a total ionic strength of 0.15 or 0.16 M, which is the approximate ionic strength of ductular bile. As bile undergoes concentration by the gallbladder, the total ionic strength increases, so that in fully concentrated bile the sodium concentration may exceed 300 mM. As we have noted previously (1), the term "total ionic strength" has no clearly defined meaning in such samples, because bile salt anions aggregate to form micelles and the activities of Na^+ and K^+ (32), and also Ca^{++} (12) are suppressed by virtue of binding to the micelle. In gallbladder bile, it is therefore likely that K'_{sp} varies somewhat from sample to sample, so that the saturation indices for gallbladder samples (Fig. 6) should be viewed as approximate estimations. Since gallbladder samples were unsaturated by a factor of ~25, minor variations in K'_{sp} due to ionic strength effects would not diminish the striking differences between ductular and gallbladder bile, and would not alter the conclusion that gallbladder bile is unsaturated with respect to calcite.

Also note that three different polymorphs of $CaCO_3$ have been identified in human gallstones: calcite, aragonite, and vaterite (10, 33). Vaterite is rarely found elsewhere in nature, and the physicochemical significance of the various polymorphs in gallstone disease is not known. Insofar as relative solubility of the various polymorphs is concerned, Broman and Hastings (31) found aragonite to be somewhat more soluble than calcite; we know of no studies of vaterite solubility under physiologic conditions. Should future studies show that vaterite is less soluble than calcite, then resulting saturation indices would be higher than those depicted in Fig. 6. On the other hand, if vaterite is more soluble than calcite, then K'_{sp} for calcite will determine $CaCO_3$ solubility, since limiting solubility is determined by the least soluble species present. The relative solubilities of aragonite and vaterite are worthy of future investigation.

We believe these studies have provided new insights into some of the factors that may lead to calcium precipitation and gallstone formation. The results support our previous hypothesis (1) that in canine bile, as in canine pancreatic juice, a nucleating factor is necessary for $CaCO_3$ precipitation. In addition, it is proposed that normal gallbladder mucosal function plays an important role in reducing calcium carbonate lithogenicity in gallbladder bile. Similar studies are now needed in humans.

Acknowledgments

We gratefully acknowledge the technical assistance of Ms. Kelli Mosher, Ms. Inge Moore, and Ms. Fran Keith.

This work was supported by grant 32130, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases.

References

1. Moore, E. W. 1984. The role of calcium in the pathogenesis of gallstones: Ca^{++} electrode studies of model bile salt solutions and other biologic systems. *Hepatology (Baltimore).* 4:228S–243S.

2. Sutor, D. J., and L. I. Wilkie. 1977. Calcium in bile and calcium salts in gallstones. *Clin. Chim. Acta.* 79:119–127.

3. Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.* 47:1043–1052.

4. Holzbach, R. T., M. Marsh, M. Olszewski, and K. Holan. 1973. Cholesterol solubility in bile. Evidence that supersaturated bile is frequent in healthy man. *J. Clin. Invest.* 52:1467–1479.

5. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relation to gallstone formation and dissolution in man. *J. Clin. Invest.* 61:998–1026.

6. Hofmann, A. F., S. M. Grundy, J. M. Lachin, S.-P. Lan, R. A. Baum, R. F. Hanson, T. Hersh, N. C. Hightower, Jr., J. W. Marks, H. Mekhjian, R. A. Shaefer, R. D. Soloway, J. L. Thistle, F. B. Thomas, M. P. Tyor, and the National Cooperative Gallstone Study Group. 1982. Pretreatment biliary lipid composition in white patients with radiolucent gallstones in the National Cooperative Gallstone Study. *Gastroenterology.* 83:738–752.

7. Holan, K. R., R. T. Holzbach, R. E. Hermann, A. M. Cooperman, and W. J. Claffey. 1979. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology.* 77:611–617.

8. Gollish, S. H., and S. M. Strasberg. 1981. Nucleation of cholesterol monohydrate crystals from hepatic bile and gallbladder bile in persons with cholesterol gallstones. *Hepatology (Baltimore).* 1:512*a*. (Abstr.)

9. Burnstein, M. J., R. G. Ilson, C. N. Petrunka, et al. 1983. Evidence for a potent nucleating factor in the bile of cholesterol gallstone patients. *Gastroenterology.* 85:801–807.

10. Been, J. M., P. M. Bills, and D. Lewis. 1979. Microstructure of gallstones. *Gastroenterology.* 76:548–555.

11. Wosiewitz, U. 1980. Limy bile and radiopague, calcified gallstones: a combined analytical, radiographic and micromorphologic examination. *Pathol. Res. Pract.* 167:273–286.

12. Moore, E. W., L. Celic, and J. D. Ostrow. 1982. Interactions between ionized calcium and sodium taurocholate: bile salts are important buffers for prevention of calcium-containing gallstones. *Gastroenterology.* 83:1079–1089.

13. Williamson, B. W. A., and I. W. Percy-Robb. 1980. Contribution of biliary lipids to calcium binding in bile. *Gastroenterology.* 78:696–702.

14. Bateson, M. C., I. A. D. Bouchier, D. B. Trash, D. P. Maudgal,

and T. C. Northfield. 1981. Calcification of radiolucent gallstones during treatment with ursodeoxycholic acid. *Br. Med. J.* 283:645–646.

15. Schoenfield, L. F., J. W. Lachin, the NCGS Steering Committee, and the NCGS Group: National Cooperative Gallstone Study. 1981. A controlled trial of the efficacy and safety of chenodeoxycholic acid for dissolution of gallstones. *Ann. Int. Med.* 95:257–282.

16. Moore, E. W., and H. J. Verine. 1985. Pathogenesis of pancreatic and biliary lithiasis: the solubility product (K'sp) of calcite determined with the Ca^{++} electrode. *J. Lab. Clin. Med.* In press.

17. Tera, H. 1960. Stratification of human gallbladder bile *in vivo*. *Acta Chir. Scand.* 256(Suppl.):1–85.

18. Talalay, P. 1960. Enzymatic analysis of steroid hormones. *Meth. Biochem. Anal.* 8:119–143.

19. Edsall, J. T., and J. Wyman. 1958. Biophysical Chemistry. Vol. 1. Academic Press, Inc., New York. 558.

20. Rege, R. V., and E. W. Moore. 1984. Pathogenesis of $CaCO_3$-containing gallstones: I. The solubility coefficient (Q') of CO_2 in canine bile. *Hepatology (Baltimore).* 4:1078. (Abstr.)

21. Moore, E. W., and M. J. Zimmerman. 1982. Pancreatic ionized and total calcium secretion: a test of the thermodynamic model for pancreatic lithogenicity. *Gastroenterology.* 82:1134*a*. (Abstr.)

22. Sarles, H. 1974. Chronic calcifying pancreatitis-chronic alcoholic pancreatitis. *Gastroenterology.* 66:604–616.

23. Verine, H. J. 1973. Reserches sur la lithogenese pancreatique: apports de la pathologie comparee et de la pathologie experimentale. Thesis in Human Biology, Lyon, No. 8, Université de Lyon, France.

24. Perinet, G., H. J. Verine, and R. Lafont. 1972. Etude thermique de calculs pancreatiques humains et bovins. *C. R. Seances Acad. Sci.* 274D:3303–3305.

25. Mallet-Guy, P., M. Perrin, and A. Thozet. 1969. Etude radiocristallographique de calculs pancreatiques humains. *C. R. Seances Soc. Biol.* 163:1838–1841.

26. Perrin, M., A. Thozet, and H. J. Verine. 1969. Etude radiocristallographique de calculs pancreatiques bovins. *C. R. Seances Soc. Biol.* 163:187–189.

27. Verine, H. J., and H. Lafont. Existence d'une matrice proteique dans les calculs pancreatiques de vertebres superieurs comparaison avec les matrices proteiques à calcite des mollusques. *Haliotis.* 2:215–219.

28. Weinman, S. A., and L. Reuss. 1982. Na^+-H^+ exchange at the apical membrane of *Necturus* gallbladder. *J. Gen. Physiol.* 80:299–319.

29. Edsall, J. T., and J. Wyman. 1958. Biophysical Chemistry. Vol. 1. Academic Press, Inc., New York. 563.

30. Hastings, A. B., C. D. Murray, and J. Sendroy. 1926. Studies of the solubility of calcium salts: I. The solubility of calcium carbonate in salt solutions. *J. Biol. Chem.* 71:723–781.

31. Broman, A. A., and A. B. Hastings. 1937. Solubility of aragonite in salt solutions. *J. Biol. Chem.* 119:241–246.

32. Moore, E. W., and J. M. Dietschy. 1964. Na and K activity coefficients in bile and bile salt solutions determined with glass electrodes. *Am. J. Physiol.* 3:1426–1433.

33. Bogren, H., and K. Larsson. 1963. Crystalline components of biliary caluli. *Scand. J. Clin. Lab. Invest.* 15:457–462.