Microradiographyand light microscopy of mineralization in the pulp of undemineralized human primary molars

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This study was undertaken to investigate the prevalence, location and histologic features of the different types of mineralization observed in the pulp of human primary tnolars. Microradiography and light microscopy of undetninetalized material disclosed that 95% of ptiinary molars contain pulp calcifications. Histologically, their structure may be classified into four diiTerent types; (1) pulp stones, (2) diffuse calcifications, (3) ebumoid tissue and (4) .spherulitic calcifications.

Numerous studies have provided data showing the high occurrence of pulp calcifications in patients over 10 years old (1-3). However the majority of these have teported on the prevalence and histologic features of pulp calcifications found in permanent human teeth examined after detnineralization.

Until recently the literature failed to disclose any information on the prevalence, location, histologic nature and ultrastructute of calcifications in the pulp of the pritnary dentition. Pulp calcifications in primary teeth have been studied only by light tnicroscopy (4, 5). They have been classified <s false or true denticles, free or attached to the dentinal wall, and as diffuse calcifications (5),

The purpose of this investigation was to study, by tnicrotadiogtaphic analysis and light microscopy of uiidemincralized material, the prevalence and nature of ptilp calcifications affecting human primary molats.

Material and methods

Primary molars were obtained fresh from extractions for orthodontic purposes from 42 healthy children of both sexes, aged 5 to 13 years. They were immediately fixed in 10% formalin. Twenty-three carious non-treated teeth, 14 tteated with amalgatn fillings and 5 caries-free primary molars were selected, having less than one third of their roots resorbed.

After immersion in tnethanol for dehydration, iti chlorofonn for defatting, and in toluene for clearing, the molars

were finally embedded in methyl methacrylate (6). Sections 120 nm thick were prepared using an automatic saw with a diamond disc (type 32, Safag, Bienne, Switzerland). Each section was reduced to a uniform thickness of 80 (im by manual grinding on a ground glass plate under methanol. A micrograph of the sections was taken on a fine grain Kodak .spectroscopic plate 649.0, exposed to long wavelength x-radiations produced by a Machlett tube with a tungsten anode and I mm thick beryllium window (Baltograph BF 5020, Liege, Belgium). The exposure at 13 kV and 18 mA lasted 15 min for a film focus distance of 61 mm. In order to complete the microradiographic observations, the undemineralized sections were stained with methylene blue (0.5% pH 4,3), The samples were mounted and studied under natural or polarized light. Histologic observations were made on an average of 10 non-demineralized slices per tooth.

Results

Histologically, four different types of mineralization were observed in the dental pulps. They were identified as free and attached pulp stones (1), diffuse calcifications (2), ebumoVd tissue (3) and spherulitic calcifications (4).

Ninety-five percent of the molars examined contained pulp calcifications. The prevalences of the four different types of mineralized material in primary molars are summarised in Fig. 1, and their relative frequency of occurrence is shown in Fig. 2. The percentages of teeth with pulpal calcifications were correlated with different parameters such as age, sex, treatments and preferential localization in the pulp (Table 1 4, Fig. 3). The microradiographic prevalence of pulpal calcifications was closely similar for males and females, as well as for maxillary and mandibular molars, and for first and second molars. In the treated group, the percentage of teeth showing pulp calcifications was lower than in the non-treated molars (Fig- 4).

Pulp stones were contained in 33 *(lH'7i<)* of the molars (Fig. 1). They were masses of calcified bodies showing homogeneous radiopacity, or heterogeneous globular structures of highly mineralized material generally more opaque than the orthodentin (Fig. 5A), In some instances, concentric laminations were observed (Fig. 5B). Fine microcanalicular structures were sometimes see erossing the concentric layers at random. In normal light some calcified bodies were observed surrounded by a layer of fibroblasts (Fig. 5C). In non-demineralized ground sections examined with polarized light, concentric pulp stones showed birefringence (Fig, 5D). Occasionally, pulp stones exhibited a central cavity filled with remnant of unidentified material. Pulp stones were found in coronal as well as in radicular me.senchyme (Fig. 3).

Pulp stones were observed occurring freely in 64*y;. of teeth in the treated group (T) and in 787o of the carious non-treated group (CNT). Etnbedded pulp stones were found in 35% of the treated molars and in 73'[A]i of the earious non-treated molars (Fig. 6).

The diffuse calcifications were present as numerous foci of mineralized material scattered throughout the pulp or as unorganized strands of calcified de-

posits (Fig. 7A, B). In polarized light tnicroscopy the ground sections showed diffuse calcifications close to blood vessels and collagen fibers. They were observed in 67% of the exaniiiicd molars (Fig. I) and were most frequently found iti the radictilar pulp (Fig, 3).

Our use of the tet in "ebumoid tissue" refers to extensive masses of calcified deposits comprising featureless homogeneous material where irregular lacunae were present; iti other cases coticentric laminations were ob.served (Fig. 8). The masses were distinctly sepatate from the physiological secondary dentin and tended to obliterate most of the pulp chamber. They were observed in I2";i of the molars (Fig. I).

Spherulitic calcifications were found in 30% of the .samples (Fig. I). Generally, the spherulites were fomied by the association of rhombic crystals converging towards a radiolucent center. In some cases the crystals were tightly packed, disclosing a fine radial pattern (Fig. 9A, B). The polarized light examination *of* the corresponding undemineralized ground sections showed birefringence and a striped pattern in the spherulites. The micrographs showed that the spherulites contained nutnerous pyknotic nuclei (Fig. *9C).*

Discussion

In the microradiographic study, thick .serial sections were made (120 (.itn), then reduced to 9D |,iin. Thus a large fraction of the pulp area was not examined. This method does not lend itself to an accurate statistical survey of the prevalence of pulp calcifications. Nevertheless, we attempted to assess the occurrence in the pulp of one or the other types of inincr;ili/cd tissue by histologic observation of an average of 10 non-demtneral-i/ed slices per tooth. We noted the inimber of teeth containing each type of pulp mineralization. In this way, the total number of positive observations provided an evaluation of the representation of the dilTerent types of microealcifications in the pulp and of their relative occurrence in the dilTerent molar groups. However, as interpretation of the results is limited by the histologic nature of the inquiry, they have not been submitted to statistical tests.

In our findings almost all pritnary molars (95" o) contained pulp calcifications. The prevalence is many times higher than that usually described in the literature based only on histological studies (5, 6). This dilTerence may be explained by the use of non-demineral-

i7ed ground sections, which are supposed to preserve most of the calcified loci revealed by microradiography of the samples.

Studying 120 primary teeth, YAAfOR & HAMtt) (5) reported that pulpal calcifications increase with age and that the presence of caries did not have any significant influence.

In contrast we found that age does not have any influence. On the other hand the occurrence of pulpal calcifications in treated and non-treated groups varied significantly. If treatment is taken into account, the large amount of secondary dentin usually observed in the treated molars must be emphasized in order to understand the lower incidence of pulpal calcifications. This fact trtay reflect an itiductive interaction between treatment and dentinogenesis, with regulation of this latter instead of heterotopic mineralization.

We distinguished lour types of pulp calcifications: pulp stoned (PS), difluse calcifications (DC), ebumoid tissue (ET) and spherulitic calcifications (S). The most common type encountcted in our material was fiee or attached pulp stones, whereas YAACoti & HAMtt) (5) reported that difluse calcifications occur more frequently.

According to their structure, pulp stones have customarily been classified as false and true denticles. The true denticles contain dentinal tubules whereas the false are described as atubular bodies (3, 7 13), These latter have tormcd us the result of concentric laminated deposits arouinl iniiuite loci of calcified tissue.

In our material, however, the calcified masses formed of incremental layers of concentric deposits were cotnposed of irregular microscopic tubules. Our observations show that there is no evidence that they are true dentinal tubules. In agreement with sotne authors (14 16) who have studied pulp calcifications either in pemiaiient or in primary dentitions, we find that the distinction between the two types of denticles is inappropriate.

Moreover, histologic findings concerning etnbedded ptilp stones strotigly suggest that they arc formed in isolation frotn dentinogenesis and are surrounded by a growing dentinal mineral front (17).

Pulps that contain pulp stones usually exhibit difluse calcifications as well. Difluse deposits of mineral may first occur independently throughout the pulp. In sotne instances they tnay later coalesce into compact pulp stones. In our view, however, this process excltides the fortnatioti of the more complex forms of pttip stones which have grown by the addition of calcified tnaterial, including tnicroscopic tubular structures ;irrangcd with a general concentric pattern as indicated by polari/ed light microscopy.

Extensive masses of calcified tissues resembling fibrodentin or osteodentin were found in contact with the Hoor and roof of the pulp chamber. This tissue was thought to be laid down in association with the resorption process (7, 18, 19). The deposition of concentric or amorphous, tubtilar or atubular calcified material, extending lar fnnn the repair areas, leads to massive obliteration of the pulp chamber. We propose to call that deposit "ebumoid tissue", by reference to the tissue described by Ftsn (20), It is unlikely that this material reHeets degenerative ehanges in the pulpal

mesenchyme. Its lorniatioii appears to reflect the potential existing in the pulp cells to form primitive types ol denlin.

The spheruiitic calcifications di.scovered by scanning electron microscopy and by microradiography (17) cannot be classified according to llie tr;Klition;il classification systems. The mcclianisni leading to the deposition of mineral in a spheruiitic pattern in the pulp is unknown (21). Histologic and crystallographic sttidics concerning the composition ol the niiiicral phases of the spherulites revealed that well-crystalli/cd brushite is the major compound (21. 22) and that they have a close relationship with innammatory cells and degenerating tissues (23).