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OBSERVATIONS ON THE ENAMEL OF ODONTOMAS

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Abstract

The morphological study of odontomas provides an alternative model for observing the formation of dental tissues, since different maturing stages are present simultaneously. Investigations were performed on decalcified samples (using light microscopy and transmission electron microscopy) and on undecalcified samples of complex odontoma enamel (using transmission electron microscopy). Simultaneous presence of prismatic enamel at various maturing stages with different structural characteristics was observed. Such enamel was sometimes associated with layers of ameloblastic cells with characteristics of cells in functional activity. In other sites, the enamel did not present a prismatic structure but it appeared as unstructured material clusters with abundant organic component. It was concluded that the theory according to which an ecto-mesenchymal inductive failure occurs in odontomas is not confirmed. The defect seen at the beginning of the differentiated and anomalous tissue maturation may be related to latest events in the development of the enamel organ. In this regard, it was concluded that such events involve the efficiency of the ameloblasts and the possible alterations in the organic matrix.

Key Words: complex odontoma, light microscopy, scanning electron microscopy, transmission electron microscopy, enamel structure, enamel prisms, ameloblasts, enamel matrix, amorphous structure, enamel maturation.

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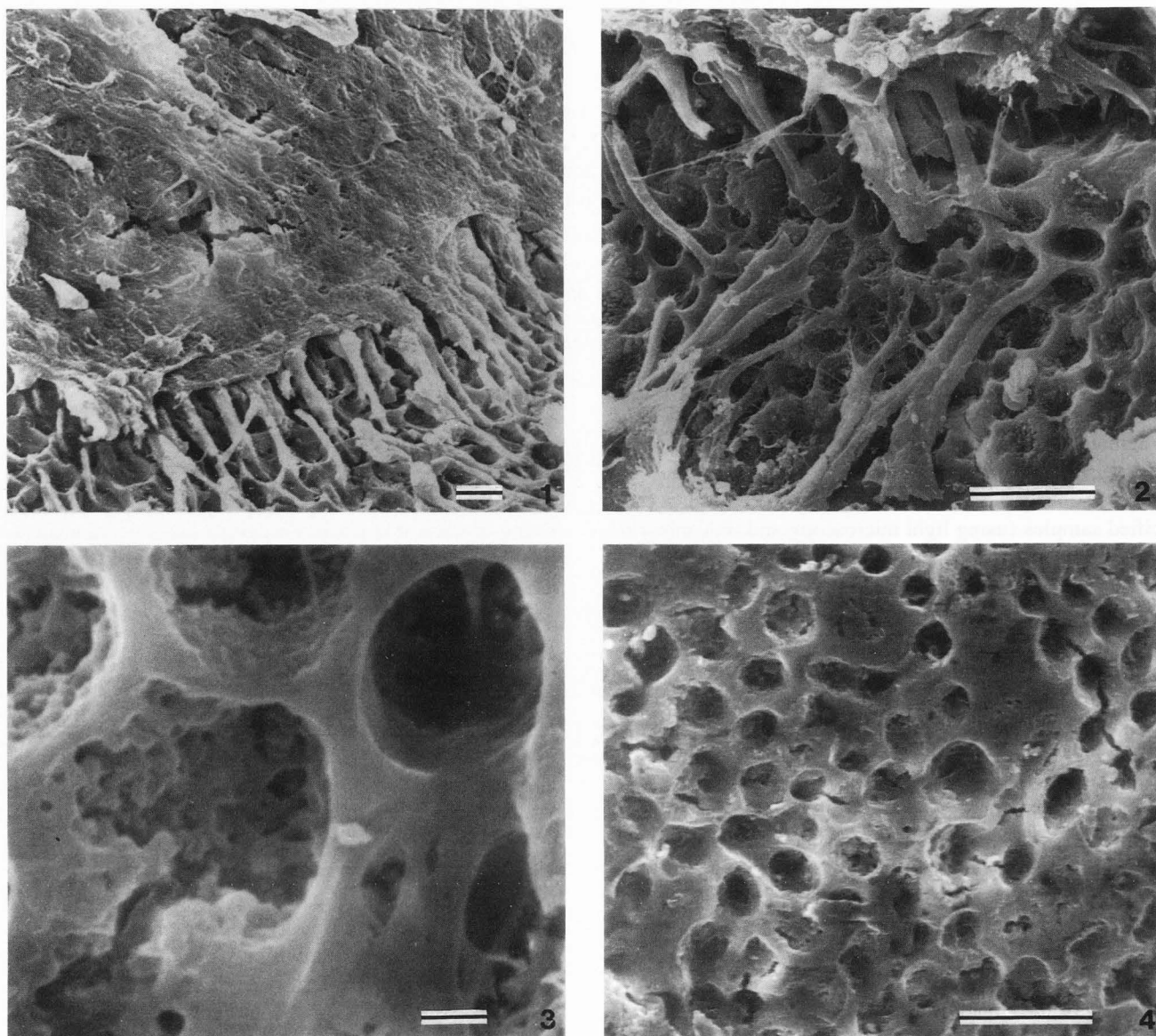
Introduction

Odontoma is a neof ormation in which all or some dental tissues are present. These tissues reach various degrees of differentiation and can have a normal appearance. The study of odontomas can be particularly interesting because it is used to highlight possible anomalies in the different phases of the complex interactions among different tissues, in particular between ectoderm and mesenchyme which guide the development of dental germ structures and the maturation of the dental tissues themselves.

Previous investigations of different types of odontoma showed that the enamel present in these structures is never completely mature but it shows numerous mineralization and structure anomalies (Sapp and Gardner, 1977; Gardner and Dort, 1979; Kerebel and Kerebel, 1984, 1985; Abati *et al.*, 1988; Piattelli and Trisi, 1992). It has been assumed that several factors may cause anomalous tissue development in odontoma. These include an unsuccessful or an altered ecto-mesenchymal interaction in the earliest phases of the dental germ development (Slootweg, 1981; Slootweg and Rademakers, 1983) and/or alterations in the subsequent phases of the development of these tissues (Eversole *et al.*, 1971; Yamamoto *et al.*, 1987). For the enamel, it has also been specifically assumed that alterations in the mineralization mechanisms with modifications of the mineral component, may lead to an incomplete maturation (Aoba *et al.*, 1980; Kerebel and Kerebel, 1984).

In our previous investigations with scanning and transmission electron microscopy (SEM and TEM) on complex odontomas, we described the presence of partially-mature prismatic enamel associated with cylindrical cells with characteristics of ameloblasts of the maturation phase. Based on these observations, we regarded this tissue as still in the active phase of maturation (Piacentini *et al.*, 1992; Marchetti, 1993).

The present investigation is an in depth microscopic study of the enamel present in odontoma to observe the different arrangements of this tissue.



Figures 1-4. Scanning electron micrographs. **Figure 1.** The enamel is covered by cylindrical cells which are overlaid by flat cellular elements. **Figure 2.** High columnar ameloblast-like cells which are in close contact with the adamantine surface. **Figure 3.** Enamel with a homogeneous and prismatic structure, with pits due to Tomes' processes of ameloblasts of a diameter of 6-8 μm . **Figure 4.** Enamel without cellular elements. The pits of Tomes' processes show different sizes and are irregularly distributed. SEM. Bars = 25 μm (Figs. 1, 2 and 4) and 2.5 μm (Fig. 2).

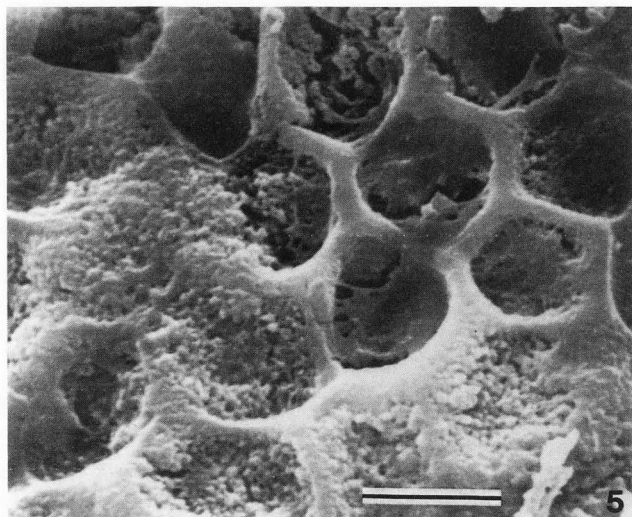
Material and Methods

Investigations were performed on odontogenic neoplasms consisting of numerous aggregates of calcified tissues that were classified histologically as complex odontomas. The samples were surgically removed from anterior areas of lower jaws (lateral incisors, cuspid and premolars) of 5 patients with ages ranging from 17 to 21 years. The samples were carefully examined in all enamel zones.

In each sample, the aggregates were fixed for 30

minutes in a mixture of glutaraldehyde (2.5%) and paraformaldehyde (2%) in sodium cacodylate buffer (0.1 M; pH 7.4) and decalcified in aqueous solution of 4 M formic acid and 0.5 M sodium formate. Following decalcification, the samples were fixed for 4 hours in the same fixative, post-fixed in OsO_4 in collidine buffer, dehydrated, and embedded in Epon for light microscopy and TEM.

Following the same double fixation procedure, other aggregates were alcohol dehydrated and critical point dried and coated with palladium for SEM. Some fixed



Figures 5-7. Scanning electron micrographs. **Figure 5.** The pits of Tomes' processes are shallow and the enamel surface is less smooth. **Figures 6 and 7.** The crystalline structure of the prisms is well organized and delimited by interprismatic enamel. Crystal aggregates (diameter 0.2 to 0.5 μm) in the central part of the prism appear to be arranged parallel to the long axis. Bars = 10 μm (Fig. 5) and 2 μm (Figs. 6 and 7).

but undecalcified samples were cut by an Isomet (Buehler) saw microtome to obtain sections of the tissues which were also processed for SEM examination.

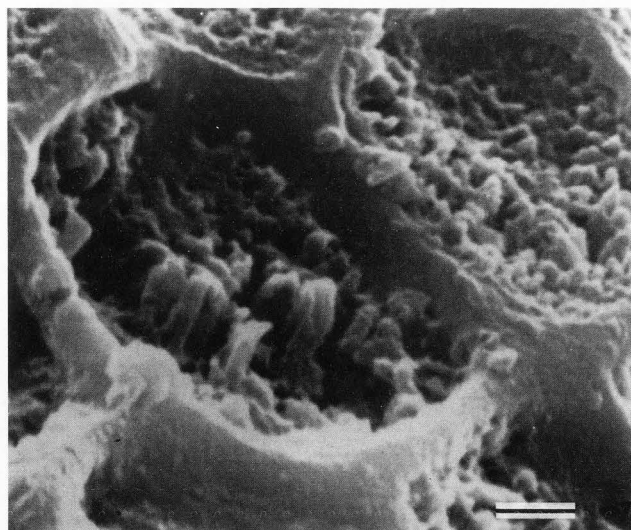
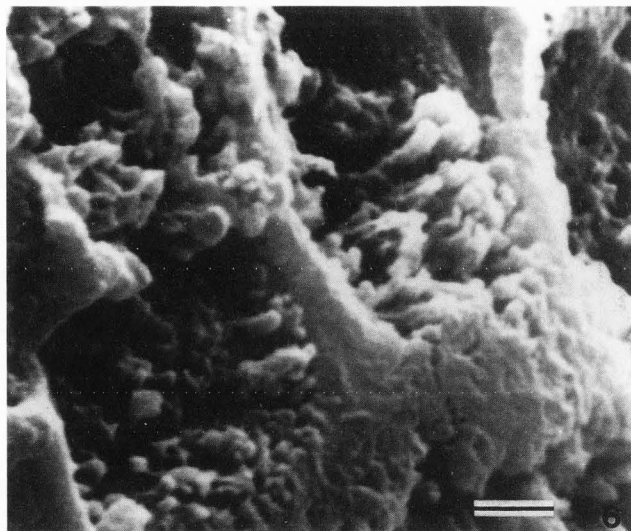
Results

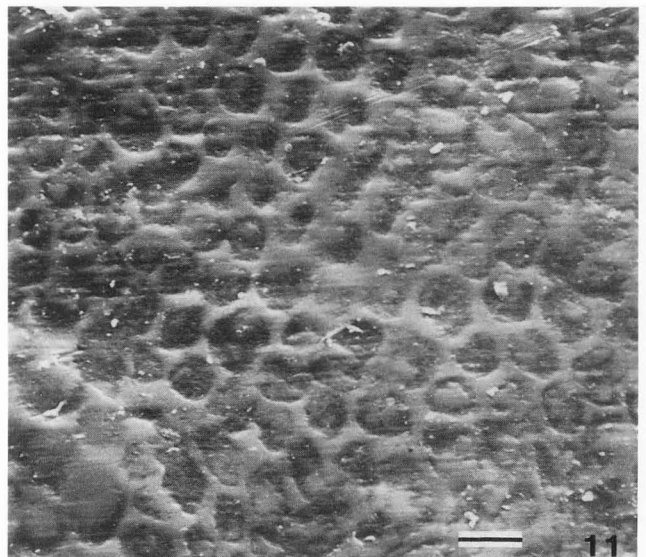
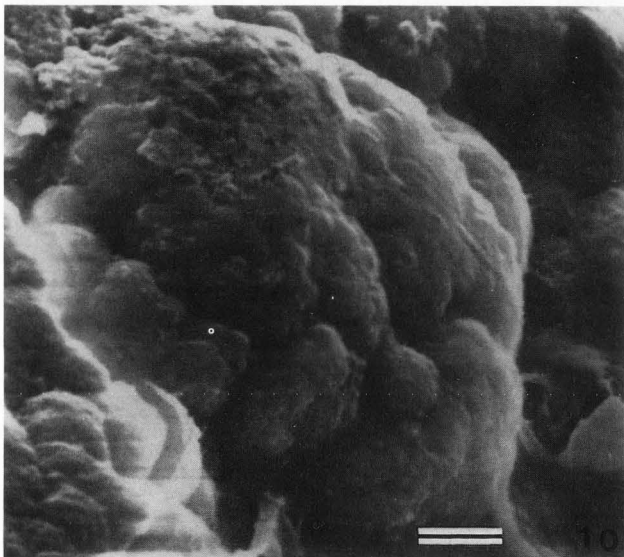
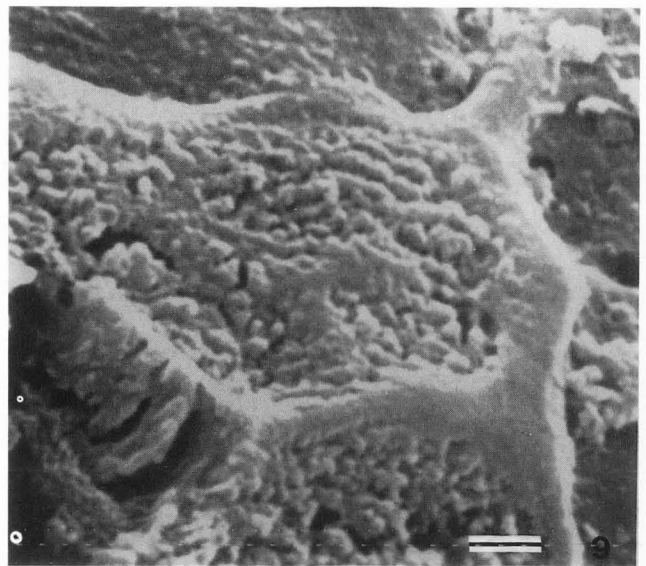
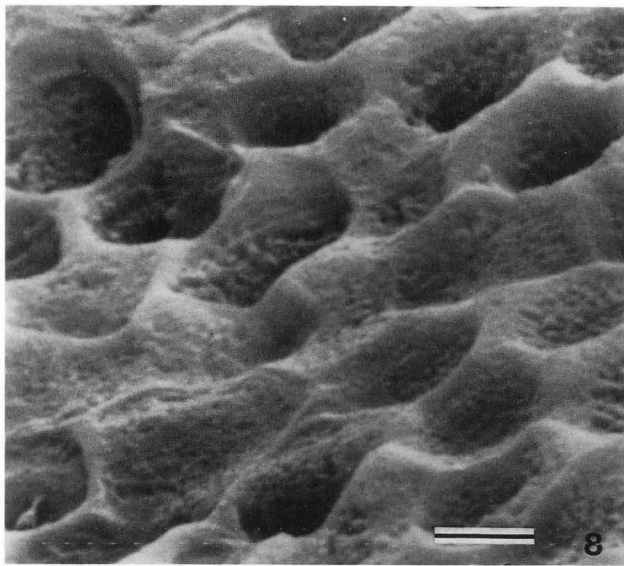
Undecalcified samples

Scanning electron microscopy In the samples examined, the superficial layer was partly composed of enamel which covered or was in relationship with variously organized components resembling dentin and cementum. The SEM investigation revealed that the enamel surface had a different appearance in various areas. In most areas of four out of five samples, the enamel was covered with cellular elements: a layer of high cylindrical ameloblast-like cells was in close contact with the enamel surface and they were related at the other end to flat cells. Pits of 6-8 μm diameter, due to the Tomes' processes of ameloblasts, could be clearly seen on the surface. The enamel between pits showed a compact and homogeneous appearance (Figures 1-3).

In other regions the enamel was lacking cellular elements and the pits of Tomes' processes showed different sizes. Furthermore, the pits of Tomes' processes were irregularly distributed in such a way that the interprismatic regions varied greatly in thickness (Figure 4). These areas bordered others in which, although the prismatic structure was still recognizable, the superficial pits of the Tomes' processes were less evident and shallow, and the enamel surface was less smooth (Figure 5). In these areas, prisms with well organized crystalline structure were delimited by inter-prismatic enamel. Crystal aggregates in the central part of the prism appeared to be arranged parallel to the long axis and had a diameter of 0.2-0.5 μm (Figures 6 and 7). In the other zones, the inter-prismatic enamel appeared looser and crystallites were evident while the pits caused by Tomes' processes were less apparent (Figures 8 and 9).

In other parts of all samples, the prismatic enamel alternated with calcified tissue which appeared uneven, irregularly organized, and with globular formations on the surface (Figure 10). These surfaces were sometimes related to round or ovular cellular elements irregularly located.





Figures 8-11. Scanning electron micrographs. **Figure 8.** The inter-prismatic enamel is quite thin. **Figure 9.** The crystallite aggregates are evident while the pits caused by Tomes' processes are less apparent. **Figure 10.** Calcified tissue which appears uneven, without arrangement, and with globular formations on the surface. **Figure 11.** In sectioned undecalcified specimen the inner enamel structure is prismatic in nature. SEM. Bars = 5 μm (Figs. 8 and 10); 2 μm (Fig. 9) and 10 μm (Fig. 11).

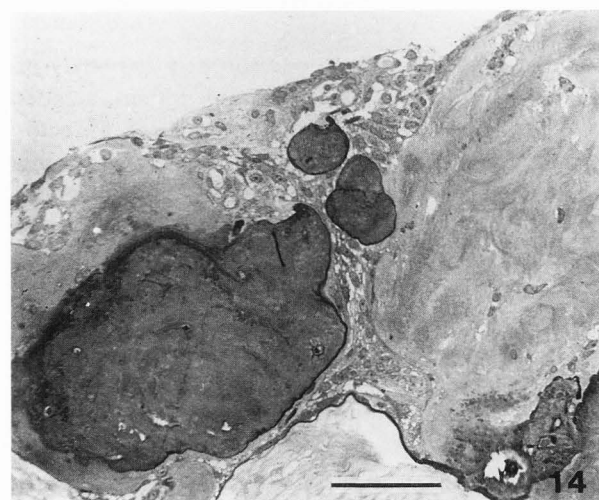
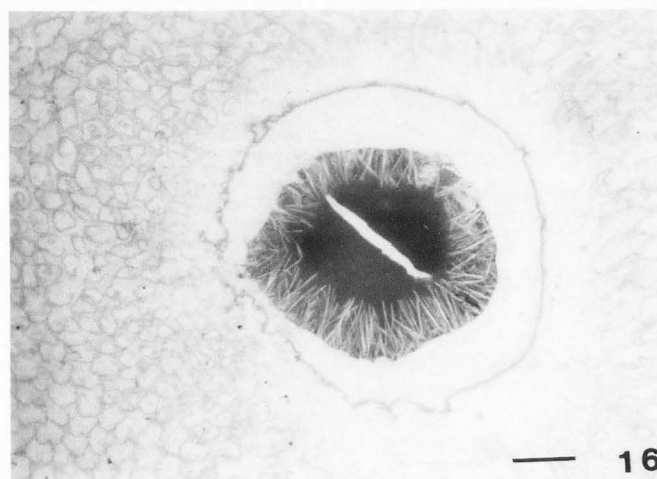
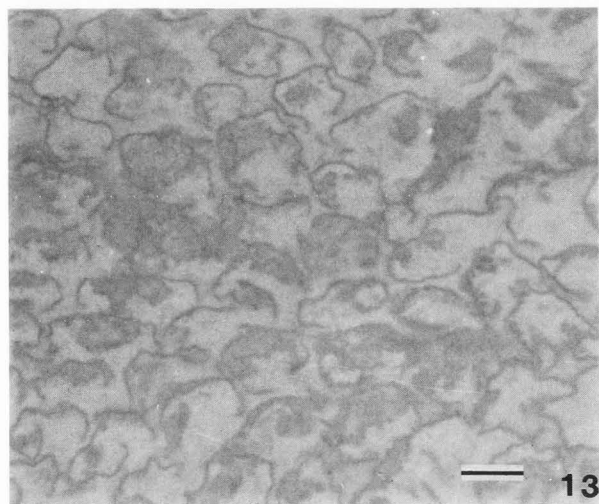
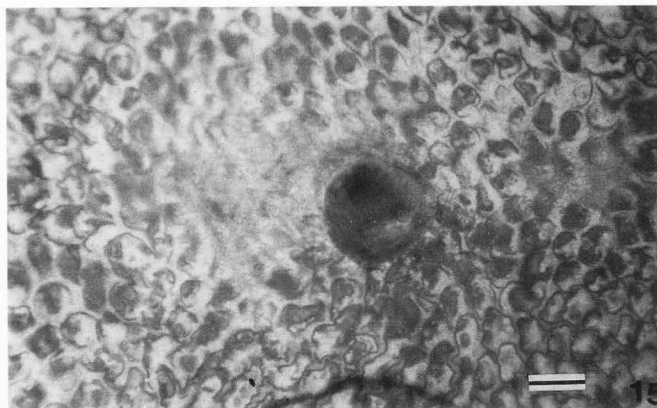
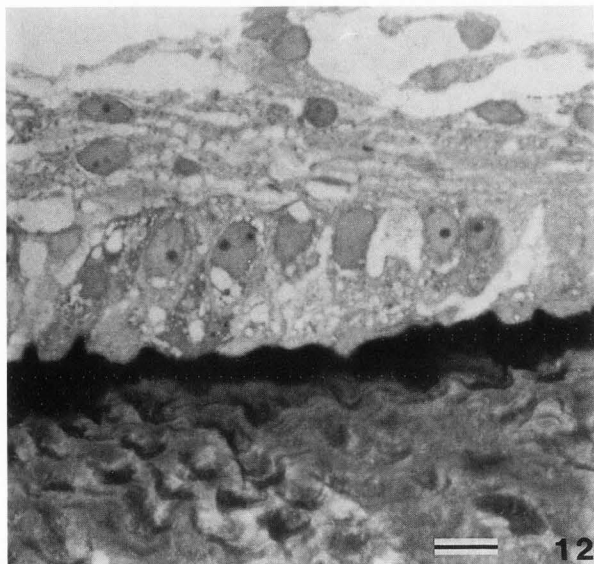
In sectioned undecalcified specimens examined with SEM, the inner enamel structure was observed to be prismatic. In cross-sections, the shape of the prisms was recognizable. The interprismatic regions appeared more homogeneous and compact than prism cores (Figure 11). The fifth sample was lacking the external cellular layer where the enamel showed the features described above.

Decalcified samples

Light microscopy In decalcified samples the enam-

el structure was preserved because of the abundance of fundamental matrix. Several regions of the enamel surface in all samples were coated with a layer of columnar ameloblast-like cells arranged in rows and overlaid by layers of flat cells. This enamel was prismatic and, in its superficial part, in contact with the cells; strongly basophilic organic matrix was more abundant (Figure 12). In the inner part, the prismatic structure was clearly recognizable, the outlines of the prisms being marked by a thick organic matrix (Figure 13). In other

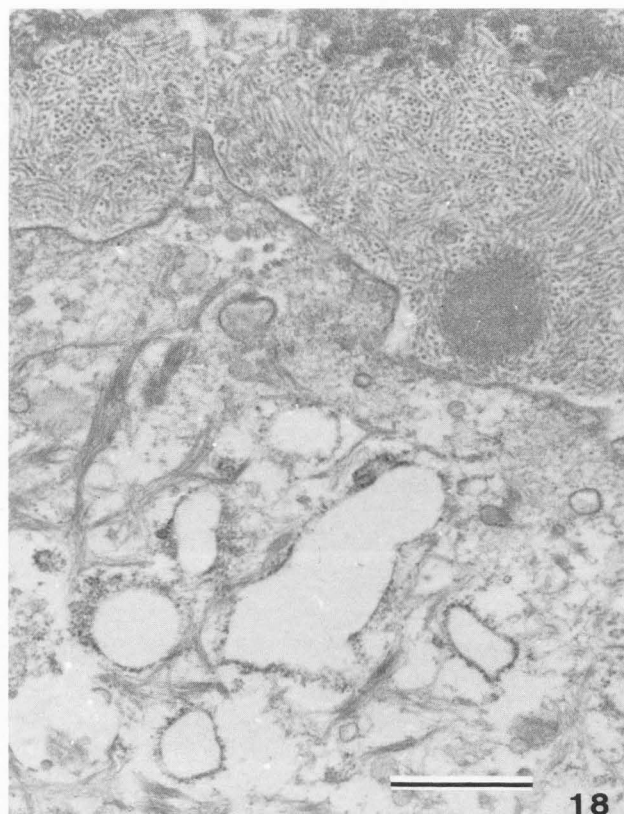
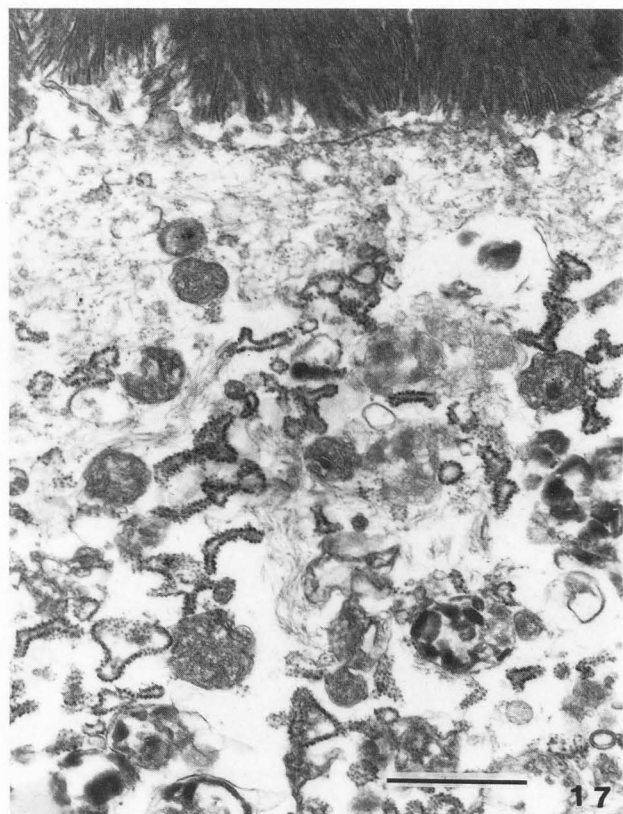
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Figures 12-16. Light microscopy. **Figure 12.** Prismatic enamel covered by a layer of ameloblastic cells overlaid by layers of flat cells. **Figure 13.** Inner enamel region. The prismatic structure is easily recognizable by accumulations of organic substance outlining the prisms (arrow). **Figure 14.** Amorphous organic matrix with basophilic globular formations associated with cells. **Figures 15 and 16.** Globular formations contained in the prismatic enamel and constituted of strongly basophilic organic material (**Figure 15**) which is sometimes crossed by light striations and surrounded by an empty appearing area (**Figure 16**). Bars = 10 μm (Figs. 12 and 13) and 50 μm (Figs. 14-16).

regions, the surface of the samples had no recognizable structure but unstructured material appeared very abundant and intensely colored. The amorphous material was intermixed with groups of irregularly distributed cells (**Figure 14**).

Inside the prismatic enamel, strongly basophilic round formations of organic substance were observed in two samples. Some of these appeared homogeneous and had no structure (**Figure 15**); others were more complex



Figures 17 and 18. Transmission electron micrographs. Apical region of ameloblasts. The cytoplasm is rich in rough endoplasmic reticulum (RER) cisternae, tonofilaments and vacuoles. The cellular surfaces are coated with floccular and filamentous enamel organic matrix. Bars = 1 μ m.

because light-colored rectilinear striations were located irregularly in the amorphous basophilic matrix. This was surrounded by an empty appearing region and was separated from the prismatic enamel by a thin coating of basophilic material (Figure 16).

Transmission electron microscopy At the ultrastructural level, ameloblasts lining part of the enamel, presented a cytoplasm rich in RER cisternae, lysosomal vacuoles and filaments. The enamel organic matrix adjacent to the cells appeared either floccular or filamentous (Figures 17 and 18). Within the enamel, the organic matrix was distributed rather uniformly in the prisms and in the inter-prismatic regions. Only the arcade shaped prism sheath revealed a denser layer of organic material (Figure 19). In other areas, the organic substance was less abundant and appeared mainly in the outer arcade shaped part of the prisms while it was practically absent in the central area (Figure 20).

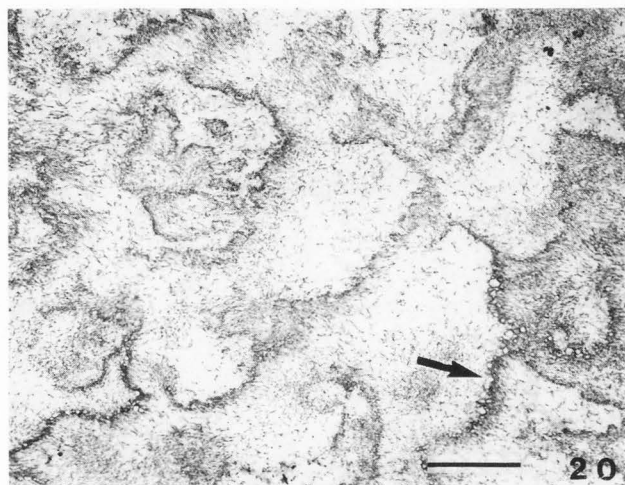
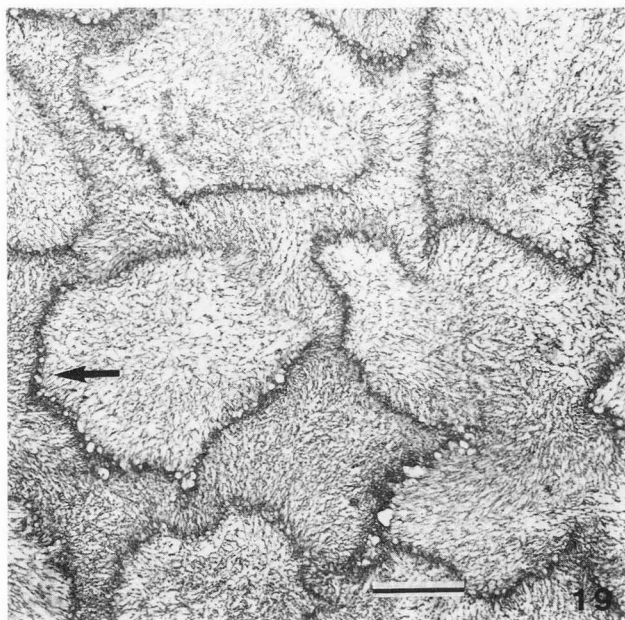
The globular structures included in the enamel consisted of an amorphous strongly electron-dense core. The striations sometimes exhibited by this material,

which were light with the light microscope, appeared empty at ultrastructural examination. They were surrounded by filamentous material resembling the enamel matrix but arranged irregularly (Figure 21).

Discussion

The anomalies of structure noticed in the study of odontomas have been attributed by other authors (Slootweg and Rademakers, 1983) to failure in the ectomesenchymal interactions which normally occur during dental germ development. The mesenchymal component should be unable to stimulate the ectodermic component with normal inductive properties. In our study, it was noticed that odontoma enamel can show a variety of structural appearances by both SEM and TEM.

In some areas, the prismatic matrix is covered by a layer of ameloblastic cells. In other zones, the prismatic structure appears irregular and incomplete; and in others, is composed of unstructured material aggregates. In decalcified samples, the irregularly arranged enamel



Figures 19-20. Cross-sections of the prisms in various enamel areas. In **Figure 19**, the organic matrix is distributed uniformly within the prisms. In **Figure 20**, it is more concentrated at the outer part of the prisms. A thicker matrix layer is always concentrated in the arcade shaped prism sheath regions (arrow). TEM. Bar = 2 μm .

regions were always composed of abundant organic substance. The latter appears as filamentous or floccular material or as homogeneous and intensely basophilic amorphous matrix. The lacking of the external cell layer in one of the samples may be due to inappropriate sampling technique during surgery.

Therefore, contrary to what is asserted by previous authors, it seems reasonable to observe that, although

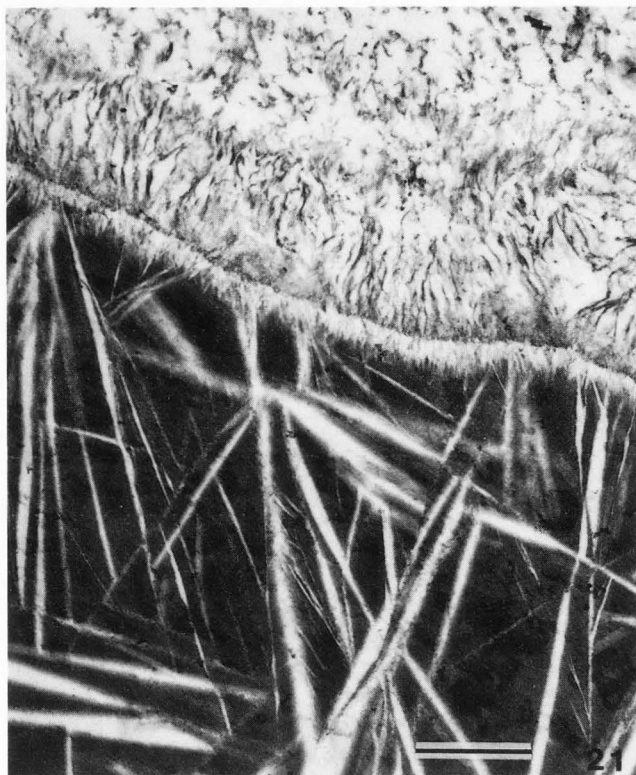


Figure 21. Detail of the ultrastructural features of the formation illustrated in Fig 16. The central portion of electron-dense matrix is crossed by light striations which seem to be without content. More externally a fine floccular matrix material can be found. TEM. Bar = 1 μm .

the mature enamel production is not achieved in all areas, a constant presence of traces of enamel in all samples demonstrates the expression of ecto-mesenchymal inductive properties followed by a differentiation of the ameloblast cells. The type of organization showed by the enamel in many different regions of the surface of the samples demonstrates as well that the ameloblastic cells achieved such a degree of differentiation to lead to the production and maturation of normal tissue. The fact that the maturation process is not complete in all areas may, therefore, result from subsequent interferences occurring in other development stages following the inductive phase.

In our samples, it is evident that the organic component is rather abundant and it is especially present in the areas showing an irregular enamel structure. The globular formations found in two samples inside the prismatic enamel and composed of accumulations of amorphous basophilic material crossed by light-colored striations

might represent areas where anomalous crystal aggregates had occurred. These crystal aggregates, removed by demineralization, may have left their print in these striations which appear clear and empty.

Another remarkable finding is that the enamel with morphological characteristics of normal tissue, although only partially-mature, seems to be related to the presence of ameloblastic cells. These, as we have previously observed (Piacentini *et al.*, 1992), have the morphological characteristics of active cells in maturation phase. The ameloblastic cells and the flat cells covering them can be identified as parts of the enamel organ. Other authors described the presence of layers of cells related to the enamel surface and showed features of reduced epithelium of the enamel organ (Kerebel and Kerebel, 1984). According to these authors, these cells were in functional rest. On the contrary, the cells we observed show morphological characteristics of active cells in the production and maturation phases of the enamel. Considering the age of the patients from whom the odontomas were removed, the enamel maturation should have been already completed.

In our opinion, the observation of incomplete maturation of the tissue and the presence of active-like ameloblasts may suggest either a delay or an interference in the enamel production and maturation. An alteration or regression in the enamel organ development due to external causes such as lack of vascularization, might have caused the modifications in the enamel development.

Acknowledgements

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Discussion with Reviewers

Reviewer 1: Can the anomalies found in the enamel be referred not only to metaplastic phenomena but also to different maturation stages?

Authors: Actually, in general, the enamel in an incomplete maturation phase shows different and apparently anomalous patterns; therefore, the degree of maturation would also depend on the chronological age of the specimen with regard to the normal cycle of enamel formation. As far as the specimen we studied are concerned, considering the age of the patients from whom the odontomas were removed, we would expect the enamel maturation to have been completed. On the contrary, we believe it is important to emphasize the ability of neoplastic tissue to so accurately produce enamel in an abnormal site.

Reviewer 2: Could disposition, organization, and size anomalies of crystalline aggregates be related to demineralization and alcohol extraction?

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Authors: Our preparation methods do not seem to be the cause of morphological alterations as the anomalies we described showed similar patterns both with TEM and with SEM on non-demineralized specimens.

Reviewer 3: Do you think that the unusual accumulation of inorganic materials which you describe in your images could be defined as a form of enamel?

Authors: We consider ameloblastic tissue as all those sites in which, after decalcification, all the typical morphological aspects of normal enamel are maintained.

Reviewer 4: Were the sections stained for light and transmission electron microscopy?

Authors: Yes. The semi-thin sections (0.5 μm) were stained with toluidine blue for light microscopy; the ultra-thin sections (80 nm) were contrasted with uranyl acetate and lead citrate.