Study on Dissolution and Disintegration of Calcium Bilirubinate Stone

YASUSHI NAKAMURA, NORIYOSHI SUZUKI, WATARU TAKAHASHI and TOSHIO SATO

Department of Surgery, Tohoku University School of Medicine, Sendai 980

NAKAMURA, Y., SUZUKI, N., TAKAHASHI, W. and SATO, T. Study on Dissolution and Disintegration of Calcium Bilirubinate Stone. Tohoku J. exp. Med., 1978, 125 (2), 121-134 — When a slice of calcium bilirubinate stone was incubated in a solution of tetrasodium salt of ethylenediamine-tetraacetic acid (EDTA-4Na), a potent chelating agent, the solution exhibited a yellow brown tint, which was spectroscopically characteristic of bilirubin. Microscopic examination of the slice revealed dissolution of granules of calcium bilirubinate, leaving behind a reticular matrix of PAS-positive substance. The effect of EDTA-4Na was influenced by pH, being fully effective only at pH 10 or more, and by temperature and concentration as well. Simultaneous application of bile salt enhanced the activity of EDTA-4Na, hydrophilizing the gallstone surface to facilitate chelating reaction and also dissolving minor fatty components of the stone. Heparin at proper concentrations also promoted disintegration of the stone, changing surface potential of its constituent particles to the dispersion-prone charge. The effect of composite EDTA-4Na-bile salt-heparin was thus significantly greater than that of single EDTA-4Na. This mixture is promising for clinical application as a means of direct dissolution of residual gallstones.

gallstone; chelating agent; ethylenediaminetetraacetic acid; bilirubine; bile salt

Management of the postoperative residual gallstone is a major problem in the biliary tract surgery. Various non-operative methods have been proposed with diverse results, including bile duct lavage, instrumental extraction, and, more recently, endoscopic papillotomy. Among them is an attempt to dissolve or decompose the residual stone by appropriate agents that are infused into the biliary tract. Although in American and European countries the cholesterol stone is the main subject of such a trial, in Japan interest has also been focused on the calcium bilirubinate stone which was once prevalent and still is not rarely encountered. Sodium hexametaphosphate (Hisatsugu 1959) and benzalkonium chloride (Maki et al. 1967) were thus reported to dissolve the calcium bilirubinate stone, yet their clinical efficacies are limited.

As published previously a theory has developed in our laboratory which elucidates the mechanism of formation of the calcium bilirubinate stone (Maki 1964; Maki et al. 1971). This theory offers a suggestion on possible process of decomposition of this gallstone, thereby indicating agents which may enhance such
process. In this study a number of substances were selected on such a theoretical basis, which were evaluated in vitro for the effect to dissolve the calcium bilirubinate stone.

**MATERIALS AND METHODS**

**Materials**

**Gallstone and related materials.** Twelve calcium bilirubinate stones of comparable sizes, selected one each from surgical specimens of 12 cases, were used. Ten of them were stratified gallstones, while two were muddy amorphous ones. Bilirubin (C33H34N406, Wako Pure Chemical Industries) and its calcium salt prepared at our laboratory (Maki et al. 1964) were also utilized for reference. These materials were preliminarily subjected to infrared absorption analysis by the KBr disk method with a Hitachi Infrared Spectroscope EPI-S2.

**Test agents.** (1) Sodium hydroxide. (2) Chelating agents — tetrasodium, trisodium and disodium salts of ethylenediaminetetraacetic acid (Dotite 4Na, Dotite 3Na and Dotite 2Na, Dojin Co. Research Laboratories, to be abbreviated in this paper as EDTA-4Na, EDTA-3Na and EDTA-2Na, respectively); sodium hexametaphosphate (Wako Pure Chemical Industries); and cyclohexanediaminetetraacetic acid (CyDTA, Dojin Co. Research Laboratories). (3) Bile salts — sodium ursodeoxycholate (Urso Injection, Tokyo Tanabe Pharmaceutical Co., to be referred to as URSO), and sodium cholate (C24H39O6Na, Nakarai Chemicals). (4) High-molecular-weight acid organic compounds — heparin sodium (for intravenous injection, J.F., 50 ml=50,000 U, Shimizu Pharmaceutical Co.), and heparin powder (1 mg=100 U, Novo Industri).

**Methods**

**Preparation of gallstone specimens.** Stratified stones were washed with water, dried at room temperature, and then prepared into serial sections of approximately 3 μm by the technique devised at our laboratory (Nakamura 1966). Muddy stones were sliced in the same way after being shaped into compressed disks by the aid of a disk molder for infrared study.

**Dissolution experiment.** A 2–4 mg aliquot of bilirubin or calcium bilirubinate was suspended in a tube in 10–30 ml test solution, stoppered tightly, and allowed to stand in the dark at room temperature (18–20°C) or, on some occasions, at 37°C. Dissolution of the material was assessed by yellow tint of the supernatant, spectrometrically with a Hitachi Spectrophotometer 139. The absorption at 430 nm was characteristic of bilirubin, and it was preliminarily confirmed that the absorbance at this wave length followed the Lembert-Beer law as regards bilirubin concentration. This enabled semiquantitative determination of dissolved bilirubin.

The gallstone slice was similarly treated with 10 ml test solution in a glass cell that was specifically designed for this purpose. Dissolution of the slice was assessed by its gross change and by sampling the solution at intervals for spectrophotometry. The slice was also examined microscopically before as well as after each treatment, as mentioned below. Effects of the test solutions were evaluated separately and in combination. In experiments with some chelating agents the influence of pH was assessed by changing pH of the solution with Palitzsch (H3BO3+NaCl–Na2B4O7) and Menzel (Na2CO3–NaHCO3) buffers and with hydrochloric acid and sodium hydroxide. The dissolution experiments were carried out with perfectly shaped slices of comparable sizes and widths, at least in triplicate for each experimental situation. The spectrometric data were analyzed statistically.

**Microscopic and histochemical examination.** The gallstone slices were washed thoroughly with distilled water, and observed microscopically. They were then stained by PAS (McManus), 1% alcian blue 8GX (3% CH3COOH) or von Kossa staining, embedded in glycerol-gelatin, and subjected to histochemical examination.
**Dissolution of Gallstone**

**Colloid-chemical experiment.** This was performed in order to evaluate the effect of surface-active substances on coagulation and dispersion of particles of gallstone ingredients. Suspension of calcium carbonate was adopted as an experimental model according to Maki and Suzuki (1964). Sodium cholate and heparin were examined exclusively, other substances of possible efficacy being not eligible to this experiment because of poor solubility in water. In a 10 ml graduated cylinder were taken 500 mg calcium carbonate and a predetermined quantity of the test agent powder, to which was added distilled water to a final volume of 10 ml while stirring gently with a glass rod. The resulting suspension was allowed to stand at room temperature for 1 hr, during which time the volume of sedimentation and the turbidity of supernatant were recorded at intervals. Electrophoretic mobility of the suspended CaCO₃ particles was also measured with a microscopic electrophoresis apparatus, and their zeta-potential calculated by the Smoluchowskii equation.

**RESULTS**

**Effect of single agents on bilirubin, calcium bilirubinate and sliced calcium bilirubinate stone**

*Sodium hydroxide.* Bilirubin dissolved well in a 0.05 M sodium hydroxide solution. A brownish yellow tint associated with a clear absorption maximum at 430–440 nm appeared in 20 min (Fig. 1a) and lasted for about 5 hr. At 6 hr the absorption maximum shifted to 420 nm while decreasing its intensity, when another gentle band, probably of denatured bilirubin, appeared at 310–320 nm. The former band then continued to decrease its intensity and eventually disappeared by 12 hr, whereas the latter became more prominent as time elapsed. In accordance with such a change the gross appearance of the solution turned from initial brownish yellow to faint yellow.

Synthetic calcium bilirubinate hardly dissolved in a 0.1 M sodium hydroxide solution, the supernatant becoming only faintly yellow without showing an absorption maximum at 430–440 nm (Fig. 1b).

The slice of calcium bilirubinate stone, when exposed to a 0.1 M sodium hydroxide solution for 24 hr, at room temperature, suffered no notable changes both grossly and microscopically. The test solution did not show a yellow tint nor an absorption maximum at 430 nm (Fig. 1c). Results were similar at 37°C.

![Absorption spectra of sodium hydroxide solutions mixed with bilirubin, calcium bilirubinate and sliced calcium bilirubinate stone](image-url)

**Fig. 1.** Absorption spectra of sodium hydroxide solutions mixed with, a, bilirubin (2 mg in 30 ml of 0.05 M NaOH, at 20 min); b, calcium bilirubinate (4 mg in 15 ml of 0.1 M NaOH, at 2 hr); and c, slice of calcium bilirubinate stone (a slice in 10 ml of 0.1 NaOH, at 6 hr) at room temperature.
EDTA. The reaction of bilirubin with EDTA·4Na was much the same as with sodium hydroxide. Results with synthetic calcium bilirubinate and a 10% EDTA·4Na solution, at room temperature, are shown in Fig. 2. An absorption band appeared at 430–440 nm in 20 min, which gradually increased in intensity for subsequent 36 hr and then decreased. At 6 days the band began to shift toward shorter wave length, while another absorption maximum appeared at 310–320 nm. The results remained essentially unaltered at an EDTA·4Na concentration of 1%. When calcium bilirubinate was incubated in a 1% EDTA·4Na solution at 37°C, dissolution of bilirubin as determined spectrometrically progressed sharply for the first 30 min and only gently thereafter (Fig. 3).

When the gallstone slice was immersed in a 10% EDTA·4Na solution at room temperature, a yellow tint appeared almost instantly and became brownish yellow and more prominent as time elapsed. The immersed slice was decolorized progressively. As shown in Fig. 4, spectrophotometry of the solution at 6 hr revealed a sharp absorption maximum at 430–440 nm. This band then became less marked, and another band appeared at 310–320 nm which became progressively prominent as in the case of sodium hydroxide reacting with bilirubin. Similar
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results were obtained with 5%, 2% and 1% solutions of this agent. In Fig. 5 is shown the influence of temperature over the effect of 1% EDTA-4Na. An increase in temperature apparently enhanced dissolution of bilirubin from the gallstone slice. On gross inspection at 30 min, decoloration of the slice was only slight at room temperature, fairly remarkable at 37°C, and almost complete at 50°C. Fig. 6 shows the results of experiments run at various concentrations of this agent while keeping temperature at 37°C. Dissolution of bilirubin appeared to be the greater the higher the concentration, but the difference was statistically insignificant between 1% and 2-5%. EDTA-3Na, EDTA-2Na and CyDTA gave essentially negative results with slices of calcium bilirubinate stone, the immersed slice suffering no gross or microscopic changes and the solutions remaining colorless and free of an absorption band at 430 nm. The aqueous solution of EDTA-4Na, EDTA-3Na and EDTA-2Na used in this series were pH 10.5, 7.6 and 4.7, respectively. The effect of changing pH on the reaction between EDTA-4Na and the gallstone slice is described later. The pH of 2% CyDTA was 11.4.

Sodium hexametaphosphate. Bilirubin, synthetic calcium bilirubinate and
sliced calcium bilirubinate stone reacted with aqueous solutions of this agent to a very slight extent. The yellow color was very weak, and the absorption at 430 nm very low, at various concentrations of the agent and at various temperatures. The pH of the sodium hexametaphosphate solution used in this experiment was 7.6. The results with modified pH are shown later.

**Bile salts.** Both bilirubin and synthetic calcium bilirubinate when mixed with 1% URSO or 1% sodium cholate at room temperature produced a perceptible yellow tint, but the absorbance at 430 nm of the supernatants remained very low.

Immersed in these solutions at room temperature, some gallstone slices faded a little and stained the solution weakly yellow, while others did not produce such a change. The yellow colored solutions, however, exhibited flat and low spectra without an absorption maximum at 430 nm. Morphologic changes of the slice were not induced by these agents.

**Heparin.** Either bilirubin or synthetic calcium bilirubinate did not produce yellow color nor a specific absorption at 430 nm on the heparin sodium solution.

Nor did the gallstone slice show an appreciable change in the heparin sodium solution, the latter remaining uncolored and free of characteristic absorptions. However, on incubation of the slice in this solution at 37°C for 2 hr or more a visible sedimentation of gallstone debris appeared on the bottom of the cell. Such a phenomenon did not occur with distilled water even on longer incubation.

**Influence of pH over activity of chelating agents**

The effect of pH was examined on EDTA-4Na and sodium hexametaphosphate. Series of 1% EDTA-4Na solutions and of 5% sodium hexametaphosphate solutions with pH adjusted to various levels by Palitzsch and Menzel buffers were prepared, in which were incubated gallstone slices at 37°C. As shown in Fig. 7, the dissolving effect of 1% EDTA-4Na was hardly perceptible over the acid and neutral range, became increasingly potent as pH was brought to 8 and over, and was maximal at pH 10 or more. The solution of 5% sodium hexametaphosphate was practically ineffective at pH below 8.5, but at higher pH it exhibited some chelating effect,
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Fig. 7. Absorbance at 430 nm of 1% EDTA·4Na and 5% sodium hexametaphosphate solutions at various pH, 30 min and 60 min after incubation with gallstone slice at 37°C. △, EDTA·4Na (60 min); ▲, EDTA·4Na (30 min); ○, sodium hexametaphosphate (60 min); ●, sodium hexametaphosphate (30 min).

Fig. 8. Change in absorbance at 430 nm of EDTA·4Na (1%)+URSO (1%) solution (●) during reaction with gallstone slice at 37°C. ○, EDTA·4Na (1%).

though much less markedly than did EDTA·4Na. Ingredients of both buffers did not in themselves interfere with chelating effects of these agents.

Effect of combined agents on sliced calcium bilirubinate stone

EDTA·4Na and URSO. EDTA·4Na was dissolved in a 1% URSO solution to a final concentration of 1% (pH 10.5). Incubated in this composite solution at 37°C, the gallstone slice faded more rapidly than in a single 1% EDTA·4Na solution. As shown in Fig. 8, the absorbance at 430 nm was higher at 10, 20 and 30 min than corresponding data with single EDTA·4Na, though the differences were not statistically significant. Gross and microscopic structure of the slice after immersion in this mixture was similar to the case of single EDTA·4Na.
Fig. 9. Change in absorbance at 430 nm of EDTA•4Na (1%)+heparin solutions during reaction with gallstone slice at 37°C. The case of single 1% EDTA•4Na is shown for comparison (○). , 2,500 U of heparin in 10 ml; Δ, 5,000 U of heparin in 10 ml; ▲, 10,000 U of heparin in 10 ml.

Fig. 10. Change in absorbance at 430 nm of EDTA•4Na (1%)+URSO(0.5%)+heparin (5,000 U) (○) and other solutions during reaction with gallstone slice at 37°C. ○, EDTA•4Na (1%); Δ, EDTA•4Na (1%)+URSO (1%); ▲, EDTA•4Na (1%)+heparin(5,000 U).

EDTA•4Na and heparin. EDTA•4Na was dissolved to a final concentration of 1% in 10 ml portions of heparin sodium solution containing 2,500 U, 5,000 U or 10,000 U heparin. Results with these composite solutions are shown in Fig. 9. Although the effect of EDTA•4Na (1%)-heparin (2,500 U) was almost comparable to that of single 1% EDTA•4Na, EDTA•4Na (1%)-heparin (5,000 U) and EDTA•4Na (1%)-heparin (10,000 U) exhibited significantly higher absorbance.

EDTA•4Na and URSO and heparin. An EDTA•4Na (1%)-URSO (0.5%)-heparin (5,000 U) solution, pH 10.5, was prepared by dissolving 100 mg EDTA•4Na in a mixture of 5 ml of 1% URSO and 5 ml of the heparin sodium solution. When the slice was incubated in this mixture at 37°C, both decoloration of the slice and yellow coloration of the solution progressed more remarkably than in the case of single EDTA•4Na or EDTA•4Na combined with either one of URSO and heparin sodium. Fig. 10 shows the time trend of absorption at 430 nm for this and other solutions. Statistical analysis against the result with single 1% EDTA•4Na was: insignificant (p>0.05) for EDTA•4Na (1%)-URSO (1%), significant (p <0.02) for EDTA•4Na (1%)-heparin (5,000 U), and highly significant (p<0.01)
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Fig. 11. Zeta-potential of suspending particles of calcium carbonate with heparin (○) or sodium cholate (●) added to various concentrations.

Table 1. Turbidity of supernatant on sedimentation of calcium carbonate

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<th>Compound</th>
<th>Turbidity at concentrations (%) indicated below</th>
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<tr>
<td></td>
<td>10⁻⁴  5×10⁻⁴  10⁻³  5×10⁻³  10⁻²  5×10⁻²  10⁻¹</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>#         #         #         #         #</td>
</tr>
<tr>
<td>Heparin</td>
<td>+             −          #         #         #         #</td>
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for EDTA·4Na (1%)–URSO (0.5%)–heparin (5,000 U).

Results with unsliced gallstones. Three stratified calcium bilirubinate stones of comparable sizes, obtained from a single patient, were each incubated for 3 hr at 37°C in 30 ml portion of (1) distilled water, pH 6.8, (2) 1% EDTA·4Na, pH 10.5, or (3) EDTA·4Na (1%)–URSO (0.5%)–heparin (5,000 U/10 ml), pH 10.5. The distilled water was colored only slightly, in which the gallstone remained grossly unchanged. On the contrary, the latter two solutions showed a strong brownish tint. In these solutions the outer shell of the gallstone was decolorized and became so fragile that they crumbled at a touch of forceps.

Dispersion-coagulation effect of heparin and of sodium cholate on calcium carbonate suspension

Results are shown in Table 1 and Fig. 11. Heparin coagulated a CaCO₃ suspension strongly at a concentration as low as 2×10⁻⁴–5×10⁻⁴%. At higher concentrations it tended to disperse CaCO₃ particles, the sediment volume decreasing and the turbidity of supernatant increasing. These phenomena were associated with a change in the zeta-potential of the particles. When an increasing amount of heparin was applied, the potential reduced its primary positive value, turned negative at the very concentration where the strong coagulation occurred, and increased the negative charge as the concentration was further increased. Sodium cholate coagulated the suspension at a higher concentration (10⁻¹–5×10⁻¹%) and less markedly than did heparin. In this case the zeta-potential was also reversed at the above concentration.
Morphologic and histochemical findings

Untreated slice. As shown in Fig. 12-i, the untreated slice of stratified calcium bilirubinate stone on unstained examination exhibited a characteristic laminary structure. This was consistent with observation of Maki and Nakamura (1969) and it was suggested that in this gallstone granules of calcium bilirubinate deposited themselves in a concentric fashion. The lamellae were von Kossa-positive in support of the above view. Although an accurate assessment was impracticable because of existing bile pigments, PAS and alcian blue stainings were considered to be weakly positive.

After immersion in test solutions. In Fig. 12-ii is shown an example of unstained slices that were exposed to EDTA-4Na. The initial laminary configuration was almost lost, as a result of dissolution of calcium bilirubinate granules, leaving a vague structure of the matrix. The von Kossa reaction gave positive results only in limited portions. As shown in Fig. 12-iii, the matrix structure was clearly visualized by PAS staining, which became progressively manifest as decalcification proceeded. The layer a of the picture, for instance, was the portion where calcium bilirubinate granules had been originally present but had disappeared during incubation. There, PAS-positive substances were arranged in a reticular fashion. In layer b, also a deposition zone, calcium bilirubinate granules were still preserved and some of them were surrounded by fibrous (part of reticular) structure of PAS-positive substances. On the other hand, layer c was a non-deposition, or interlamellar, layer. Such zone was almost amorphous.
and showed weak PAS staining. The PAS-positive reticular structure was alcianophilic at pH 2.9. Examination of sliced muddy amorphous stones that were incubated in effective solutions also disclosed morphologic evidence for dissolution of granules of calcium bilirubinate.

**DISCUSSION**

The calcium bilirubinate stone is produced mainly in ductal portions of the biliary system and occasionally involves the intrahepatic ducts, whereas the cholesterol stone is usually confined to the gallbladder or to the common duct at most. Accordingly, when an incomplete surgical removal occurs, the calcium bilirubinate stone is more likely to be the case. If a residual gallstone, of whichever type, is identified during an immediate postoperative phase where a bile duct drain is still in place, endeavors are usually made to remove it non-operatively. Among such attempts is dissolution of the stone by an agent administered through the drain.

As far as the cholesterol stone is concerned, such an attempt has registered considerable success. Walker (1891) for the first time succeeded in dissolving the stone by infusing ethyl ether. As many as 113 agents were then tested by Best et al. (1953), who claimed that among them only ether and chloroform were effective. But these agents are not used today because of their untoward effects. Later, as the mixed micelle theory of cholesterol dissolution developed, bile acids drew attention. With sodium cholate, favorable results were obtained by Way et al. (1972) and subsequently by others. On the other hand, Gardner (1969) demonstrated that an addition of an anionic compound enhanced stability of colloidal or suspending particles of bile, and on this basis he and his associates succeeded in dissolving, or, more properly, distintegrating a residual cholesterol stone by heparin (Gardner et al. 1973). In Japan, Hisatsugu et al. (1972) advocated a natural limonene preparation as being clinically effective.

On the other hand far less success has been attained so far in direct dissolution of the calcium bilirubinate stone. As mentioned before, sodium hexametaphosphate and benzalkonium chloride are in practical use, only with limited results. Further fundamental studies are thus awaited in order to develop a successful means of dissolving this type of gallstone.

Developmental mechanism of the calcium bilirubinate stone has been elucidated by Maki and associates of our Department (Maki 1964; Maki et al. 1971). While the stone consists mainly of water-insoluble particles of calcium bilirubinate and partly of cholesterol and other minor components, it also contains a small amount of high-molecular-weight acid organic compounds, above all sulfated glycoproteins, which bridge those components to form the skeleton or matrix of the stone. Accordingly, decomposition of this gallstone may be achieved by (1) removing calcium bilirubinate with the aid of a chelating agent, (2) enzymatically decomposing the high-molecular-weight organic compounds, (3) dissolving components other than calcium bilirubinate, and/or (4) dispersing the constituent particles by some
Among the chelating agents examined, tetrasodium salt of ethylenediaminetetraacetic acid (EDTA-4Na) was the most effective. While free bilirubin dissolved rapidly in a sodium hydroxide solution and colored the latter brownish yellow, synthetic calcium bilirubinate and slices of the calcium bilirubinate stone did not. On the other hand, all these three materials dissolved well in an EDTA-4Na solution, producing an apparent yellow color. In each case the yellow solution showed an absorption maximum at 430–440 nm, a characteristic band of bilirubin. These facts suggest that calcium bilirubinate is decalcified by the chelating effect of EDTA-4Na, and liberated bilirubin dissolves as sodium salt. Morphologic and histochemical examination of the EDTA-4Na-treated gallstone slices disclosed loss of granules of calcium bilirubinate, visualizing a reticular network of residual high-molecular-weight acid organic compounds.

Although calcium bilirubinate granules are hydrophilic, the surface of a natural calcium bilirubinate stone is rather hydrophobic because of co-existing fatty substances. Accordingly, a combinatory use of surface-active material with the chelating agent may facilitate contact of the latter with calcium bilirubinate and thus promote their reaction. A preparation of sodium salt of ursodeoxycholic acid (URSO) was examined for such an adjuvant effect, which actually enhanced the effect of EDTA-4Na though not to the level of statistical significance. Furthermore, instances were observed in which the gallstone slice was dissolved slightly by URSO alone, probably as a result of dissolution of cholesterol and other minor components. Combination of the bile salt is thus considered to give dual benefit to the dissolution of calcium bilirubinate stone.

Dispersion of the constituent particles, the fourth of the above-mentioned principles, may be achieved by adding a proper material which changes the surface potential of the particles from the coagulation-prone to the dispersion-prone side. Heparin is known to have such a property and on experiment actually enhanced the activity of EDTA-4Na to dissolve the stone. Such an adjuvant effect of heparin became manifest in as soon as 10 min, a reaction time short enough for clinical application. Heparin when applied singly resulted in a slight disintegration of the gallstone slice only after 2 hr.

On these theoretical and experimental bases, the triple combination of EDTA-4Na–URSO–heparin was finally established and examined. As was expected, this combination showed a strong activity to dissolve calcium bilirubinate from the gallstone slice, being more effective than any or any other combination of the agents tested. The effect of a chelating agent is largely influenced by pH of the reacting system, generally being fully effective only in an alkaline reaction. In fact it was shown in the present experiment that when pH of 1% EDTA-4Na (10.5 in distilled water) was progressively lowered using buffer, the activity to decalcify the gallstone slice decreased considerably. It is expected that supplementation of the agent with URSO and heparin may partly compensate for such a loss of activity.
in the neutral range.

Solutions to be infused into the biliary tract for the purpose of gallstone dissolution must be harmless to the organism. At the same time, their reaction products with gallstone constituents must also be harmless. Although EDTA is in itself non-toxic, at a large dose it may possibly cause disturbance by interfering with metal metabolism. The LD_{50} of EDTA varies according to the species and to the route of administration, reportedly from 60 mg/kg to 7,000 mg/kg (Toyoda 1960; Foreman 1963). However, clinical experience with this agent (to remove radioactive substances, to dissolve directly urinary calculi, to inactivate habu snake venom and as an anticoagulant, to mention examples) has revealed its relative safety. Ca·EDTA, the reaction product of EDTA with the calcium bilirubinate stone, is essentially harmless; it is believed that a healthy adult can tolerate oral intake of some 10 g of this substance. Among chelating agents EDTA has a particularly large stability constant with Ca^{++}, and in this point is much advantageous over sodium hexametaphosphate (LD_{50}=140 mg/kg, intravenous to dog*) and other agents.

Clinical success in dissolving a calculous concrement by direct application of the chelating agent was first registered on the urinary stone by Gehres and Raymond (1951) using Calsol (EDTA·2Na). The gallstone was challenged by Best et al. (1953) with Versenes (EDTA·3Na) and by Hisatsugu et al. (1959) with sodium hexametaphosphate and EDTA·2Na. However, adjusting pH of the solution to physiological near neutrality, these authors failed to recognize the efficacy of the sodium salts of EDTA. The conditional stability constant of EDTA+Ca^{++}→Ca·EDTA increases as pH of the reacting system increases, attaining a maximal value at pH 10 or more. A strongly alkaline condition is thus mandatory for EDTA to exert a full activity, but too high a pH may cause injury to the biliary tract where bile is normally only weakly alkaline. Therefore, a pH adjustment is necessary for clinical application, which inevitably results in a loss of its activity. It has been the author's idea to recoup this loss by combinatory use of other substances. URSO and heparin were shown by the present study to meet such purposes — the former facilitating contact of EDTA with calcium bilirubinate and also by itself dissolving minor components of the stone, while the latter acting to disperse constituent particles of the stone. It is reasonably expected that the EDTA·4Na–URSO–heparin solution may be applied within a pH range of clinical compatibility while keeping its effect to an acceptable level. Although bile salts sometimes cause diarrhea, and a case was reported in which intrabiliary application of heparin induced bile duct hemorrhage (Gardner et al. 1973), these substances are already in wide clinical use. The composite solution may be brought to clinical trial after an optimum pH, at which maximal effect is attained with minimal injury to the biliary mucosa, has been established by further studies.

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References