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Crystalloids in salivary duct cysts of the human parotid gland

Scanning electron microscopical study with electron probe X-ray microanalysis

Yasunori Takeda[1] and Goro Ishikawa[2]

[1] Department of Oral Pathology, School of Dentistry, Iwate Medical University, Uchimaru 19-1, Morioka, Iwate 020
[2] Department of Oral Pathology, Faculty of Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

Summary. Crystalloids found in salivary duct cysts of the human parotid gland were examined by scanning electron microscopical observations with electron probe X-ray microanalysis. The cystic spaces were filled with numerous crystalloids which had a variety of forms with slight eosinophilic and glassy appearance. Scanning electron microscopically, crystalloids were hexagonal and rhombohedral in shape, and cutting the surface showed a polycyclic structure or regular parallel lamination. By electron probe X-ray microanalysis, sulphur was the only detected element. The present study suggests that crystalloids resulted from deposition from supersaturated saliva containing sulphur containing compounds into the cystic lumen or into epithelial cytoplasm.

Key words: Crystalloids – Salivary duct cyst – Parotid gland – Scanning electron microscope – X-ray microanalysis

Crystalline structures (usually referred to as crystalloids) in the duct lumen have occasionally been found in routine postmortem sections of normal parotid glands (Thackray and Lucas 1974) and in cystic lesions of the parotid glands (Seifert and Waller 1982). These crystalloids are eosinophilic and variable in shape and size, but their nature and the mechanism of crystallization are poorly understood. This paper reports the results of scanning electron microscopical examination of crystalloids in the salivary duct cysts of the parotid gland with electron probe X-ray microanalysis in an autopsy case.

Materials and methods

In an autopsy case of 61-year-old female who had been suffering from pulmonary carcinoma with widespread metastases, a small ($2 \times 2 \times 3$ mm) salivary duct cyst containing numerous crystalloids in its cystic spaces was found in the right parotid gland. For the histopathological

Offprint request to: Y. Takeda at the above address

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study of the crystalloids, serial sections were made and the following stains were employed: hematoxylin and eosin, periodic acid-Shiff, Prussian blue, von Kossa, Alcian blue (pH 0.4, 1.0, 2.5), mucicarmine, toluidin blue, orcein, van Gieson, Congo red and phosphotungustic acid-hematoxylin. The specimen was also examined with polarized light.

For scanning electron microscopical examination, 5 μm thick formalin fixed and paraffin-embedded unstained tissue sections were placed on graphite specimen mounts, and were deparaffinized in xylene and dehydrated in alcohols. The specimens were coated with carbon, and were viewed in Hitachi 430 and 560 scanning electron microscopes fitted with an electron probe X-ray microanalizer.

Results

Histological findings

The epithelial element consisted of columnar to cuboidal cells arranged in a single- or double-layer which sometimes showed papillary projections into the cystic spaces. These epithelial cells had fine granular and pronouncedly eosinophilic cytoplasm and round vesicular nuclei. An outermost layer of cells disposed like myoepithelial cells were present, in part. In the stroma, there was dense accumulation of lymphoid cells, but lymph follicles and other inflammatory cells were not found. The lesion was partly surrounded by thin fibrous connective tissue capsule. The surrounding parotid gland tissue showed marked atrophy with fatty replacement and very mild lymphoid cell infiltration. The cystic spaces were filled with numerous crystalloids which had a variety of morphological forms including granular, rod-like, square and polyhedral shapes with a slight eosinophilic and glassy appearance (Fig. 1a). Some of these crystalloids fused together forming large masses. A few tiny crystalloids were located within the linning epithelia (Fig. 1b). There were no inflammatory cells in any cystic spaces. These crystalloids were unstainable with following stains: periodic acid-Shiff, Prussian blue, von Kossa, Alcian blue (pH 0.4, 1.0, 2.5), mucicarmine, toluidin blue, orcein, van Gieson, Congo red and phosphotungustic acid-hematoxylin. Birefringency was not observed, by polarized light.

Scanning electron microscopical findings

Crystalloids were divided into two types according to their size on scanning electron microscopy, i.e. they were large or small crystalloids. The former were more than 10 μm in size, and the latter less than 5 μm in size. Of course, intermediate size between them also existed. The most common shapes of large crystalloids were the hexagon and rhombohedron that show in Fig. 2a and b. Numerous small crystalloids around the large crystalloids varied in shape, and aggregated to form irregular clusters (Fig. 3a). The cutting surface of some crystalloids showed a homogeneous appearance with polycyclic structure, but an obvious central core was not found (Fig. 2a). The other cutting surface showed regular parallel lamination (Fig. 3b). On the surfaces of large crystalloids which showed lamination, various-sized tiny crystalloids were seen (Fig. 4a and b). This finding suggested new crystalloids developed from the surface of large crystalloids.

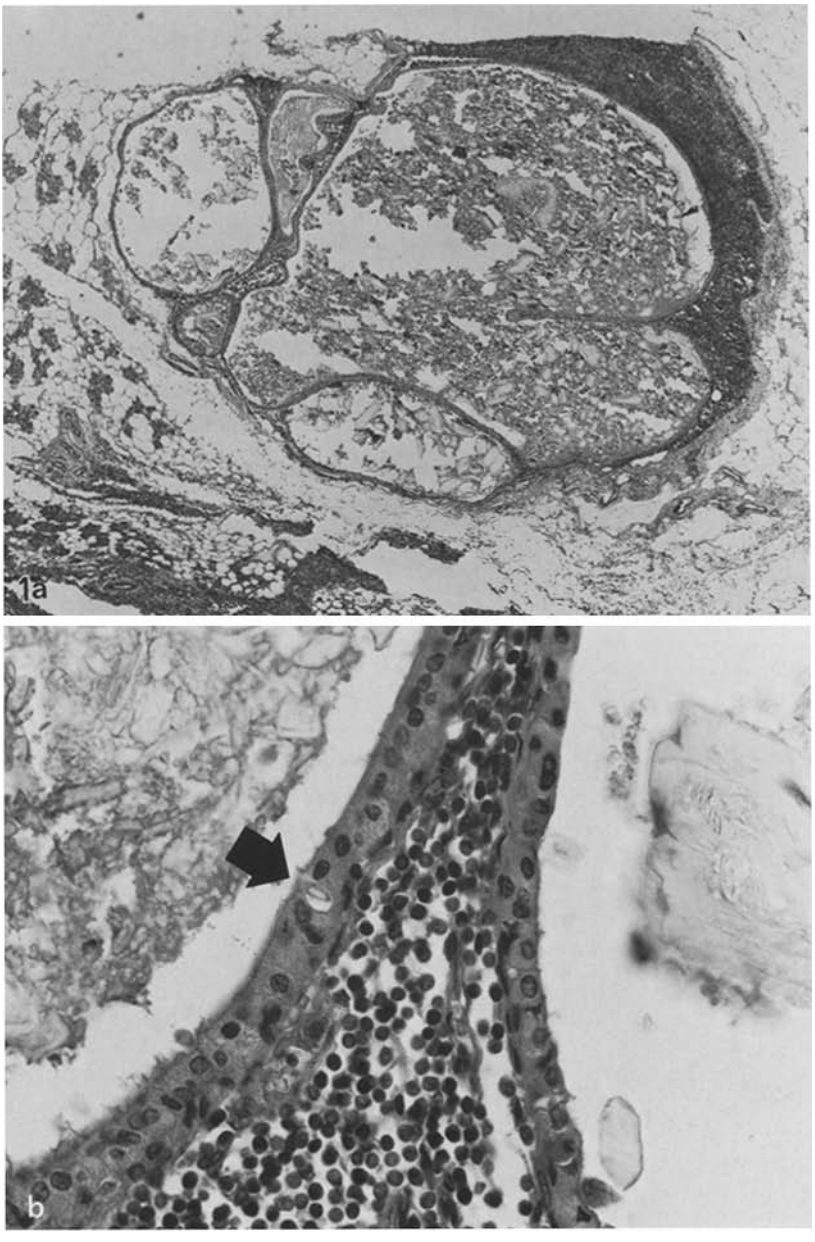

Fig. 1 a, b. Light microphotographs of crystalloids found in salivary duct cysts of the parotid gland. **a** showing whole cut-view of the specimen. **b** showing a tiny crystalloid found in lining epithelium (*arrow*). Hematoxylin and eosin, (**a**) × 35 and (**b**) × 400

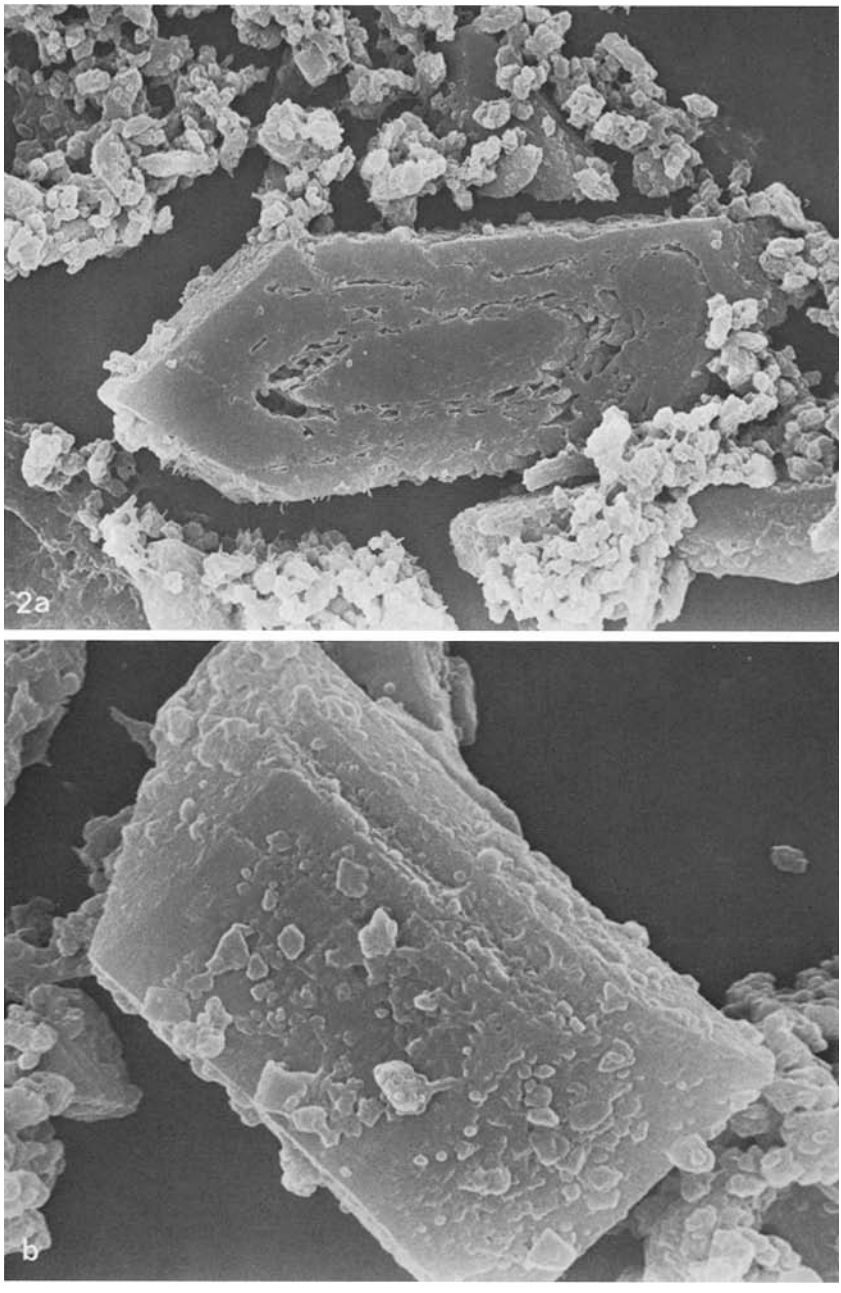

Fig. 2a, b. Scanning electron microphotographs of crystalloids. Large crystalloids are hexagonal and rhombohedral in shapes, and numerous small crystalloids surround large ones. The cut surface of a large crystalloid shown in (**a**) is polycyclic. (**a**) × 3,300 and (**b**) × 8,800

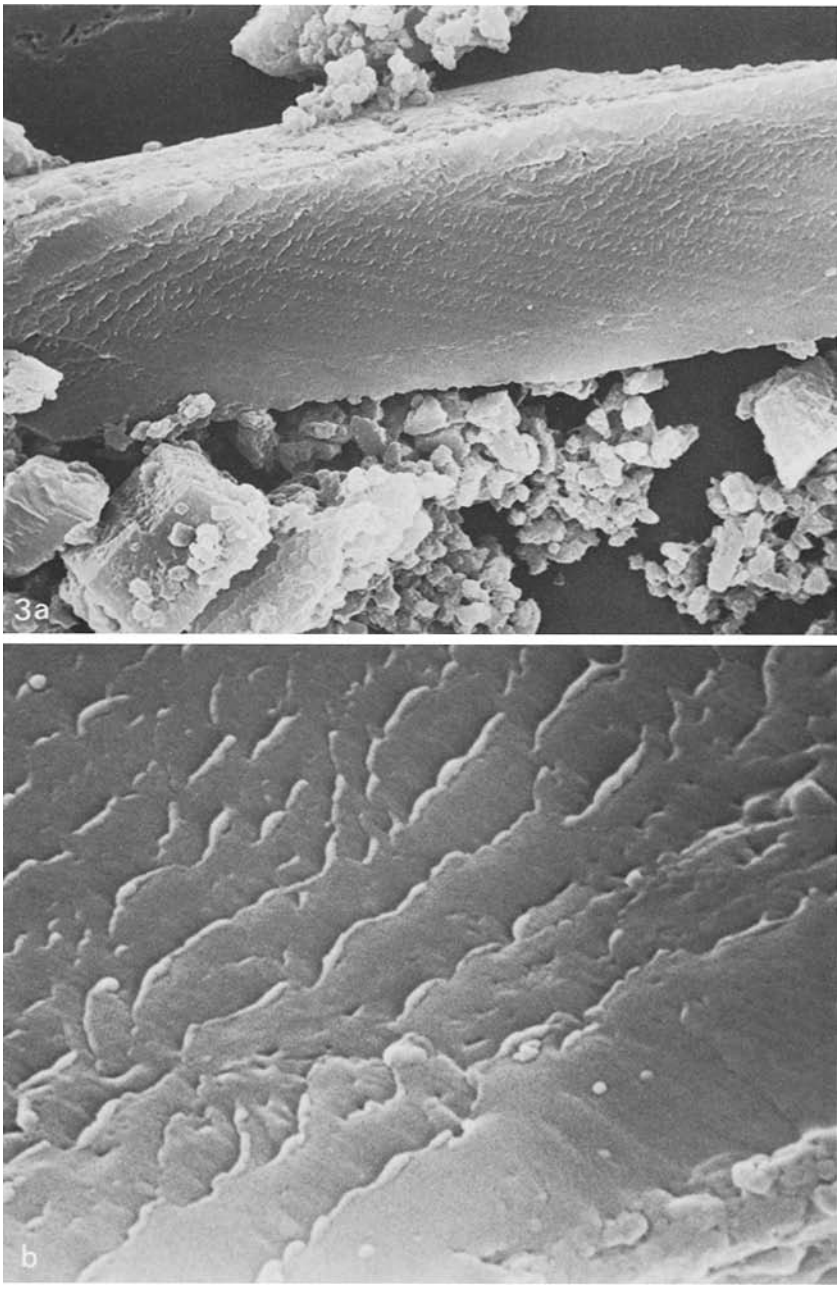

Fig. 3a, b. Scanning electron microphotographs of crystalloids. **a** showing the cut surface of a large crystalloid displaying regular parallel lamination, and numerous small crystalloids varied in shapes, around a large one. **b** is a high magnification of large crystalloid shown in (**a**). (**a**) × 4,400 and (**b**) × 22,000

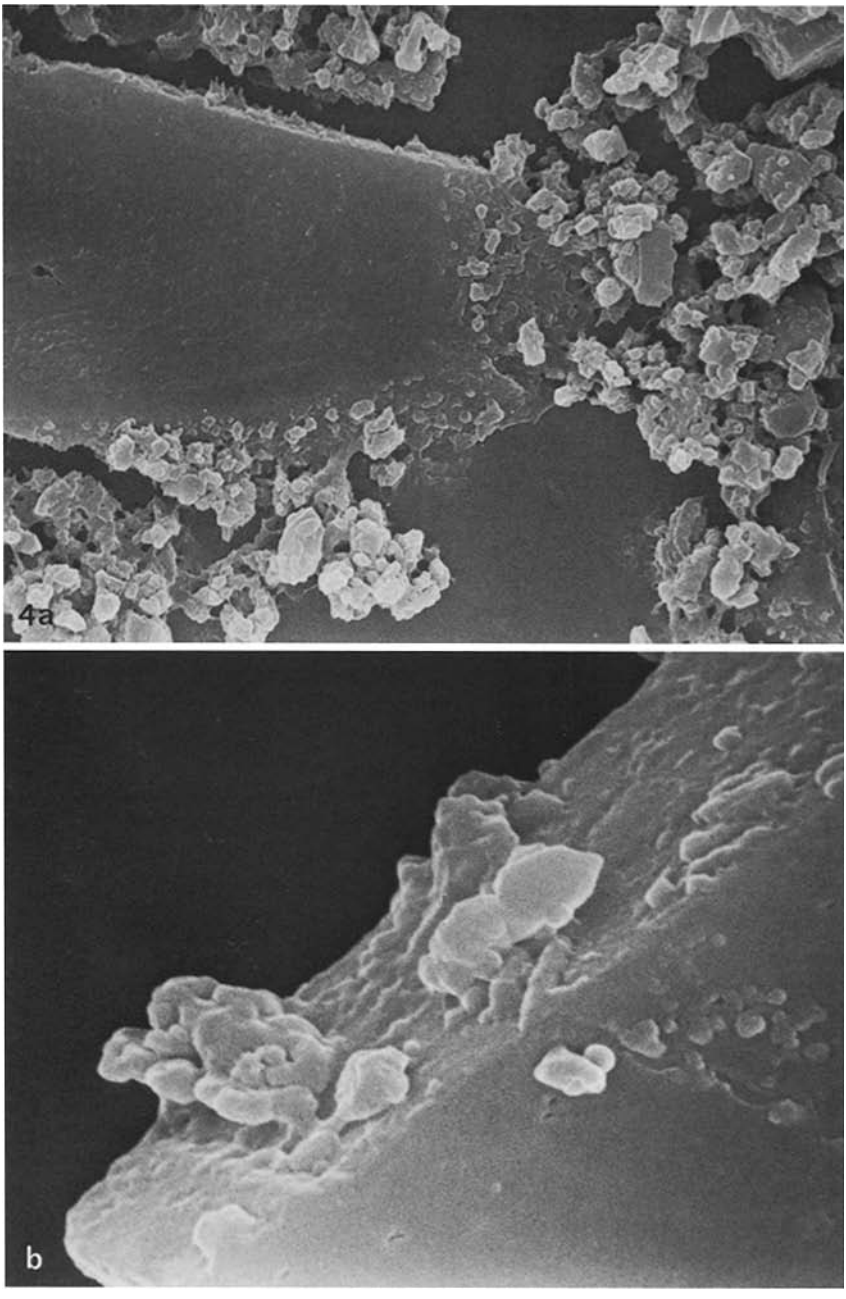

Fig. 4a, b. Scanning electron microphotographs of crystalloids. Various-sized and -shaped tiny crystalloids are seen on the surfaces of large and laminated crystalloids. (**a**) × 4,400 and (**b**) × 22,000

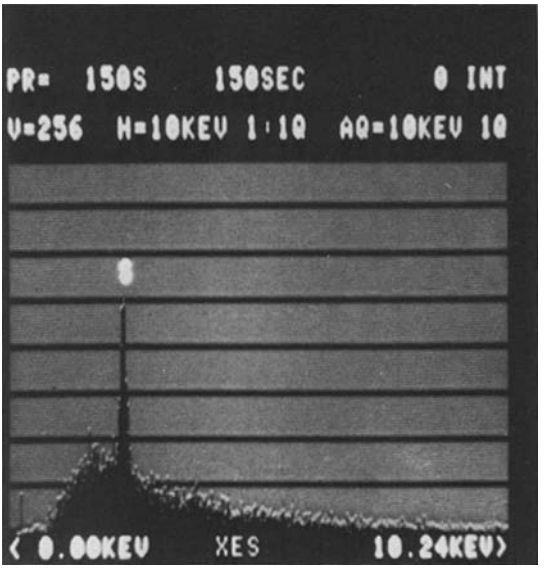

Fig. 5. Electron probe X-ray microanalysis of crystalloids. The detected element is sulphur (*S*) alone

Electron probe X-ray microanalysis

The electron probe was directed at several parts of the crystalloids, and detected element was sulphur only (Fig. 5).

Discussion

It is well known that an occasional duct containing crystalloids may be found in postmortem examination of the parotid glands, but little work has been done to study their nature and the mechanism of crystallization. Thackray and Lucas (1974) suggested that crystalloids would be found in about 5% of routine postmortem sections of the parotid glands, and commented that crystalloids were probably produced by the duct epithelial cells. Crystalloids were found rarely in parotid cystic lesions. Seifert and Waller (1982) showed crystalloids (kristallinen Sphärolithen) in the cystic spaces of parotid salivary duct cyst, and they considered those crystalloids to be derived from secretory substance (kristallinen Sekretpartikeln). If artifacts in the processing of tissue-section can be satisfactorily excluded, the results of the present study suggest that some crystalloids are produced from the epithelial cells, since a few tiny crystalloids are found in the linning epithelia by light microscope.

Although the detailed chemical nature of the crystalloids is not clarified by the present examination, it is thought that sulphur compounds are one of the main components of the crystalloids as shown here by electron probe X-ray microanalysis. The saliva is composed of various organic and inorganic components, and some of them sulphur containing compounds, e.g. thiocyanate, chondroitin sulfuric acid, thiamine, glutathione, cysteine, etc. Then

crystalloids may result from the supersaturation of saliva in the cystic lumen or epithelial cytoplasm.

On the basis of the present findings, the following hypothetical process for crystalloid growth in the salivary duct cysts is proposed. When the secretory substance is supersaturated in closed cystic spaces, single fine crystalloids appear and subsequent growth of extruded crystalloids begins. Larger crystalloids are formed in the cystic spaces in the subsequent steps; (1) Enlargement: Each crystalloid grows in the supersaturated secretory substance. (2) New crystalloid formation: On the surface of the larger crystalloids new fine crystalloids develop and grow, another new crystalloid then develops on the surface, and there is progressive increase in size and number by repeating this process. (3) Fusion of crystalloids: Single crystalloids may fuse and are then arranged in a laminated pattern. Although the mechanism of early crystallization is not obvious from the present study, two methods are conventionally used for the artificial production of crystals in vitro; the first is used when a supersaturated solution is presented with an anchored seed, the second is supersaturation of the solution by evaporation (Holden and Singer 1968). Saliva is a colloid solution or gel and experimental crystallization occurs more effectively in a gel than in solution, since gels slow down the molecular movement and favor crystal growth (Henisch 1970). It is thought that crystalloid-formation and its subsequent growth in the cystic spaces of the lesion may be contributed to and accelerated by the conditions discussed above, by a concentration gradient and by additional unknown factors.

Crystalloids in parotid cystic lesions may be formed by supersaturation of retained secretory products which contain components characteristic of the parotid saliva. Some of the crystalloids may be formed in lining epithelia which cannot release their secretory products.

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