From:
The Dental Institute of Experimental Research and
Department of Anatomy, Dental Faculty,
University of Oslo, Norway

THE INTERFACE BETWEEN DENTINE AND IRREGULAR SECONDARY DENTINE

by

IVAR A. MJÖR
KJELL KARLSEN

INTRODUCTION

Different terms are in common use to denote various types of secondary dentine, such as physiologic, regular, irregular, functional and reparative. However, the different terms seem to be merely descriptive or are based on presumed functional differences. In fact the term »secondary dentine» itself is not a well-defined structural or functional entity. *Scott* and *Symons* (1967) have stated that »the dentine which is formed after the dentine (primary dentine) producing the typical form of the tooth has been laid down is known as secondary dentine». On the other hand, *Sicher* (1962) and *Provenza* (1964) have described secondary dentine as the dentine which is found pulpally to a demarcation line caused by an altered direction of the tubules as a result of crowding and loss of some of the odontoblasts as the pulp cavity becomes smaller.

Symons (1968) has distinguished between two types of secondary dentine. One type forms on the entire pulpal surface of the coronal dentine. It is a direct continuation of the primary dentine, but differs from it by a scarcity of highly mineralized peritubular areas and by differences in the organic matrix of the intertubular areas. The other type forms in restricted areas, shows more irregularity in the course of the dentinal tubules, and appears later than the first described type. Both types were considered as physiologic secondary

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dentine, and they were not related to destructive processes as attrition or caries.

However, destructive processes may affect the rate of formation and the structure of secondary dentine. The present paper will deal with this type of secondary dentine, and the term »irregular secondary dentine» will be used to denote well-delimited, locally increased formations of dentine found subjacent to lesions in the coronal portion of teeth.

The formation of irregular secondary dentine is maintained to be a main defence mechanism of the pulp-dentine organ. The initial changes occurring at the dentine/irregular secondary dentine interface, are essential for an understanding of the nature of the processes involved. Furthermore, knowledge of the structure and composition of the junctional zone are important in order to establish criteria for an evaluation of tissue changes in the dentine. In the present paper these features will be discussed on the basis of findings obtained from human and monkey material as a result of caries, attrition and certain experimental procedures.

MATERIALS AND METHODS

Three human teeth from young adults with extensive carious lesions were fixed in 10% neutral, buffered formalin, decalcified in 5.2% nitric acid, embedded in paraffin and serially sectioned. Sections passing through the lesions and the corresponding part of the dentine and pulp, were stained with hematoxylin and eosin (HE), or 0.1% alcian blue (AB) at pH 3.6, or they were microradiographed using a technique which is considered to show the mass distribution of organic material (*Shackleford*, 1965; *Mjör & Shackleford*, 1966).

In addition, several teeth from four monkeys were employed. The animals were anesthetized with nembutal, and buccal class V cavities were prepared with steel burs in a slow speed dental engine. The cavities were either left unfilled or restored with various filling materials; zinc/oxide eugenol cement, $Ca(OH)_2$, Hydrex (Kerr Dent. Manuf. Co.), selfcuring acrylic resin, zinc phosphate cement or amalgam. Some of these cavities had been left unfilled for 7 days after preparation prior to inserting the restorations. The distribution of the experimental material and the observation periods employed are outlined in Table I.

After varying observation periods (Table I) the monkeys were again anesthetized with nembutal, and fixation was performed by perfusion with 10% neutral, buffered formalin. The heads were then removed and stored in the same type of formalin solution.

Table I

The monkey material and the observation periods

	DECALCIFIED			UNDECALCIFIED		
	Exposed cavities	Restored cavities*	Untreated teeth	Exposed cavities	Restored cavities	Untreated teeth
No. of teeth	18	11	2	1	5	6
Obs. period (days)	7—210	7—81	—	7	7	—

*) Some of these cavities had been left unfilled for 7 days prior to the insertion of restorations.

The aim of the experimental procedure was to initiate irregular secondary dentine formation. The reason for using different filling materials was to evaluate possible differences under varying experimental conditions, but the series are too small to allow a comparison between any reactions produced by the various restorative materials.

Judged by the dental status, three of the monkeys were adults showing typical attrition on all teeth, while one was considered to be young, because the permanent canines had not erupted. Those teeth which exhibited attrition allow studies of the irregular secondary dentine commonly found subjacent to such lesions.

Most of the monkey teeth were decalcified and prepared histologically in the same way as the human material mentioned above, i.e. the sections were either stained or microradiographed. In addition, some undecalcified teeth were sectioned longitudinally through the cavities and the areas showing attrition (Table I). The ground sections thus obtained were microradiographed using Cu k-radiation in order to evaluate the distribution of mineral salts. Details of the method employed to prepare the ground sections and the microradiographs have been described by *Mjör* (1966a).

OBSERVATIONS

Human carious teeth. The irregular secondary dentine was limited to the dentinal tubules corresponding to the carious lesion. It stained lighter than the dentine, and contained fewer and more irregularly arranged dentinal tubules than the primary dentine (Figs. 1 and 3). Some irregularities in the dentine structure at the dentine/irregular secondary dentine junction were found in two teeth (Fig. 1). The predentine was wider than normal.

A hematoxyphilic band (Figs. 1 and 3) was found at the dentine/irregular secondary dentine junction. This interface did not usually stain with AB. It should be noted that the hyperchromatic band was not delimited to the dentinal tubules affected by the lesion, but extended beyond this area and was continuous with the hyperchromasia normally found at the dentine/predentine junction (*Mjör*, 1966b).

The most striking feature in the microradiographs of the decalcified sections was the increased radiodensity of the irregular secondary dentine as compared to the rest of the dentine (Figs. 2 and 4). The areas with irregular structure close to the hyperchromatic band, appeared as radiolucent areas (Fig. 2).

Monkey material — attrition. Localized masses of irregular secondary dentine were found subjacent to areas showing attrition (Figs. 5 and 11). A distinct hematoxyphilic line separated the irregular secondary dentine from the adjoining dentine, and it was in direct continuity with the similarly stained band at the dentine/predentine junction in unaffected areas (Figs. 5 and 11). This hematoxyphilic band was either AB positive or negative (Figs. 6 and 12). Similar variations in the staining reaction with AB at the dentine/predentine junction have been observed in normal material (*Mjör*, 1968). It should be noted, however, that even if the dentine/predentine junction in unaffected areas was AB positive, the dentine/irregular secondary dentine interface could be AB negative (Fig. 6). In one tooth, from which half the sections were cut longitudinally and the other half transversely to the long axis of the tooth, an eosinophilic area was found at the dentine/irregular secondary dentine interface (Fig. 7), i.e. the staining reaction was similar to that seen in the predentine. This junction was apparently weak, because a separation between the dentine and irregular secondary dentine frequently occurred in this location.

The decalcified sections of the monkey material employed for microradiography were more often folded or loosened than was the case with those from the human material. It appeared, however, that the dentine/irregular secondary dentine junction could contain more organic material than the dentine on either side of it (Fig. 8).

The microradiographs of the undecalcified sections showed that the irregular secondary dentine subjacent to attrition defects varied more in mineral content than the primary dentine (Fig. 9). At the junction between the two, a slightly more mineralized band was found (Figs. 9 and 10), and it was continuous with the more mineralized dentine normally found close to the predentine in such teeth (*Mjör*, 1966a).

Monkey material — experimental cavity preparations. The reactions subjacent to the exposed or restored cavities varied considerably, and irregular

secondary dentine formations were usually associated with unfilled cavities (Fig. 13). The junction between the dentine and irregular secondary dentine also varied. Cellular inclusions or predentine-like tissue could sometimes be observed in this location (Figs. 14 and 15), while in other instances the irregular secondary dentine had been deposited on the dentine in a manner similar to that seen subjacent to attrition. In some teeth with exposed cavities as well as in some of those which had been restored, only minor alterations could be noticed, while in others a predentine zone could not be discerned in the area corresponding to the cavity tubules (Fig. 16). Accompanying the change in the stainability of the predentine a higher content of organic material (Fig. 17) than that normally found (Fig. 18) was observed. An inflammatory response was only rarely detected, but, when present, it did not inhibit the formation of irregular secondary dentine (Fig. 15).

The microradiographs of the ground sections from restored teeth were only from cases with short observation periods, and no differences in radiodensity from that seen normally, could be observed. However, this evidence is not considered conclusive, and extended studies are required.

Plate I

Fig. 1. Irregular secondary dentine (SD) subjacent to a carious lesion in a human tooth. Note the hematoxyphilic (dark) line separating the irregular secondary dentine from the adjacent dentine. Arrows indicate areas with defective structure. Microradiograph of a section from the same tooth is shown in Fig. 2. HE stained section. × 110.

Fig. 2. Microradiograph of a decalcified section from the same tooth as shown in Fig. 1. Note that the irregular secondary dentine (SD) is more radiodense than the rest of the dentine. Arrows indicate radiolucent areas found in the dentine close to the dentine/irregular secondary dentine interface (compare with arrows in Fig. 1). × 110.

Fig. 3. HE stained section showing irregular secondary dentine (SD) subjacent to a carious lesion in a human tooth. Hematoxyphilic (black) line is found at the dentine/irregular secondary dentine interface. Note the irregular course of the dentinal tubules in the secondary dentine. Microradiograph of a section from the same tooth is shown in Fig. 4. × 275.

Fig. 4. Microradiograph of a decalcified section from the same tooth as shown in Fig. 3. The irregular secondary dentine (SD) is more radiodense than the adjacent dentine. × 275.

Plate II

Fig. 5. HE stained section showing irregular secondary dentine (SD) in the pulp horn subjacent to attrition. Note that the hematoxyphilic line separating the irregular secondary dentine from the rest of the dentine is continuous with a similarly stained line at the dentine/predentine junction in unaffected areas. AB stained section from the same tooth is seen in Fig. 6, and a section taken at right angles to this one from part of the same irregular secondary dentine, is shown in Fig. 7. × 110.

Fig. 6. AB stained section from the same tooth as shown in Fig. 5. Note that the AB positive line (L) at the dentine/predentine junction is not found at the dentine/irregular secondary dentine interface. The irregular secondary dentine stains lighter than the rest of the dentine. × 110.

Fig. 7. HE stained section from the same irregular secondary dentine as shown in Fig. 5. The section was cut at right angles to the one shown in Fig. 5, and cross-sectioned dentinal tubules are seen at the dentine/irregular secondary dentine interface. Note that the hematoxyphilic band in this location is caused by an intense staining of the intertubular areas. A light staining area (at arrows), with stainability similar to that of the predentine, is found interposed between part of the irregular secondary dentine and the adjacent dentine. × 275.

Fig. 8. Microradiograph of a decalcified section showing irregular secondary dentine (SD) in a pulp horn subjacent to attrition. Note the radiodense line at the dentine/irregular secondary dentine interface. × 110.

Fig. 9. Microradiograph of an undecalcified section showing irregular secondary dentine (SD) in the pulp horn subjacent to an attrition defect. A radiodense line (at arrows) separates part of the irregular secondary dentine from the rest of the dentine. Higher magnification is shown in Fig. 10. C: Crack in section. × 110.

Fig. 10. Higher magnification of an area from the microradiograph shown in Fig. 9. Note the variation in radiodensity of the irregular secondary dentine (SD), and the relatively radiodense band separating it from the rest of the dentine. × 275.

Plate III

Fig. 11. HE stained section showing irregular secondary dentine (SD) with cellular inclusions subjacent to attrition. Note that the hematoxyphilic (black) band at the dentine/irregular secondary dentine interface is continuous with the similarly stained dentine/predentine junction in unaffected areas. AB stained section from same tooth is shown in Fig. 12, and a section showing an experimental cavity in this tooth is found in Fig. 13. × 110.

Fig. 12. AB stained section from the same area as shown in Fig. 11. Note that the dentine/irregular secondary dentine interface is AB positive and that it stains in the same manner as the dentine/predentine junction in unaffected areas. × 110.

Fig. 13. HE stained section from a tooth with an experimental cavity (EC) which had been left unfilled for 210 days. Note irregular secondary dentine (SD) formation corresponding to the cavity tubules. S: Separation of the tissue at dentine/irregular secondary dentine interface. × 50.

Fig. 14. Irregular secondary dentine (SD) with numerous cellular inclusions subjacent to an unfilled cavity. 67 days observation time. HE stained section × 275.

Fig. 15. Irregular secondary dentine (SD) subjacent to *part of* the cavity tubules. Predentine (PD) stains either lightly or darkly at the dentine/irregular secondary dentine interface; both areas corresponding to the cavity tubules. Marked inflammatory reaction in the pulp tissue. Cavity had been left unfilled for 67 days. HE stained section. × 275.

Fig. 16. HE stained section showing altered stainability of the predentine (PD) subjacent to a cavity which had been left unfilled for 7 days. The area where predentine should normally be found, stains like the rest of the dentine under part of the cavity tubules. Microradiograph from an area in this tooth which lacks predentine is seen in Fig. 17. × 275.

Fig. 17. Microradiograph of a decalcified section from the tooth shown in Fig. 16. No predentine can be discerned (Normal appearance of predentine in Fig. 18). × 275.

Fig. 18. Area from unaffected dentine showing the radiolucent part of the predentine (PD) adjacent to the odontoblasts. × 275.

PLATE I

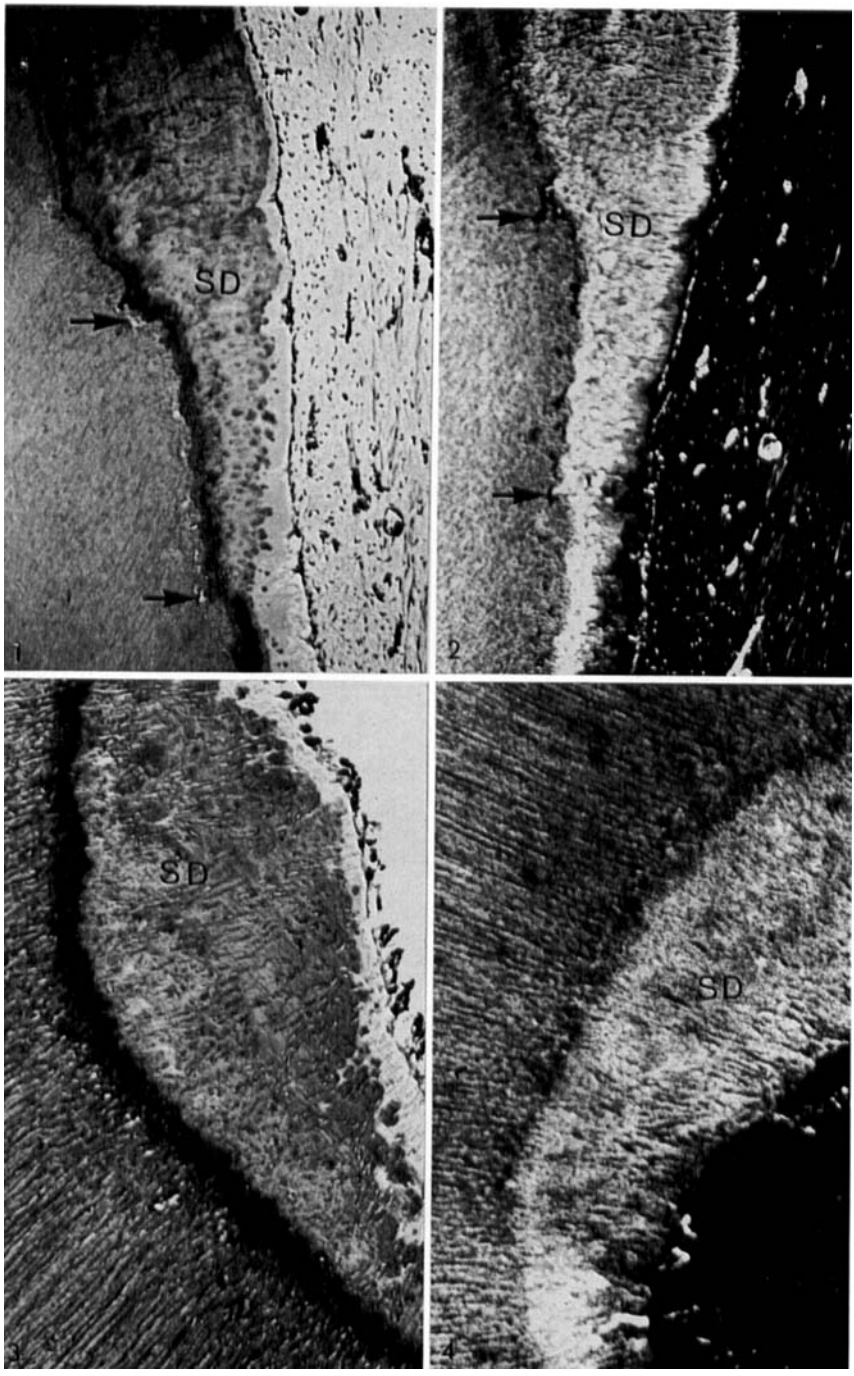


PLATE 11

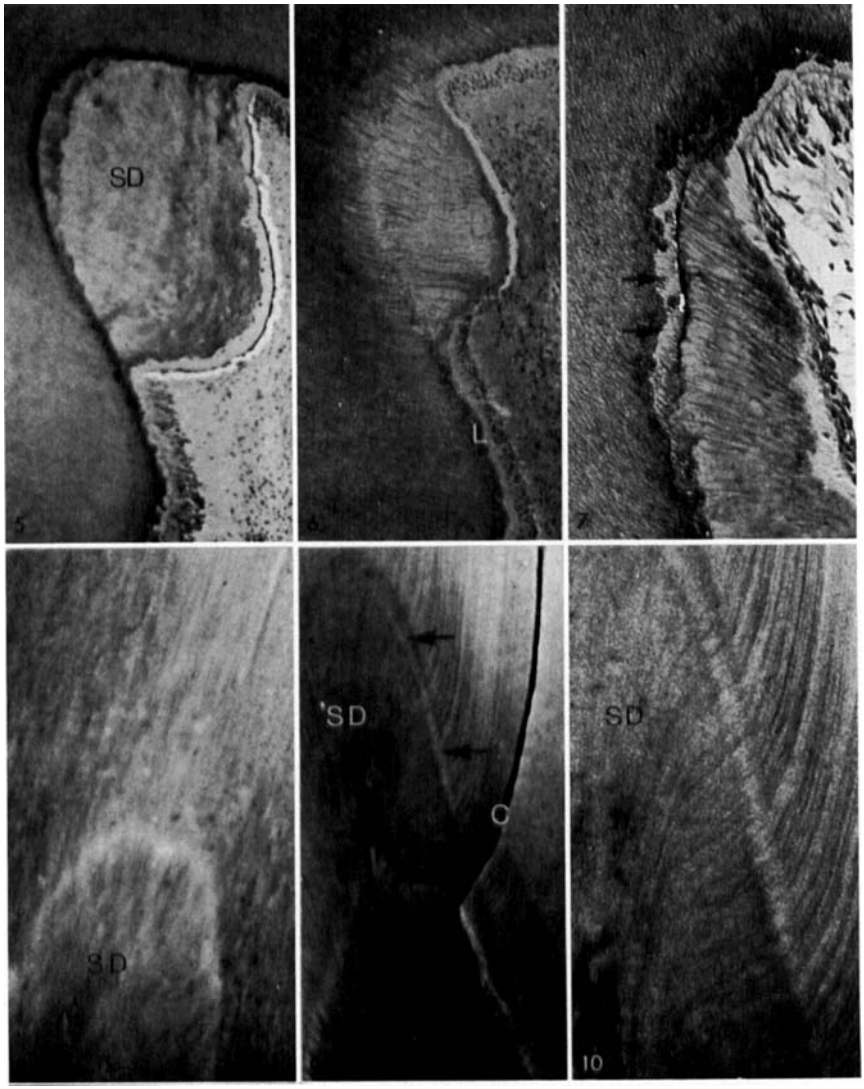
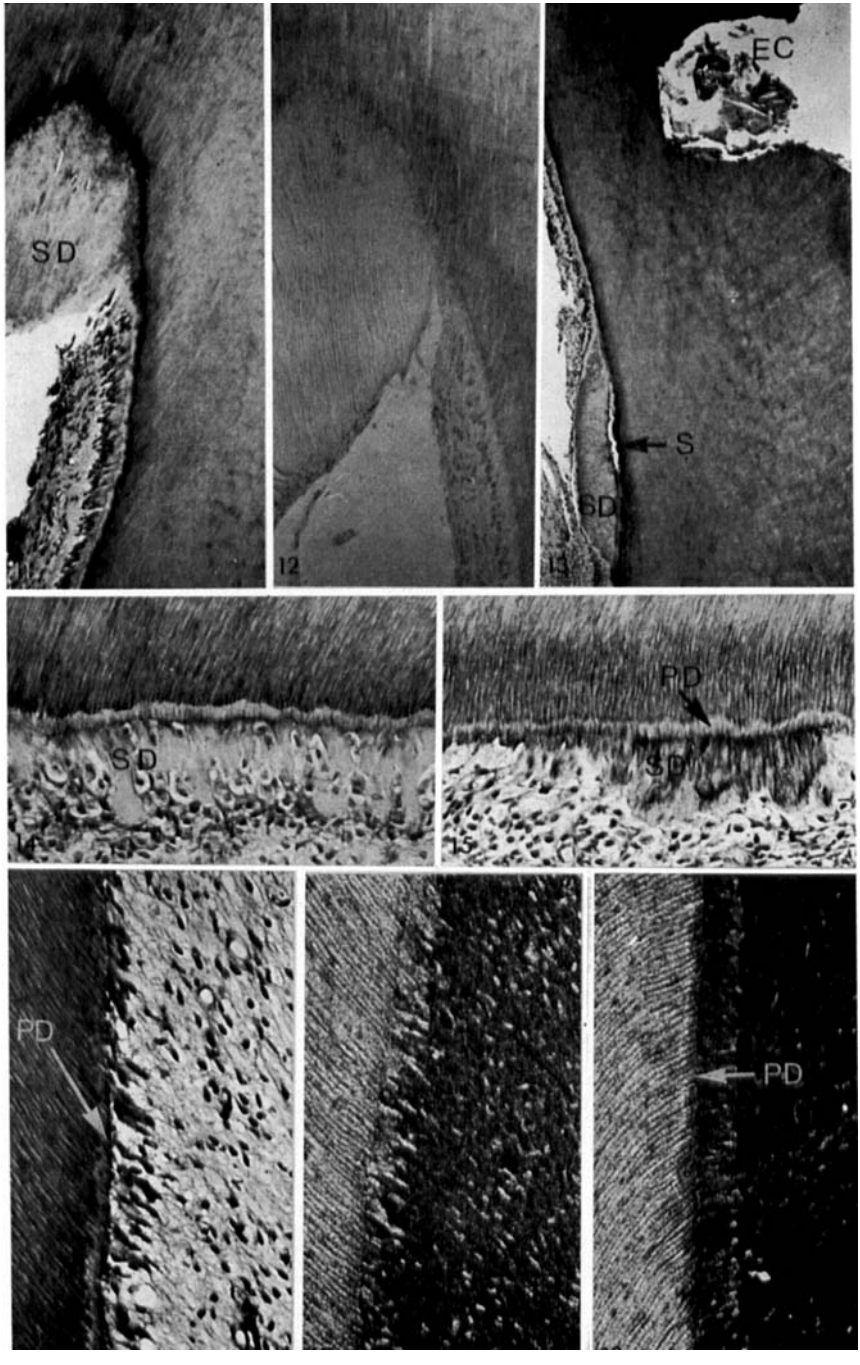


PLATE III



DISCUSSION

The present observations indicate that marked structural variations may be found at the dentine/irregular secondary dentine interface. These variations are so numerous that they cannot be directly related to the different types of destructive processes or experimental procedures. Apparently, similar conditions resulted in different types of structural alterations, both at the dentine/irregular secondary dentine interface and in the irregular secondary dentine *per se*.

Formations of irregular secondary dentine have previously been shown to be delimited from the rest of the dentine by a narrow band with staining properties and microradiographic appearances which differ from those in the adjacent dentine (e.g. *Sicher*, 1962; *Anneroth, Bergman & Welander*, 1966; *Nakamura*, 1967). This demarcation zone has been referred to as a calciotraumatic line by *Mitchell* and *Jensen* (1957) on the basis of its hematoxyphilia, and by *Nakamura* (1967), because of its stainability and increased mineral content. They imply, in other words, that it represented an alteration similar to that in the calciotraumatic response described in the rodent incisor (*Irving & Weinmann*, 1948; *Yeager & Eisenmann*, 1963). This response involves marked changes both in stainability and mineralization of the dentine; it affects the entire dentine which is formed at a given time following various systemic disturbances, and it may vary depending on the experimental conditions. It has not been conclusively demonstrated that such a response may be induced locally in the dentine. In *Koppang*'s (1967) material, for example, no such demarcation line appears to be present between the primary dentine and the irregular secondary dentine formed in local defects created by irradiation of rodent incisors. Some of the reactions observed in the present study are similar to those described for the calciotraumatic response. However, due to the great number of variations noted, it is not considered justified to classify the localized alterations found at the dentine/irregular secondary dentine interface as a calciotraumatic response.

It was of particular interest that the hematoxyphilia at the dentine/irregular secondary dentine interface was continuous with the similarly stained dentine/predentine junction in unaffected areas. The intertubular areas were markedly stained in both these regions. Similar observations have been made in normal material (zone 1 — *Mjör*, 1966 b). Considering that the dentine close to the predentine is the only area where the intertubular matrix normally stains distinctly hematoxyphilic (*Mjör*, 1966 b), it seems likely that a relationship exists between the hematoxyphilia in the two locations. Furthermore, the AB stainability of the dentine/irregular secondary dentine interface varied, a con-

dition which is also found in normal material (*Mjör*, 1968). This evidence points to the hypothesis that destructive processes may initially affect the odontoblasts and predentine to such an extent that further development is inhibited.

In support of this hypothesis, it may be pointed out that predentine-like tissue was sometimes found between the dentine and the irregular secondary dentine. In these cases the destructive processes may have resulted in a severe reaction which prevented transformation of predentine into dentine before irregular secondary dentine formation was initiated. This latter observation may explain the band of low mineral content observed at the dentine/irregular secondary dentine interface (*Nakamura*, 1967).

The localized, altered staining reactions and increased amount of organic material in the predentine present at the start of the experiments, apparently accompained by a lack of formation of new predentine, has also been noted in corticosteroid-covered dentine (*Mjör*, 1967) and in orthodontically intruded teeth (*Mjör & Stenvik*, 1969). It is likely that if irregular secondary dentine formation is subsequently initiated, the continuity between the two types of dentine will be weak, resulting in a predisposition for histologic artefacts.

The present study has demonstrated that the irregular secondary dentine subjacent to carious lesions contains more organic material than primary dentine. This is in disagreement with the observations made by *Anneroth, Bergman* and *Welander* (1966), who indicate that it is similar in both types of dentine. The permeability of dentine is particularly dependent on the amount of organic material (*Blake*, 1958), and its distribution is, therefore, important from a clinical point of view. Even though irregular secondary dentine formation must be considered as one of the main defence mechanisms of the pulp-dentine organ, it should be kept in mind that the »barrier» formed may be of inferior quality as compared to primary dentine as far as the ingress of toxic agents is concerned.

SUMMARY

The material comprised human and monkey teeth with caries or attrition. Experimental cavities were prepared in some of the monkey teeth and they were either restored or left unfilled. Stained decalcified sections as well as microradiographs of decalcified and undecalcified sections have been studied.

Marked structural variations were found at the dentine/irregular secondary dentine interface. These variations could not be directly related to the types of destructive processes or experimental procedures. A hematoxyphilic line observed at the interface was found to be continuous with the similarly stained

zone at the dentine/predentine junction in unaffected areas. A more mineralized band in this location was also continuous with a similar band in unaffected areas. Predentine-like tissue sometimes intervened between localized formations of irregular secondary dentine and the primary dentine. In some teeth with experimental cavities, the predentine had apparently mineralized without the formation of new predentine. The irregular secondary dentine varied more in mineralization than primary dentine, and generally it contained more organic material.

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RÉSUMÉ

INTERFACE ENTRE LA DENTINE ET LA DENTINE SECONDAIRE IRRÉGULIÈRE

Le matériel de cette étude comprenait des dents humaines et des dents de singes, présentant des caries ou de l'attrition. Des cavités expérimentales ont été préparées dans quelques unes des dents de singe, et certaines ont été obturées tandis que d'autres restaient ouvertes. Des coupes décalcifiées et colorées et des microradiographies de coupes décalcifiées ou non ont été étudiées.

Des variations marquées dans la structure ont été trouvées au niveau de l'interface dentine/dentine secondaire irrégulière. Ces variations ne pouvaient être directement mises en relation avec les types de processus destructifs ou de procédés expérimentaux. On a constaté qu'une ligne présentant une affinité pour l'hématoxyline et observée au niveau de l'interface, continuait la zone présentant une coloration semblable au niveau de la jonction dentine/prédentine dans les régions indemnes. Une bande plus fortement minéralisée à cet endroit continuait aussi une bande semblable dans les régions indemnes. Un tissu analogue à la prédentine était parfois interposé entre des dépôts localisés de dentine secondaire irrégulière et la dentine primaire. Dans quelques unes des dents à cavités expérimentales, la prédentine s'était apparemment minéralisée sans formation de nouvelle prédentine. La dentine secondaire irrégulière présentait plus de variations dans la minéralisation que la dentine primaire, et contenait en général plus de substances organiques.

ZUSAMMENFASSUNG

DER ÜBERGANG ZWISCHEN DENTIN UND IRREGULÄREM SEKUNDÄRDENTIN

Das Material bestand aus Menschen- und Affenzähnen die Karies oder Attrition aufwiesen. In einigen Affenzähnen wurden experimentelle Kavitäten prepariert und entweder gefüllt oder offenstehend gelassen. Dekalzinierte Schnitte wurden gefärbt und untersucht, und auch Mikroradiogramme von solchen Schnitten sowie von undekalzinierten Schnitten wurden studiert. Markierte strukturelle Variationen in der Übergangszone zwischen Dentin und irregulärem Sekundärdentin wurden observiert. Diese Variationen schienen von der Art der destruktiven Prozesse oder experimentellen Vorgänge unabhängig zu sein.

Eine hämatoxyphile Linie an der Übergangszone Dentin/Sekundärdentin war mit einer ähnlich gefärbten Linie an der Dentin/Prädentingrenze nichtaffizierter Teile zusammenhangend. Weiter konnte observiert werden, dass ein stärker mineraliziertes Band dieselben Verhältnisse aufwies. Prädentinähnliches Gewebe erschien sporadisch zwischen dem Dentin und dem irregulären Sekundärdentin.

Einige Zähne in welchen experimentelle Kavitäten präpariert worden waren, zeigten mineraliziertes Prädentin ohne Bildung von neuem Prädentin auf. Das irreguläre Sekundärdentin variierte mehr bezüglich der Mineralization als das reguläre Dentin, und enthielt im allgemeinen mehr organisches Material.

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Address:
Ivar A. Mjör,
Department of Anatomy,
Dental Faculty,
University of Oslo,
Blindern, Oslo 3, Norway

Kjell Karlsen,
Clinic for Prosthetics,
Geitmyrsveien 71,
Oslo 4, Norway