CHEMICAL AND CRYSTALLOGRAPHIC STUDIES ON 33 CASES OF CALCIUM CARBONATE GALLSTONE (SO-CALLED LIMY BILE)


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Summary

Chemical analyses by atomic absorption spectrophotometry and crystallographic studies by the X-ray powder diffraction method and infrared spectrometry (KBr-disk method) were made on 33 cases of calcium carbonate gallstone or so-called limy bile.

Chemically, calcium carbonate was the major constituent, ranged from 33.7 to 91.6% and averaged 77.8%.

Crystallographically, calcium carbonate has three different polymorphic crystalline forms; calcite, aragonite and vaterite. In nature the most stable calcite (hexagonal) is most commonly found and aragonite (rhombic) is next. On the other hand vaterite, which is unstable hexagonal modification, rarely occurs in biological systems.

But in our gallstone series in man, aragonite was most commonly found, with an occurrence rate of 90.6%, while that of calcite was 62.5%. Even vaterite was found in 28.1%. Moreover three cases contained all three forms of calcium carbonate polymorphs; calcite, aragonite and vaterite. This was a very unusual condition.

Some environmental factors controlling the growth of these crystals, such as specificity of the bile, are suggested.

Key Words: Aragonite, Calcite, Calcium carbonate gallstone, Limy bile, Vaterite.

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Chemical

- dried in electric oven at 105°C

Crystallographic

- infrared spectroscopy (KBr-disk method)
  - powder X-ray diffraction analysis (CuKα, Ni-filter)
  - calculation of crystalline compositions, by Davies and Hooper's method
  - analysed by atomic absorption spectroscopy
  - Ca²⁺

Fig. 1. Methods of analysing the calcium carbonate gallstones.

**Materials and Methods (Fig. 1)**

These 33 calcium carbonate gallstone (limy bile) specimens were obtained at cholecystectomy at our surgical department and other affiliated hospitals.

Specimens were washed with deionized distilled water, air-dried, pulverized and desiccated to a constant weight in an oven at 105°C.

For chemical analysis, calcium was determined with a Shimadzu AA-646 atomic absorption spectrometer after ashing the stone powder in an electric muffle furnace at 550°C for one hour.

For qualitative analysis or infrared spectroscopy, 1 to 2 mgs of desiccated stone powder was finely ground with an agate mortar and pestle, then mixed and reground with 200 mgs of spectral grade potassium bromide (KBr). The resulting fine homogenous powder was placed in a stainless steel die, vacuumed and pressed at 400 kgs per square centimeter for eight minutes. A spectrum was obtained with a Hitachi-295 infrared spectrometer.

For quantitative or crystallographic analysis, the X-ray diffraction method was performed on finely powdered samples of 100 mgs using a nickel-filtered CuKα radiation with Shimadzu VD-12 X-ray diffractometer.

For the determination of the calcite, aragonite and vaterite ratio, Davies’ method (2) by X-ray diffraction techniques was applied. The reference materials were duck's egg shell for calcite, coral for aragonite. Vaterite was experimentally precipitated by dropwise addition of calcium chloride (2 M, CaCl₂) to sodium carbonate (2 M, Na₂CO₃) as described by Lowenstam (3). The characteristic spectral peaks used for the determination of each crystalline forms were 3.04 Å (104) for calcite, 3.40 Å (111) for aragonite and 3.58 Å (110) for vaterite respectively. The calibration curves were constructed by mixing reference calcite and aragonite in different proportions, measuring the integrated intensity of calcite : aragonite ratio: IC/(IC + IA), plotting these values against weight percent calcite. Then the calcite : vaterite calibration curve was also constructed in the same way (Fig. 2).