The morphology of salivary calculi
A scanning electron microscopic study

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Salivary calculi are a common disorder in the submandibular gland duct system, but the etiology and pathogenesis of the concrements remain unclear. Ten submandibular gland calculi were examined in a scanning electron microscope after critical-point drying, fracturing, and gold coating. The surface of the calculi was built up of numerous knobs covered by a smooth and occasionally filamentous substance, probably of mucinous origin. Microorganisms were commonly found at the surface. The nuclei of the calculi, which were rather homogeneous, were surrounded by lamellated structures. Between the lamellae spheroid bodies, 20–100 μm in diameter, were present. These spheroid bodies are probably of mucinous origin, which together with an amorphous granulated substance builds up the matrix. Microorganism-like structures occasionally appeared at the interface between lamellae. Microorganisms can be an important factor by providing an organic matrix in the later phase of the pathogenesis of salivary calculi. However, the nuclei did not show morphological evidence of any particular factor explaining the etiology of salivary calculus. □ *Microorganisms; pathology; salivary glands; structure*

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Salivary calculus, which is a concretion formed in the salivary gland duct system, is one of the commonest disorders affecting salivary glands (1). The pathogenesis of salivary calculus has for a long time been debated in the literature. Fungi (2, 3), bacteria (4, 5), or foreign bodies (6, 7) have been suggested as provoking factors in the formation of the concretion. It has also been proposed that desquamated epithelial cells form a nidus, acting as a focus for the development of the calculus (8, 9). Using electron microscopy, Vahl et al. (10), Höhling et al. (11), and Anneroth et al. (12) found bacteria mineralized to various degrees in the peripheral part of salivary calculi, whereas no bacterial structures were present in the central part. Spheroid bodies with low electron density, which had a diameter varying from 1 to 100 μm, were also a common finding. These bodies were assumed to be composed of a mucoprotein substance (12).

Despite extensive research, the knowledge of salivary calculi is incomplete, and contradictory opinions about the pathogenesis exist. The aim of the present investigation was to study the morphology of salivary calculi by scanning electron microscopy to elucidate further the complex background of the concretions.

Materials and methods

Ten submandibular duct salivary calculi from 10 patients were examined. Immediately after surgery the calculi were put in 10% neutral buffered formalin with an effective osmolality of 300 mosM (Histofix®).

The calculi were prepared for examination with a scanning electron microscope (SEM) by the critical-point drying method (13). The drying made the calculus very brittle and therefore easy to split with a scalpel, thus creating a fracture surface throughout all levels of the specimen. It was then possible to examine the peripheral surface of the calculus as well as the fracture surface.

After being dried and fractured, the specimens were glued to specimen holders and coated with a 50-nm-thick gold layer by the sputter technique (Polaron®). SEM was per-

formed with an accelerating voltage of 15 kV (Philips 501). After viewing the concretions in the scanning electron microscope, fragments of special interest were carefully dissected out from two calculi. The fragments were then embedded in a self-curing resin (Epon 812®), cut in 1-µm sections, stained with toluidine blue, and examined in a light microscope.

Results

A feature the examined calculi had in common was a homogeneous nucleus surrounded by lamellae (Fig. 1). About one third of the diameter of the calculus was occupied by the nucleus and two thirds by the lamellar structures.

A general image of the calculus surface at low magnification was the presence of numerous 200-µm- to 3-mm-wide knobs (Fig. 2). Fractured knobs consisted of a granular mineralized substance and also of 20- to 100-µm-wide spheroid bodies. The surface of the calculi was usually covered by a smooth film of organic character (Fig. 2). On the surface of all specimens, 0.5–1.0-µm-wide, rod-like and spherical structures appeared similar to bacterial elements in different stages of mineralization (Fig. 3).

The lamellae often appeared heterogeneous in shape and size, and adjacent lamellae were usually separated from each other, making it possible to examine the interface. Spheroid bodies with a size of 20–100 µm often dominated the surfaces of the exposed lamellae. In these instances, the facing lamella showed distinct spheroid impressions (Figs. 4 and 5). The fracture usually followed the surface at the spheroid bodies. In one specimen, however, some spheroid bodies were fractured, disclosing a fibrous interior texture (Fig. 6). The toluidine blue-stained

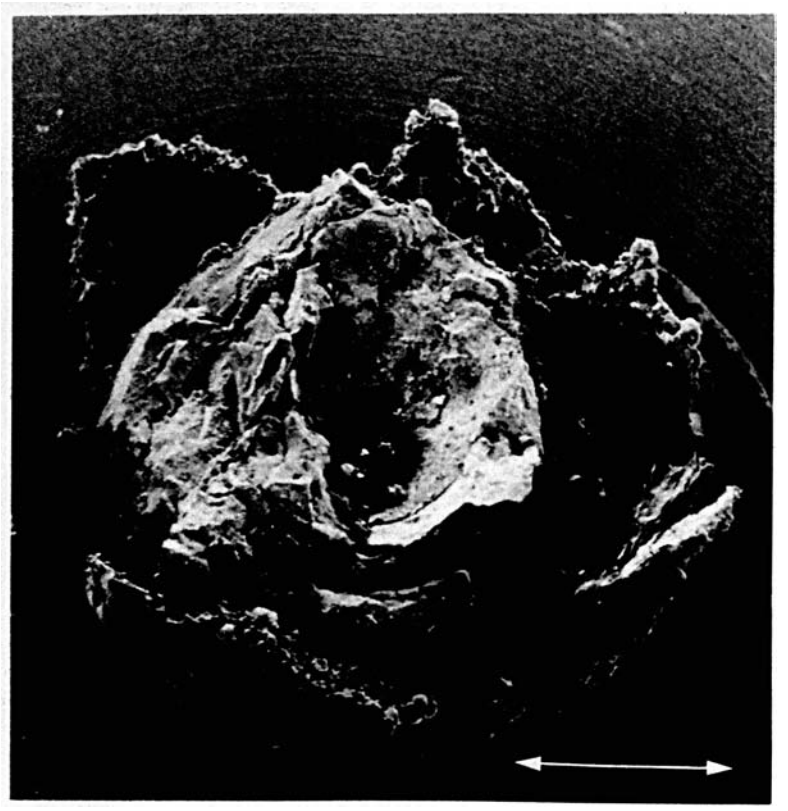

Fig. 1. The typical calculus was composed of a homogeneous nucleus (N) surrounded by lamellae (L). Arrows equal 1 mm.

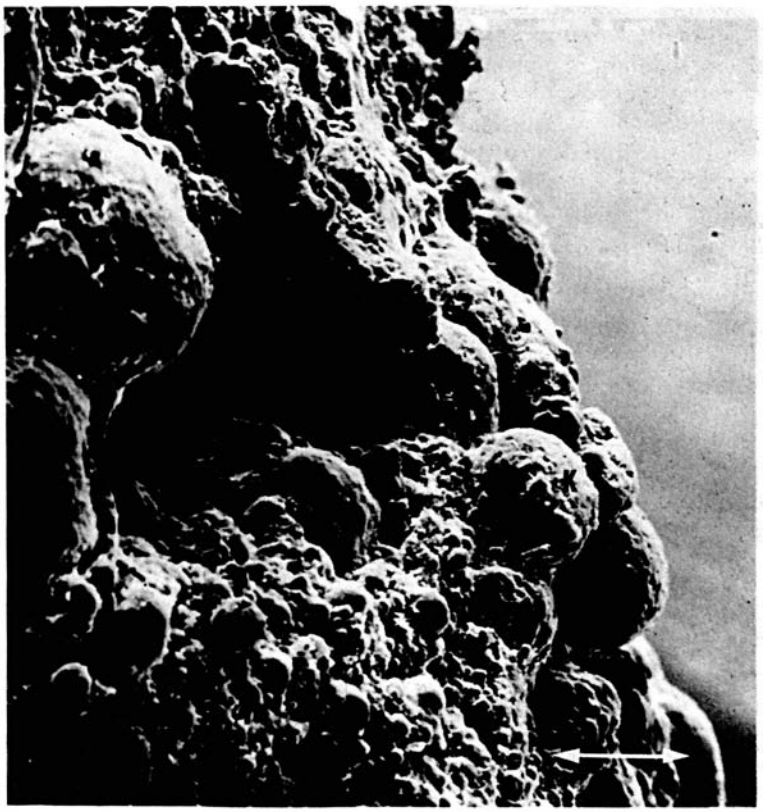

Fig. 2. The surface of a calculus composed of knobs (K) covered by mucinous-like film. Arrows equal 200 μm.

sections exhibited large, stained, spheroid bodies corresponding to the SEM findings.

Parts of the interface between lamellae of many calculi were dominated by the appearance of approximately 0.5-μm-wide, rounded structures resembling cocci (Fig. 7).

The nucleus always had a smooth or finely granulated fracture surface, which appeared to be mineralized (Fig. 8). No microorganism-like structures appeared in the nucleus. Structures resembling mammalian cells were not seen in the examined specimens.

Discussion

The results of the present study support many previous reports with regard to salivary calculi lamellation, nucleus formation, and the presence of microorganisms. The lamellated pattern found in salivary calculi has been thought to represent the morphological expression of a rhythmical deposition of material or a so-called Liesegang's phenomenon (14). In the present study the drying procedure probably caused the separation of lamellae, which made it possible to examine the lamella interface.

Microorganisms have been reported to occur in the intermediate and peripheral parts of calculi (11, 12, 14–17) but not in the nucleus, the oldest part of the concrement. These findings are confirmed in the present study, in which microorganism-like structures were found throughout the peripheral two thirds of the specimens. However, exceptions have been described (18). This peripheral localization might be explained by a secondary infection due to stasis in the excretory ducts, favoring a retrograde infection (16). Microorganisms are therefore presumably not usually present in the initial formation of the salivary calculi, as suggested

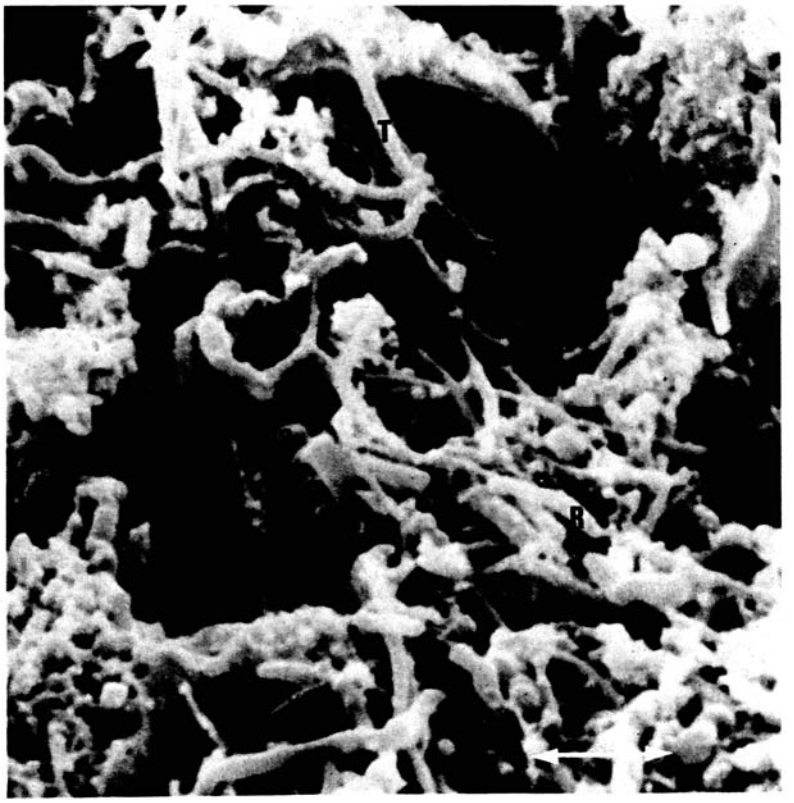

Fig. 3. On the surface of a calculus streptococcus-like (S) and rod-like (R) structures appeared together with a shrunken organic film with a thread-like character (T). Arrows equal 2 μm.

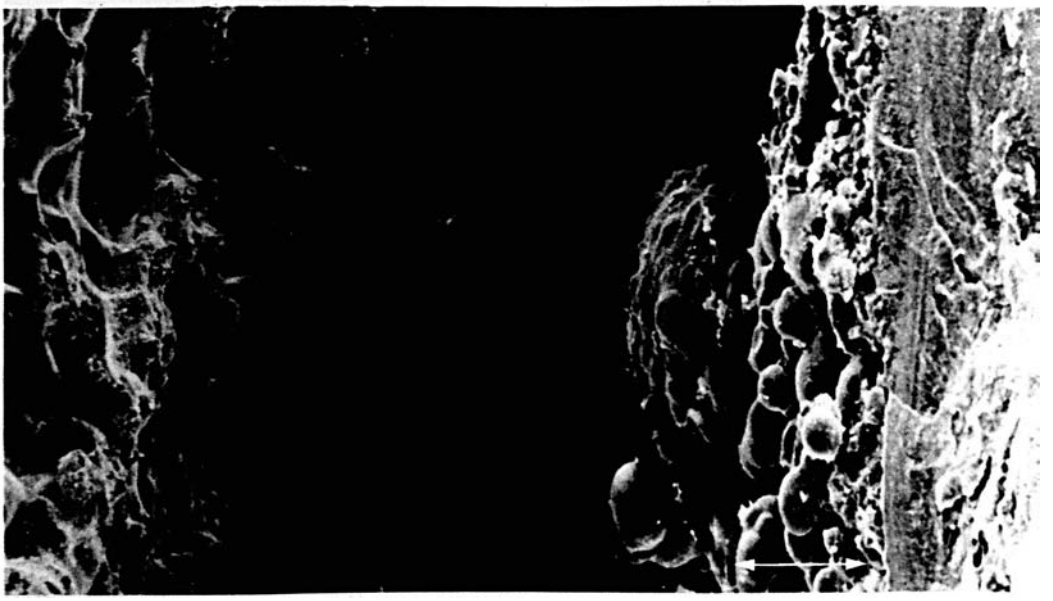

Fig. 4. In the intermediate part of a calculus two lamellae are separated from each other, disclosing spheroid bodies (S). Facing lamella shows obvious spheroid impressions (I). Arrows equal 250 μm.

Fig. 5. Detail of the spheroid bodies (S) shown in Fig. 4. The shrunken appearance is probably caused by the preparative dehydration. Arrows equal 50 μm.

Fig. 6. Fractured spheroid bodies showing a fibrillar and apparently non-mineralized substance. The matrix surrounding these spheroid bodies has a fine, granulated, calcified appearance. Arrows equal 45 μm.

Fig. 7. A lamella in the intermediate part of a calculus covered with sphere-like structures similar to bacteria. Arrows equal 2 μm.

by Vahl et al. (10) and Höhling et al. (11). However, the possibility that microorganisms are hidden in the compact material of the nuclei cannot be entirely excluded.

The spheroid bodies, which may be present in most parts of the calculus, with exception of the nucleus, are smooth and have a low density when viewed in the transmission electron microscope (11, 12, 17). Höhling et al. (11) assumed that the spheroid bodies were composed of a mucoprotein substance originating from dissolved bacterial plasma. However, the size of the spheroid bodies does not correspond to that of bacteria, and no morphological likeness has been reported. Hiraide & Nomura (19), in a SEM study, interpreted the spheroid bodies as crystalline structures. The observations contradict the results of the TEM study by Anneroth et al. (12), who reported that these bodies became mineralized at a very late stage in the concretion mineralization. The spheroid bodies probably correspond to mucin aggregates of saliva origin (12).

The fibrous-looking fracture surface of spheroid bodies was obviously not crystalline but rather a mucinous one, made brittle by the specimen drying procedure. The spheroid bodies and the surrounding amorphous matrix have been assumed to be the so-called mucoid gel postulated by Harrill et al. (20). In this 'gel' the initial formation of salivary calculi presumably takes place. The 'gel' gradually mineralizes and grows into contact with the wall of the salivary duct and cellular debris from surrounding duct epithelium, and connective tissue contributes with organic matrix (12). However, the saliva seems to contribute with the major bulk of organic material. Microorganisms can be an

Fig. 8. In the nucleus the fracture surface had a fine granulated mineralized structure with crystallization centers with crystals radiating from a core (C). Arrows equal 5 μm.

important factor by providing an organic matrix in the later phase of the pathogenesis of the calculus (12), but bacteria can be ruled out in the etiology of the calculi.

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