A microradiographic and electron microscopic study of tertiary dentin in human deciduous teeth

Randi Furseth Klinge
Department of Oral Biology, Dental Faculty, University of Oslo, Oslo, Norway

Furseth Klinge R. A microradiographic and electron microscopic study of tertiary dentin in human deciduous teeth. Acta Odontol Scand 1999;57:87–92. Oslo. ISSN 0001-6357.

This study deals with the structure of the inorganic phase of tertiary dentin, and is limited to dentin formation caused by mild or moderate stimuli, e.g., attrition or initial or shallow carious lesions. Ground sections were prepared from 20 human deciduous teeth extracted mainly because of orthodontic treatment, and contact microradiographs were produced. Based on visual inspection of the ground sections in reflected and transmitted light and microradiographic findings, small areas were dissected out and processed for electron microscopy. In the tertiary dentin formed in the pulp horns the number of tubules was reduced, while in that on the side walls of the pulp there was often no marked reduction in the number of tubules. Several tubules could be followed from the physiological (primary and secondary) dentin into the tertiary dentin, and a change in the direction of the tubules was often noted. Interglobular dentin was frequently observed, and in some teeth incremental lines with alternating high and low mineral content were seen, indicating that tertiary dentin, like other mineralized tissues, is subject to biological rhythms during formation. When studied in the electron microscope, tubules with varying size and distribution as well as occluded tubules with a high mineral content were seen. The tubules often had an irregular circumference with projections of mineralized tissue protruding into the lumen. Highly mineralized peritubular dentin was rarely observed. The present results show that orthodentin is formed when dentin in primary human teeth is exposed to mild or moderate stimuli. ☐ *Dentinal tubules; incremental lines; inorganic phase; interglobular dentin*

Randi Furseth Klinge, Department of Oral Biology, Dental Faculty, University of Oslo, PO Box 1052, Blindern, N-0316 Oslo, Norway. Tel. +47 22 85 60 13, fax. +47 22 85 60 25, e-mail. klinge@odont.uio.no

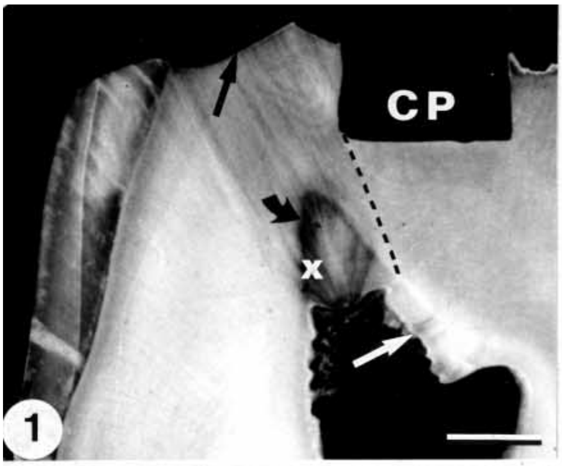
Tertiary dentin has been defined as the dentin, more or less irregular in structure, deposited on the pulpal aspects of primary or secondary dentin, corresponding to areas of external irritations (1). A number of terms have been used to describe this dentin, including irregular secondary dentin, reparative dentin, reactionary dentin and irritation dentin, all reflecting its varied etiology. Smith et al. (2) differentiate between reactionary and reparative dentinogenesis. Reactionary dentinogenesis is defined as the secretion of a tertiary dentin matrix by surviving odontoblast cells in response to an appropriate stimulus. Whilst this stimulus may be exogenous in nature, it may also be from endogenous tissue components released from the matrix during pathological processes (2). This type of dentin is usually the result of a mild or moderate stimulus. Reparative dentinogenesis is tertiary dentin usually secreted by a new generation of odontoblast-like cells; this type of tertiary dentin often has an irregular structure (2, 3).

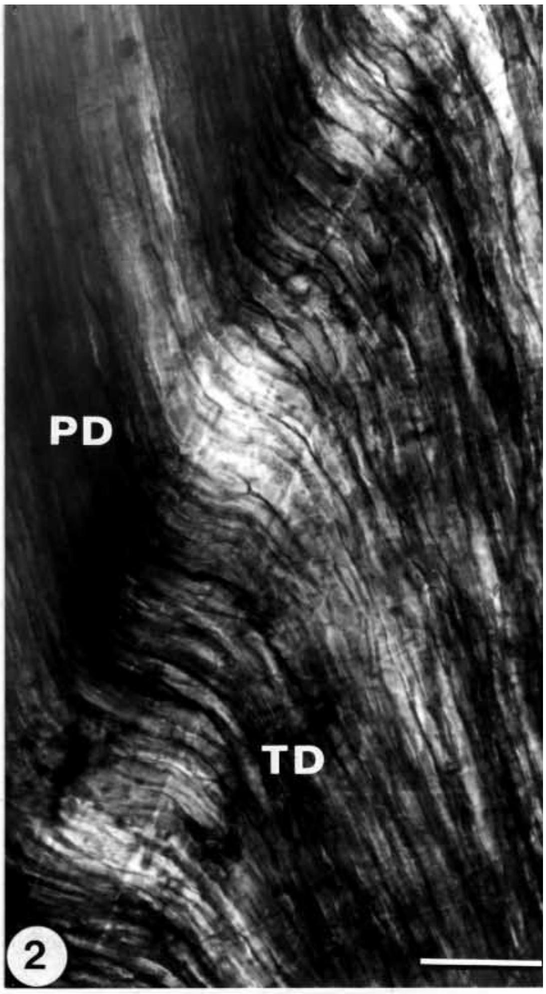
Several studies have dealt with the structure of tertiary dentin in permanent teeth (2, 4, 5). Studies in primary teeth, however, are scarce, and for comparative purposes a study of tertiary dentin in primary teeth was therefore carried out. Particular emphasis was placed on the inorganic phase. The observations in this study are limited to tertiary dentin caused by mild or moderate stimuli, like attrition and incipient or shallow carious lesions. Primary and secondary dentin are often referred to as physiological dentin, and this common denominator will be used in the present paper.

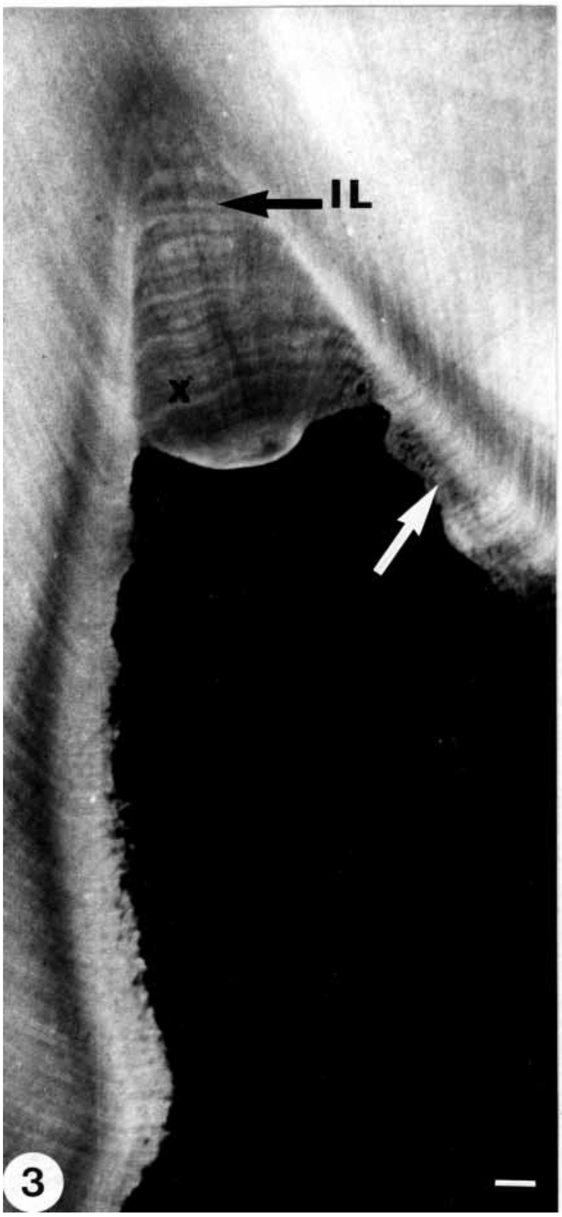
Materials and methods

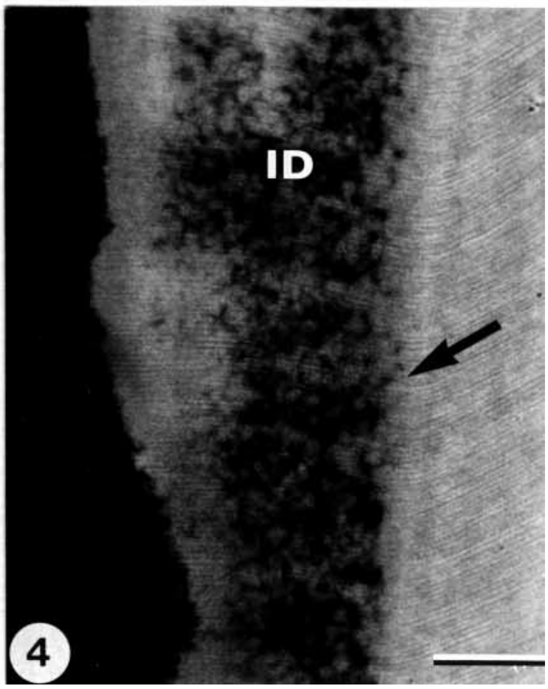
Twenty human deciduous teeth were included in the study. The teeth were extracted mainly because of orthodontic treatment and had amalgam restorations and/or attrited cusps, and often the roots were partly resorbed. Immediately after extraction, the teeth were placed in 2.5% cacodylate-buffered glutaraldehyde or 4% formaldehyde buffered to pH 7.2. After fixation for at least 48 h, ground sections parallel to the long axis of the teeth were cut on a Gillings-Hamco Thin Sectioning Machine. The thickness of the ground sections varied between 150 and 400μ. Some teeth were dehydrated in graded solutions of acetone and embedded in Vestopal W (6) prior to sectioning. Contact microradiographs were produced on Kodak Spectroscopic plates with a Philips X-ray diffraction unit supplied with a fine focus tube. Nickel-filtered copper radiation at 20 kV and 20 mA was employed.

Based on visual inspection of the ground sections in reflected and transmitted light and microradiographic findings, small areas were dissected out and processed for


CP
x
1


PD
TD
2


IL
x
3


ID
4

electron microscopy. Ultrathin sections were cut with a diamond knife and collected on formvar- and carbon-coated grids. The sections were examined unstained in a Philips 400T electron microscope operated at 80 kV.

Results

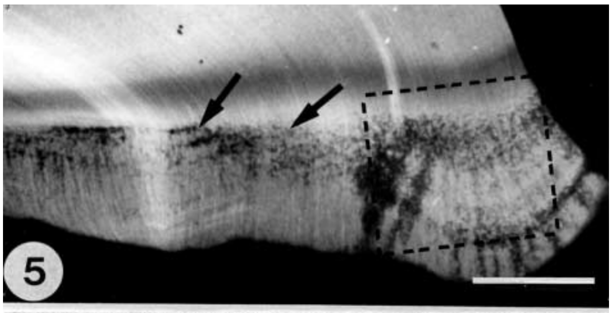
Light microscopy and microradiography

Attrition of cusps resulting in exposure of dentin to the oral environment was a common finding (Fig. 1). This stimulus had resulted in formation of dentin with a relatively normal structure (orthodentin) subjacent to the exposed tubules (Figs 2, 3). Orthodentin was also found subjacent to restorations and incipient or shallow carious lesions (Figs 1, 4–6).

When sections were cut parallel to the long axis of the dentinal tubules, several tubules could be followed from the physiological dentin into the tertiary dentin (Figs 2, 4, 6). At the transition between the two types of dentin, a marked change in the direction of the tubules was often noted (Figs 2, 4), but not always, not at least in the plane of sectioning (Fig. 6). In the incisal or cuspal part of the teeth, the number of tubules was often reduced and the course of the tubules was frequently more irregular than in the physiological dentin (Fig. 2). In tertiary dentin in other parts of the pulp chamber no marked reduction in the number of tubules was observed when passing from physiological into tertiary dentin (Figs 4, 6).

Some teeth, particularly those which had been subject to cavity preparation and insertion of restorative materials, showed wide zones of interglobular dentin (Figs 4–6), while interglobular dentin was rarely noted subjacent to attrited dentinal tubules. Larger areas almost devoid of mineral were sometimes seen in the tertiary dentin (Figs 5, 6). The mineral content in the tertiary dentin seemed slightly lower than in the physiological dentin (Figs 3–6). In some of the teeth, incremental lines with alternating high and low mineralization were observed, particularly in the tertiary dentin underlying attrited dentin (Fig. 3).

5



6

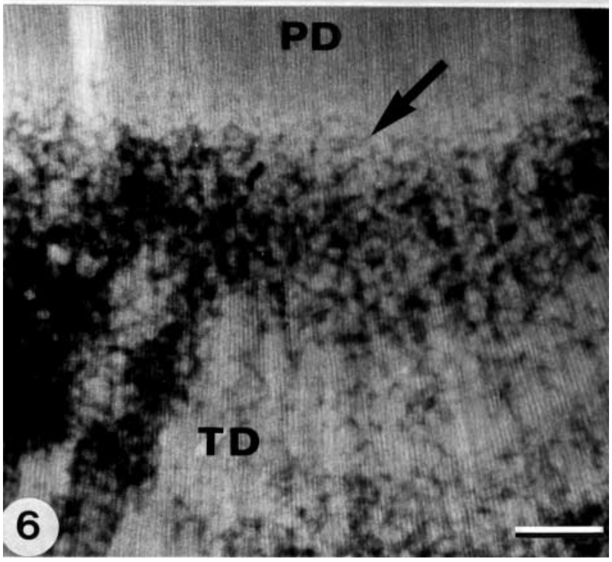


Fig. 5. Microradiograph of a ground section from lower second molar showing wide zone of tertiary dentin subjacent to an amalgam restoration. The tertiary dentin has a lower mineral content than the physiological dentin, and numerous areas of interglobular dentin can be noted. Some areas are almost devoid of mineral. Arrows mark transition between physiological and tertiary dentin. Higher magnification of outlined area is shown in Fig. 6. Scale bar = 0.5 mm.

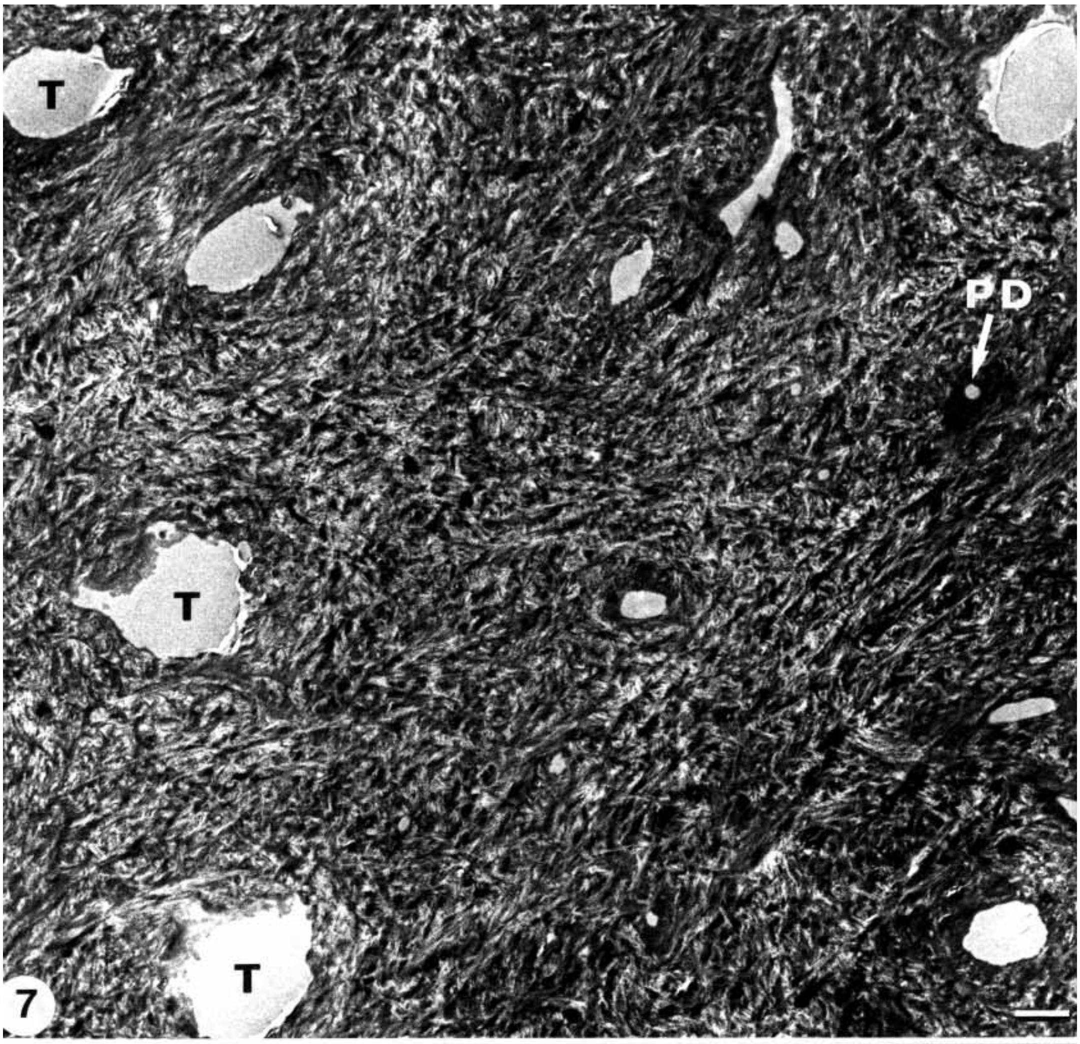
Fig. 6. Higher magnification of area outlined in Fig. 5 showing tertiary dentin with numerous interglobular areas. Some areas are almost devoid of mineral. The dentinal tubules can be followed from the physiological into the tertiary dentin, and there is no change in the direction of the tubules in the plane of section. Arrow marks transition between physiological dentin (PD) and tertiary dentin (TD). Scale bar = 0.1 mm.

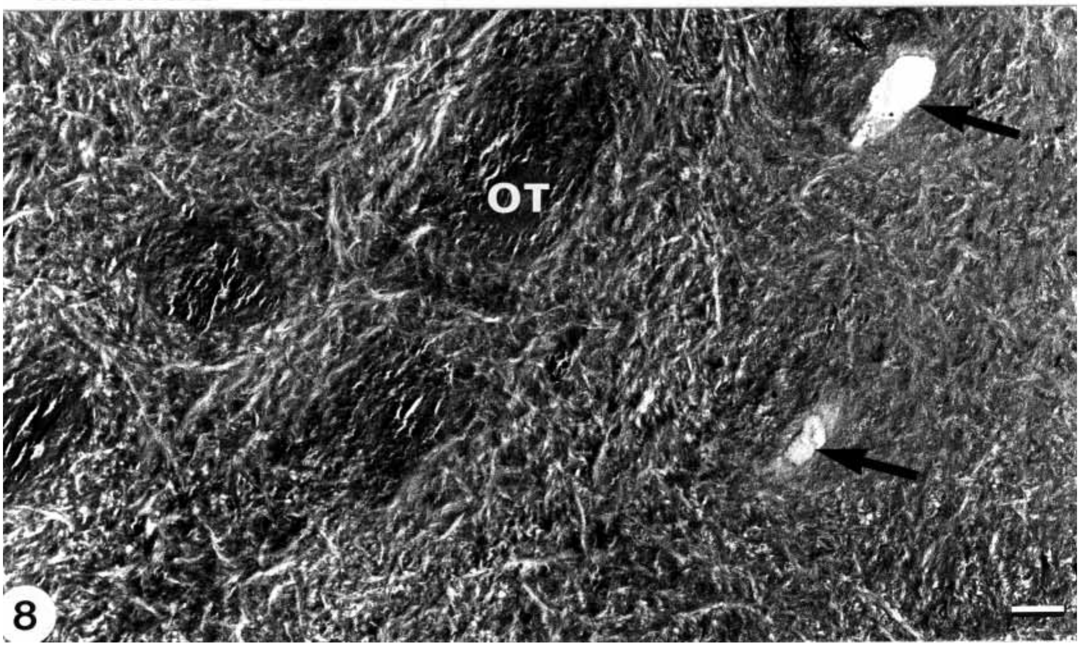
Fig. 1. Photomicrograph of a ground section from a primary lower molar viewed in reflected light. Attrition of enamel with exposure of dentin to the oral environment (*arrow*) has resulted in formation of tertiary dentin subjacent to the affected dentinal tubules. Transition between physiological and tertiary dentin (*curved arrow*). Area marked with a cross viewed in transmitted light at higher magnification is shown in Fig. 2. An occlusal restoration inserted because of a carious lesion has resulted in the formation of tertiary dentin (*white arrow*). Note that the amalgam was lost in preparing the specimen. The transition between the dentinal tubules subjacent to attrition and dentinal tubules subjacent to the restoration is marked by a dotted line. Cavity preparation (CP). Microradiograph of tertiary dentin is shown in Fig. 3. Scale bar = 1 mm.

Fig. 2. Area marked with a cross in Fig. 1 showing transition between physiological dentin (PD) and tertiary dentin (TD) viewed in transmitted light. Several of the dentinal tubules can be followed from physiological dentin into tertiary dentin, and a change in direction of the tubules, a reduction in number and a more irregular course can be noted. Scale bar = 50 μ.

Fig. 3. Microradiograph of the ground section shown in Fig. 1. The overall mineral content in the tertiary dentin seems slightly lower than that in the physiological dentin. Incremental lines (IL) with alternating degrees of mineralization can be noted. Area marked with a cross is shown in the electron micrograph in Fig. 8. White arrow points to tertiary dentin subjacent to cavity tubules. Scale bar = 0.1 mm.

Fig. 4. Microradiograph of a ground section from primary upper central incisor showing a regular type of tertiary dentin where the dentinal tubules can be followed from the physiological into the tertiary dentin. At the transition between the two types of dentin (*arrow*) there is a change in the direction of the tubules, but the number of tubules does not seem to be reduced. Large areas of interglobular dentin (ID) are present in the tertiary dentin. Scale bar = 0.1 mm.


T
T
T
PD
7


OT
8

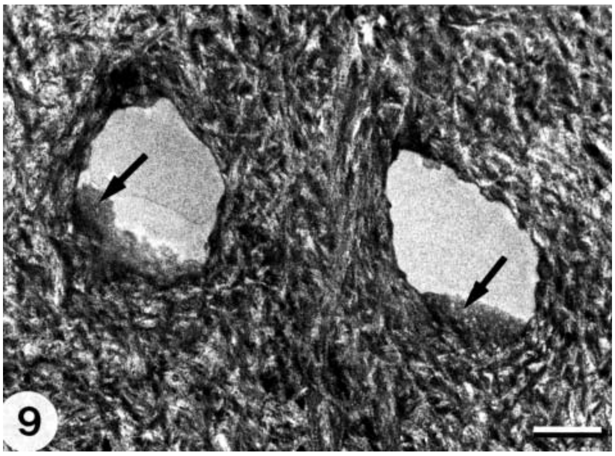


Fig. 9. Electron micrograph of tertiary dentin from an area close to the predentin showing tubules with projections of mineralized tissue protruding into the lumen (*arrows*). The projections have approximately the same electron density as the intertubular dentin. Scale bar = 1 μ.

Electron microscopy

When studied in the electron microscope, tertiary dentin had a varied appearance. Comparatively large areas with no tubules (Figs 7, 8) alternated with areas with occluded tubules (Fig. 8) and areas with tubules of varying size and distribution (Figs 7–9). Areas devoid of tubules had the same appearance as regular intertubular dentin with mineralized fibers running in a criss-cross manner and the typical 64 nm cross-banding of the collagen fibers could be discerned (Figs 7, 9). The occluded tubules had a higher mineral content than the intertubular dentin (Fig. 8). Tubules with an irregular circumference and tubules with projections of mineralized tissue protruding into the lumen were often noted (Figs 7–9). These projections had approximately the same electron density as the intertubular dentin (Fig. 9). With the exception of an occasional narrow tubule with highly mineralized peritubular dentin (Fig. 7), highly mineralized peritubular dentin lining the tubules was rarely observed.

Discussion

Attrition of cusps and incisal edges with exposure of dentin to the oral environment is often observed in the primary dentition, even though those teeth function for a few years only before they are shed. This finding may be related to the thickness of the enamel layer, since it is thinner in primary than in permanent teeth (7). The hardness of the enamel may also play a role. Attrition resulted in the formation of tertiary dentin and this is in agreement with findings in permanent teeth (4, 8). Attrition is a slow, gradual wear and, therefore, a mild stimulus, and usually resulted in orthodentin formation subjacent to the exposed tubules. Dentin with a more irregular structure termed reparative dentin, usually formed by a new generation of odontoblast-like cells in response to an appropriate stimulus after the death of the original odontoblasts (2), was not observed.

Tertiary dentin formation in the pulp horns subjacent to attrition will result in crowding and reduction in the number of odontoblasts and therefore a reduction in the number of tubules. This is illustrated in Fig. 3, where the surface of the tertiary dentin bordering the odontoblasts is only one third of the length of the border between the physiological and the tertiary dentin. A marked change in the direction of the tubules and a reduction in number were often noted, as in permanent teeth (9, 10). However, a change in the direction and a reduction in the number of the tubules were sometimes absent, particularly in tertiary dentin subjacent to restorations on the side walls of the pulp chamber, where there is more space for the odontoblasts to continue dentin production. When the plane of sectioning was parallel to the long axis of the dentinal tubules, the tubules could often be followed from the physiological into the tertiary dentin, indicating that the dentin was formed by increased activity of the original post-mitotic odontoblasts in response to an appropriate stimulus (2). Tubular communication between physiological and tertiary dentin is significant for the permeability of the tissue. The interface will act as an impermeable barrier only if there is no tubular communication between the two tissues (5).

The tertiary dentin underlying attrited surfaces seldom exhibited interglobular areas, whereas interglobular dentin was quite extensive and was often observed in areas subjacent to restorations. The occurrence of interglobular areas is one of several features which has been related to the rate of hard tissue formation (11). Alternating bands with high and low mineral content were observed in several specimens, and this is in agreement with findings in physiological dentin (9) and cementum (12). These bands corresponded to incremental lines and were considered to represent alternating periods of activity and quiescence during cementogenesis and thus a result of biological rhythms.

The cross-sectioned occluded tubules observed in tertiary dentin morphologically resembled age changes observed in permanent teeth (4). Ingrowth of mineralized tissue protruding into the tubular lumen and occluded

Fig. 7. Electron micrograph showing tertiary dentin from an area close to the predentin. Tubules (T) with a regular size alternate with an area containing small tubules with varying size and distribution. An occasional narrow tubule with highly mineralized peritubular dentin (PD) can be noted. Scale bar = 1 μ.

Fig. 8. Electron micrograph of area marked with a cross in Fig. 3 showing two partly occluded dentinal tubules (*arrows*), four occluded tubules (OT) and an area devoid of tubules. The occluded tubules have a higher mineral content than the intertubular dentin. Scale bar = 1 μ.

tubules, as seen in the present study, have also been observed in Cu-amalgam covered primary dentin (13) and corticosteroid-covered dentin in permanent teeth (14, 15). The ingrowth of mineralized tissue may be the beginning of a closure of the dentinal tubules eventually resulting in complete occlusion. The similarity to age changes indicates that a gradual closure is brought about, probably under cellular control.

Highly mineralized peritubular dentin was observed lining only a few tubules in the present study. This observation disagrees with findings by Robertson et al. (16), who describe a dense layer of peritubular matrix on the tubule wall and in the hypermineralized peritubular region in tertiary dentin in traumatized primary incisors. However, these authors only looked at EDTA-demineralized tissue. Since the organic matrix of the peritubular dentin is scarce and usually disintegrates during demineralization, demineralized sections are not suitable for studying peritubular dentin.

The present study shows that the pulp reacts to mild or moderate stimuli, like for example attrition or insertion of restorative materials, by the formation of tertiary dentin on the pulpal aspect of the stimulated dentinal tubules in human deciduous teeth. In another attempt to wall off the irritating agent, the pulp responded by obturation of some of the tubules, thus reducing the permeability of the dentin. These findings are in agreement with observations in permanent teeth (1, 4). The tertiary dentin was of the orthodentin type, and the structure varied somewhat in different areas of the pulp. The presence of incremental lines with high and low mineral content indicates that tertiary dentin, like physiological dentin, can be subject to biological rhythms during formation.

References

1. Baume LJ. The biology of pulp and dentine. In: Myers HM, editor. Monographs in oral science. Basel: Krager; 1980;8:57–66.
2. Smith AJ, Cassidy N, Perry H, Bègue-Kirn C, Ruch J-V, Lesot H. Reactionary dentinogenesis. Int J Dev Biol 1995;39:273–80.
3. Linde A, Goldberg M. Dentinogenesis. Crit Rev Oral Biol Med 1993;4:679–728.
4. Stanley HR, Pereira JC, Spiegel E, Broom C, Schultz M. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. J Pathol 1983;12:257–89.
5. Mjör IA. Detin-predentin complex and its permeability: pathology and treatment overview. J Dent Res (Spec issue) 1985;64:621–7.
6. Ryter A, Kellenberger E. L'inclusion au polyester pour l'ultramicrotomie. J Ultrastruct Res 1958;2:200–14.
7. Kraus BS, Jordan RE, Abrams L. Anatomy of the individual teeth. In: Dental anatomy and occlusion. Baltimore: Williams and Wilkins; 1969. p. 1–115.
8. Tronstad L. Optical and microradiographic appearance of intact and worn human coronal dentine. Arch Oral Biol 1972;17:847–58.
9. Mjör IA. Dentin and pulp. In: Mjör IA, Fejerskov O, editors. Human oral embryology and histology. Copenhagen: Munksgaard; 1986. p. 90–129.
10. Avery JK. Pulp. In: Bhaskar SN, editor. Orban's oral histology and embryology. 11th ed. St.Louis: Mosby Year Book; 1991. p. 106–38.
11. Furseth R. The resorption processes of human deciduous teeth studied by light microscopy, microradiography and electron microscopy. Arch Oral Biol 1968;13:417–31.
12. Furseth R, Johansen E. A microradiographic comparison of sound and carious human dental cementum. Arch Oral Biol 1968;13:1197–206.
13. Furseth Klinge R. The ultrastructure of copper amalgam-covered dentin from human deciduous teeth. Acta Odontol Scand 1993;51:223–28.
14. Mjör IA, Furseth R. The inorganic phase of calcium hydroxide- and corticosteroid-covered dentine studied by electron microscopy. Arch Oral Biol 1968;13:755–63.
15. Furseth R, Mjör IA. The fine structure of corticosteroid-covered, human dentine. Arch Oral Biol 1972;17:719–28.
16. Robertson A, Lundgren T, Andreasen JO, Dietz W, Hoyer I, Norèn JG. Pulp calcifications in traumatized primary incisors. A morphological and inductive analysis study. Eur J Oral Sci 1997;105:196–206.

Received for publication 7 October 1998
Accepted 29 January 1999