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Choledocolithiasis. Scanning Electron Microscopy and Infection

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ABSTRACT

A scanning electron microscope study was made of 23 gallstones from the human common bile duct. A number of crystalline structures were observed, including not only cholesterol in its hydrated and dehydrated forms, but also calcium palmitate, different calcium carbonate polymorphs, calcium phosphate and calcium bilirubinate. Emphasis is placed on the high incidence of pigment gallstones observed, within which were seen a great many casts occupied or previously occupied by germs such as E. coli, Enterococcus, etc. This lends support to the idea that germs play an important role in the formation of pigment gallstones. Other data, including the appearance of whiskers or the presence of a number of calcium carbonate polymorphs, seem to indicate that accelerated growth processes may take place. Regarding pathogeny, infection and stasis (both possibly coinciding) are fundamental to the development of pigment gallstones.

Key words: Gallstones. Scanning electron microscopy. Infection. Stasis.

INTRODUCTION

The study of primary or secondary gallstone formation in the common bile duct reveals a marked incidence of pigmentary lithiasis, as confirmed by X-ray diffraction (1), and a diminished cholesterol concentration.

Infectious factors seem to be important in the origin of pigmentary lithiasis, and may determine certain clinical conditions, including postoperative infectious problems. In this sense, Maki in 1966 demonstrated that β-glucuronidase secreting bacteria are able to precipitate calcium bilirubinate due to the agglomeration of bilirubin glucuronide; brown gallstones form as a result, contrary to black stones, which are related to hemolytic processes.

In the present study we examined human gallstones with the scanning electron microscope, in an attempt to correlate composition with the incidence of germs within the stones.

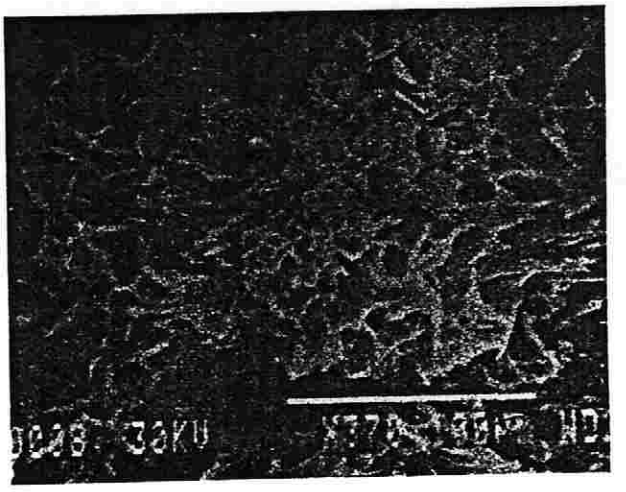

Fig. 1. Calcium palmitate under SEM. x370.

MATERIAL AND METHODS

A JSM-820 scanning electron microscope (SEM) affording a magnifying range of x10-30,000 was used to examine 23 gallstones removed by laparotomy from the common bile duct, or by retrograde pancreatic cholangiography.

Fig. 2. Polymorph form of calcium carbonate (calcite). x3700.

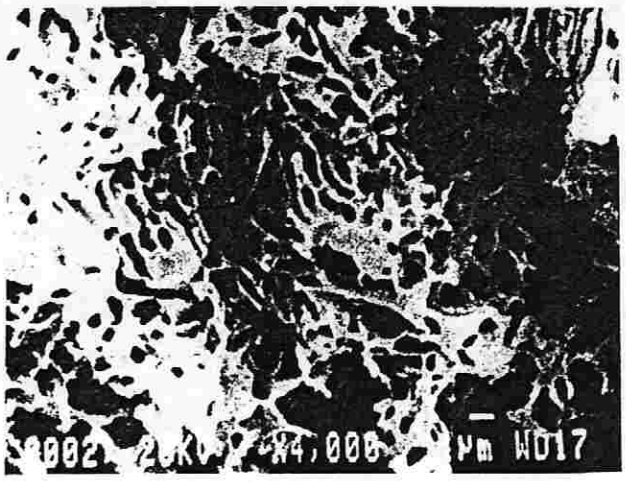

Fig. 3. Stone interior showing numerous bacterial casts devoid of bacteria. x4000.

The stones were washed in water and dried in an oven at 60°C for two hours. Once dry, they were cut in halves, one half being used for the SEM study. The specimens were gold sputter-coated to 25 nm thickness to turn them into conductors and thus avoid electrical charging by the electron beam.

RESULTS

Of the 23 gallstones from the common bile duct examined by SEM, the presence of cholesterol hydrate was established in 73%, preferably at nuclear level, but sometimes also on the surface. Cholesterol hydrate was easily recognized by its presentation in the form of needle-like slab-form crystals, frequently detached from each other at their most intricate or developed surfaces. Crystal shape was in the form of monoclinic crystals. On other occasions these crystals were detected isolatedly, or piled up in close proximity to the joining points. Less frequently, they appeared as needle or fiber-like shapes.

Hydrated cholesterol could not be detected in 6 stones; in four of these, not even X-ray diffraction was able to identify cholesterol hydrate. In the others, it was detected by diffraction, but only in minute quantities, as reflected by the peak heights in the diffractogram.

Dehydrated cholesterol is somewhat more difficult to detect; nevertheless, it was found to be present in 47% of the stones. It offers no uniform appearance, and may be seen in needle form, or as rosettes with warped laminas that frequently form crossed intersections. Dehydrated cholesterol was mainly encountered in the intermediate surface region of the section, and near the periphery. Its presence could not be demonstrated in 12 stones.

Calcium palmitate was detected in 30.4% of the specimens investigated, and was identified without difficulty. Calcium palmitate normally appears in the form of curved crinkled leaves, generally in the core or its immediate vicinity, and never in the shell or external surface region. This component lost its form as a result of contraction during the study, because of the heat generated by the electron beam - thus reflecting its lipidic character. The presence of calcium palmitate was confirmed by X-ray diffraction in all cases (Fig. 1).

It proved more difficult to identify any of the three polymorph forms of calcium carbonate. Nevertheless, they were encountered in 30.4% of the stones examined. Generally, calcium carbonate was found as rounded, egg-shaped forms with a smooth surface and lying in the midst of the hydrated cholesterol crystals. Such structures could correlate with vaterite crystals. Occasionally, romboid crystals with superimposed hexagonal bases were seen, piled up in a way suggestive of calcite. Finally, in two cases, the crystals took on the appearance of polyhedron-formed agglomerates. Aragonite was not observed (Fig. 2).

These findings agree with the X-ray diffraction observations, and with the exception of a single case, the structures appeared either in the core or very close to it.

Calcium phosphate is thought to have been detected in three cases as rounded or spherical forms with some irregularities, and located peripherally to the nuclear region. This could feasibly be whicklokite. On one occasion calcium phosphate was seen in laminar form.

Calcium bilirubinate formations were seen in the form of a network or matrix exhibiting trabecular structures. Diffraction in turn confirmed the presence of calcium bilirubinate.

The pigmentary network exhibited a great many elongated depressions approximately 0.5-1.0 µm in width and 4-5 µm long, and arranged in close proximity to each other not only in the core region but also in its immediate surroundings. SEM revealed a few elongated, wormy calcified objects 0.5-4 µm in size within the cavities. These may have been calcified microorganisms; this hypothesis was later confirmed by cultures of pieces removed from the common bile duct, where different types of Enterococci were isolated (Fig.s 3,4).

SEM was unable to identify either colic acid or calcium oxalate or montmorillonite - all of which were detected by diffraction. At this point it should be mentioned that "whiskers" were frequently observed either on the gallstone surface or in its section, these components being often bizarre is shape and structure.

Generally, the whiskers were arranged as hollow or tubular filaments or thin wires; at high magnification

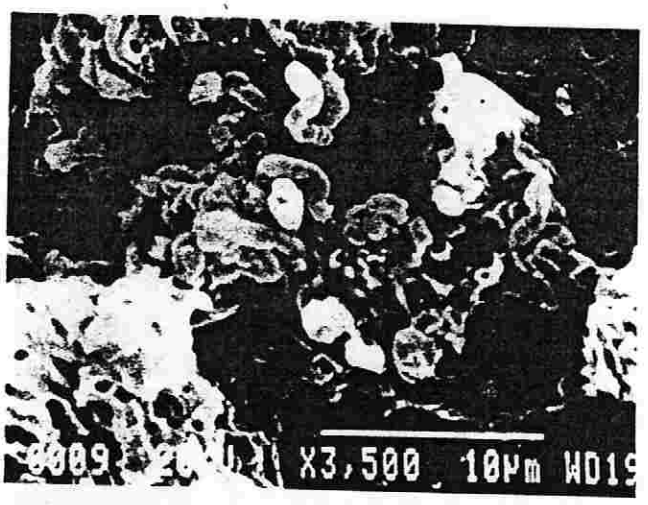

Fig. 4. Stone interior showing numerous bacteria. x3500.

crystals were seen both inside them and on the surface. A well-developed crystal was often noted at one extreme end of the structure.

In three cases suture material was identified within the gallstones, respresenting remains from previous surgical operations.

DISCUSSION

A number of different crystals were observed in the present study, generally in correspondence with those detected by X-ray diffraction. However, such correlation was not encountered in all cases, possibly as a result of the limited fields of vision inherent in SEM examination.

Both forms of cholesterol - hydrated and dehydrated - were usually seen, often simultaneously within the same gallstone. Their characteristic forms of crystallization made identification simple (2).

At low magnification, calcium palmitate appears as white salt particles spread out on the section surface or positioned externally; generally this component clearly contrasts within the pigmentary area of the nucleus or its immediate vicinity. Under SEM, calcium palmitate appears in the shape of curved, crinkled leaves, which other authors have described as whirls (3). In no case did we perform previous ether washing (3) to eliminate the cholesterol crystals and thus enhance calcium palmitate visibility.

The calcium carbonate polymorphs were more difficult to identify. Thus, aragonite could not be detected. Vaterite was more commonly seen, and showed a spherical shape with a smooth white surface. This appearance might be due to solution oversaturation with calcium carbonate (4), which causes an increase in the grouping rate of growth units with a simultaneous decrease in carbonate group orientation capacity. A disorder in the accumulation process results. Such a situation cannot be assimilated by structures as well arranged as calcite; in the case of vaterite, it has even been suggested that growth in impure media is possible in the presence of extraneous ions (5). In quite a few cases, when examining the nuclear area, two different crystal types were identified (as described above). A number of authors claim that the spherical shapes might correspond to wicklokite, while the laminar structures would represent apatite (6). However, this could not be definitely established due to the lack of Edax.

Of considerable interest in the present study was the observation of calcium bilirubinate aggregates in which sponge surface-like depressions were noted. These depressions or canals always appeared in the brown and black stones. Other authors (2,7,8) refer to these structures as "casts" and define them as the beds or "graves" of previously contained microorganisms. In the case of pigmented or composite gallstones, the incidence of these cavities has been rated at up to 86% (7,8). In turn, the bacteria encountered have been described as being similar to those detected in the present study (9).

The presence of bacteria within pigmented gallstones lends support to Maki's theory (10) regarding bilirubinate formation, and the idea that germs play an important role in the development of pigmented stones. On the other hand, the fact that bilirubinate is usually associated with the presence of palmitate speaks of the relevance of phospholipases - also produced by germs - in the formation of palmitate.

The microorganism usually encountered was E. coli, although other bacteria including Pseudomona aeruginosae, Klebsiella pneumoniae, Enterococci and gastrointestinal floral elements in general have also been reported (2).

These bacterial casts have not been seen in the cholesterol or calcium carbonate stones, or in those parts of composite stones in which these substances accumulate (although they were often noted at stone surface level)(7).

As regards the interpretation of the crystalline forms described as whiskers, these structures have been described as common in crystalography, and calcium in the form of vaterite has been claimed to exhibit an accelerated growth process - in this case in a single preponderant direction, and generally under atypical circumstances. These whiskers were not identified as such in gallstones or in amorphous pigmentary areas, but in cholesterol crystals and, less frequently, on calcium carbonate crystals.

The presence of extraneous matter in some gallstones points to the use of non-absorbable material in ligating the cystic duct or suturing the common bile duct, thus clearly reflecting the need for employing absorbable material.

In view of the SEM results obtained, it may be concluded that infection and stasis (probably simultaneously) play an important role in the formation of pigmented gallstones; however, other factors are probably also involved, including the presence of extraneous material as described above, and advanced patient age (even though the age range in our study was considerable).

The existence of such a correlation between gallstone formation and infection lends support to reports in the literature, according to which acute cholangitis is four times more common in patients with pigment gallstones than with cholesterol stones (11). Thus, gallstones may serve to protect bacteria against endogenous antibacterial factors.

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