reduced the ^{45}Ca net uptake from 5.38 ± 0.57 ($n = 14$) to 3.85 ± 0.18 ($n = 16$) and 3.39 ± 0.24 ($n = 16$) respectively ($p < 0.01$ in both cases). The ^{45}Ca net uptake obtained with 10^{-3} M gentamycin was very close (63% of the control) of the Ca^{2+} threshold whereas the amount of Ca incorporated in the presence of 10^{-4} M gentamycin reached supra-threshold values (71% of the control), sufficient to release twice the amount of insulin when compared to the basal value obtained in the absence of gentamycin and glucose. The inhibition of insulin release and the reduction of the ^{45}Ca net uptake induced by gentamycin is very similar to that we obtained recently using sisomycin, an aminoglycoside antibiotic of the gentamycin family[14]. The observation that increasing extracellular Ca^{2+} concentration abolished or at least significantly reduced the effect of sisomycin is in good agreement with the idea that gentamycin exerts its inhibitory effect on the insulin release mainly by lowering the uptake of Ca^{2+} by the islets. In the absence of Ca^{2+}, with or without EGTA, Ba^{2+} is the unique agent able to activate the process of insulin release[15,16]. This process can be potentiated by theophylline[16,17] and it is assumed to result from a direct activation of the effector system responsible for the extrusion of insulin-containing granules, namely the microtubules-microfilaments and membranes. Indeed Ba^{2+} plus theophylline (2 mM each) significantly stimulated insulin release from 33 ± 1.6 ($n = 14$) to 113 ± 12.3 ($n = 13$) μU/islet per 90 min ($p < 0.01$). In order to investigate possible effects of gentamycin on the effector system we tested 10^{-4} M gentamycin (which significantly reduced both insulin release and ^{45}Ca net uptake) on the insulin secretion induced by Ba^{2+} and theophylline. Under these conditions the insulin release averaged 114 ± 11.4 ($n = 14$) μU/islet per 90 min, a value not statistically different ($p > 0.95$) from the above control. In conclusion, these results suggest that gentamycin may inhibit insulin release by blocking the entry of Ca^{2+} into the B-cells, instead of having a deleterious effect on a more distant event in the secretory sequence, i.e., the extrusion of the B-granules.

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Studies on the fissure of cholesterol-pigment-calcium stone (multiple faceted stone)

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Summary. Multiple faceted gallstones with an internal fissure have been examined by gas chromatography-mass spectrometry. In vivo, the fissures probably contain only water vapor. The fissure may be produced by dehydration or water trapping during the stone formation process.

It is well known that many cholesterol-pigment-calcium stones (multiple faceted stones) possess a characteristic fissure[1-3] in the central part as shown in figure 1. However, little attention has yet been focused on the contents or causation of the fissures. In the present paper, the authors report some analytical results on the contents of the fissures of multiple faceted stones using gas chromatography-mass spectrometry (GC-MS), and discuss the formation process of multiple faceted stones with fissures in comparison with non-fissured stones.

Materials and methods. 14 multiple faceted stones, obtained from gall bladders which were not inflamed but had slightly infected bile, were studied. The average content of cholesterol in the stones was $80 \pm 12\%$ by weight. 12 stones kept under room conditions for severeral months were classified on the basis of their soft X-ray findings[4] into 2 groups: 8 stones (group A) had fissures and 4 stones (group B) did not. 2 fresh stones were placed immediately after operation in an isotonic physiological saline solution (group C).

GC/selected ion monitoring MS: A JEOL-JMS-D100 GC-MS was used for analyzing the content of the fissures. The operating conditions were as follows: ionization energy, 75 eV; ion source temperature, 200 °C; accelerating voltage, 3 kV; ionizing current, 300 μA; ion multiplier, 1.5 for m/z 28, m/z 32 and 0.3 for m/z 18. A stainless steel column (1.5 ml × 3 mm i.d.) packed with Carbosieve S100/200 mesh (Supelco, Inc.) was used, and the column temperature was held at 60 °C for 8 min and programmed to 120 °C at a rate of 15 °C/min. Each stone was placed in a teflon tube (10 cm 1 × 10 mm i.d.) for a few minutes in order to exclude air and water around the stone. GC-MS was started immediately after crushing the stone by pressing the tube. Under these conditions the calibration curves were obtained by plotting the ratio of peak areas against the injected amounts.

Results and discussion. Japanese gallstones obtained from the gall bladder are in general cholesterol stones, particularly multiple faceted stones. Many of these stones possess a fissure within the central part as shown in figure 1. The fissures can form in the human gall bladder in vivo; this was shown using soft X-rays which revealed them in group C stones.

Since the work of Admirand and Small[5], it has been generally accepted that human bile supersaturated with cholesterol is lithogenic[6] and also that the lithogenicity may be assessed as a single point on triangular coordinates relating the relative concentrations of bile salts, lecithin and cholesterol. Such a biochemical model is useful in understanding the pathogenesis of cholesterol gallstones. Nevertheless, it does not necessarily fully explain the complex processes involved in stone formation. Two diametrically opposed assumptions have thus far been presented According to Sweet's assumption[7], the peripheral part of a stone is formed first and then cholesterol crystals develop toward the center in much the same way as is observed in ice crystal formation. On the contrary, the other assumption takes the position that cholesterol crystal growth and agglomeration proceed from the center towards the periphery of the stone. The authors' results support Sweet's assumption, in the case of multiple faceted stones, and suggest that the fissures might be formed by dehydration or water trapping in the internal part of the stone during its formation. It is, therefore, of great importance to know whether or not a water passage is present from inside the fissure to the outside of the stone. An inspection of mass chromatograms in figure 2 shows that for group A stones the ratio of m/z 32 (O_2) at 5.0 min of retention time to m/z 28 (N_2) at 5.7 min was 1:4, which means the gas in the fissure showed a similar composition to air. For group B stones there was a little N_2, but no O_2 under the same attenuation, due to the low content of air. The air volume based on the N_2 peak for group A stones (7.75×10^{-2} µl/mg) is about 10 times as much as that for group B (0.80×10^{-2} µl/mg), and no trace of air was found in group C. It seems reasonable to interpret these findings as showing that atmospheric gases can enter the fissure from the outside when the stones are exposed to air.

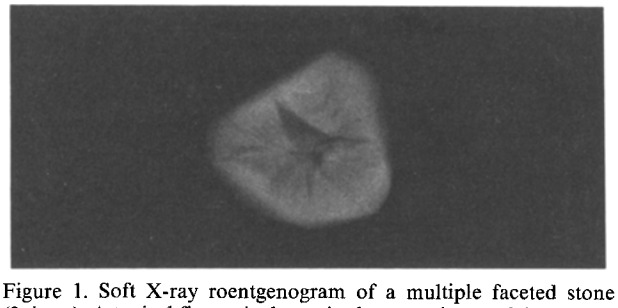

Figure 1. Soft X-ray roentgenogram of a multiple faceted stone (3 times). A typical fissure is shown in the central part of the stone.

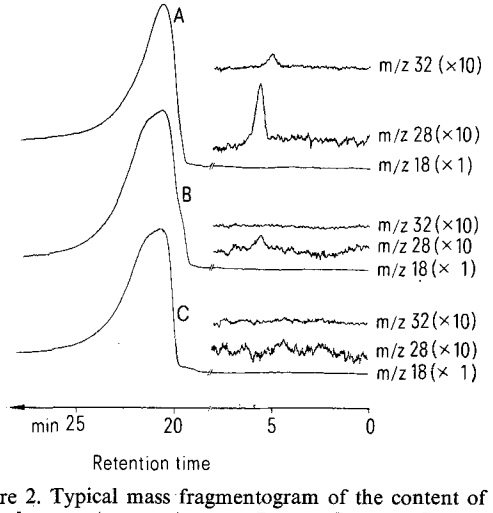

Figure 2. Typical mass fragmentogram of the content of multiple faceted stone. *A* group A stone; *B* group B stone; *C* group C stone. Values in parenthesis, amplification factors.

A constant quantity of water, m/z 18 (H_2O) at 20.9 min of retention time, is found in stones of all groups. The water volume for each group is on the average as follows: group A, 3.73×10^{-3} µl/mg; group B, 3.83×10^{-3} µl/mg; group C, 4.35×10^{-3} µl/mg. Since most of the water which is detected probably originates not from water in the fissure, which seems to be only a minor constituent, but from cholesterol monohydrate[8,9] which is a major constituent of cholesterol stones, there is no marked difference in quantity of water between the fissure group on the one hand and the non-fissure group on the other. However, the difference between group C stones and those in the other 2 groups suggests that the fissure in the stone contains some pure water in vivo.

The importance of gall bladder mucin in the formation of gallstones has long been recognized. Recently, Lee et al.[10,11] observed that cholesterol monohydrate is formed in the mucin gel secreted from the gall bladder wall, and they postulated that the stone cannot be formed without the mucin gel, namely, the gel participates. A plausible pathogenesis of the multiple faceted stone and the fissure, therefore, is considered to be as follows. A marked increase in gallbladder mucin secretion parallels the enrichment of bile with cholesterol. Gelation of mucin precedes the coprecipitation of cholesterol liquid and solid crystals with other constituents to form a pre-stone[12] in the mucin gel-like matrix. Cholesterol crystal nuclei form at the interface between the mucin gel and the surrounding bile and grow successively inwards. The pre-stone may exclude most of the water present, but some is utilized for cholesterol crystal water, and/or is retained in the form of internal water in the pre-stone. Eventually, the water is extruded, thus forming the fissure. It is likely that these processes proceed with great rapidity[13]. If very little internal water remains in the pre-stone, fissures may not be formed. Whether or not a multiple faceted stone possesses internal fissures seems to depend on the amount of water which remains trapped during the formation process of the stone.

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