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DIFFERENTIATION OF TWO LAYERS OF CARIOUS DENTIN BY STAINING

BY

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ABSTRACT

In order to differentiate the layers physiologically recalcifiable and unrecalcifiable of carious dentin, a variety of dyes in various solvents were tried and a 0.5% basic fuchsin-propylene glycol solution was found to stain specifically the superficial layer of carious dentin distinctly and not to stain the deeper layer at all. The relation of the fuchsin-staining front to the softening, natural discoloration, and bacterial invasion fronts was investigated on the sections of 41 freshly extracted human teeth with dentin caries. The softening front was always the deepest, the discoloration front the next, and the bacterial front was the most superficial. The fuchsin-stained front roughly corresponded to the bacterial front and the former was usually ahead in acute decays and behind in chronic decays.

INTRODUCTION

Complete elimination of soft dentin has so far been a widely accepted clinical requirement[1–3]. Clinical excavation by feeling hardness, however, always removes only the very soft dentin leaving the intermediately softened pathologic dentin[4]. Hardness is thus no reliable clincal criterion in caries excavation.

Fusayama, Okuse, and Hosoda[5] revealed that the start of softening or decalicification was always the most advanced in carious dentin, the discoloration the next, and the bacterial invasion the last. This finding may indicate that complete removal of discolored dentin can assure complete removal of infected dentin, but clinical differentiation of discolored and undiscolored dentin layers is difficult, particularly in acute caries with faint discoloration.

On the other hand, Kato and Fusayama[6] recognized two layers in carious dentin experimentally produced in dog teeth by using an acid. The first decalcified layer was very soft with very low calcium content and could not be recalcified although a mere calcium deposition from saliva or

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capping material could occur very slightly on it. The second decalcified layer was harder with intermediate decalcification and could be highly recalcified by vital reaction. Such a second layer which is recalcifiable is considered to be preferably saved, as many clinical investigators have recommended to save a part of softened dentin to avoid pulp exposure[7-15]. Differentiation of the two layers, however, is also difficult in clinics.

This study was planned, to find a means for differentiating the carious dentin layers to be removed and to be saved by using a dye.

Materials and Methods

Preparation of specimens. Forty-one freshly extracted human teeth with dentin decays not reaching the pulp were stored in a 10% formalin solution for 1 to 14 days and longitudinally sectioned through the center of decay by using a thin-sectioning machine with a water coolant. The section was cut into a plate approximately 2 mm thick and its surface was polished by the metallurgical technique with a dilute alumina suspension.

Hardness determination and color observation. The specimen was fixed on a black compound block and placed under the Knoop indentor of a microhardness tester loading 50 g for 15 seconds. The hardness was determined every 50 μ from the decay surface to the pulp chamber wall parallel to dentinal tubules through the center of decay (Fig. 1-left). For

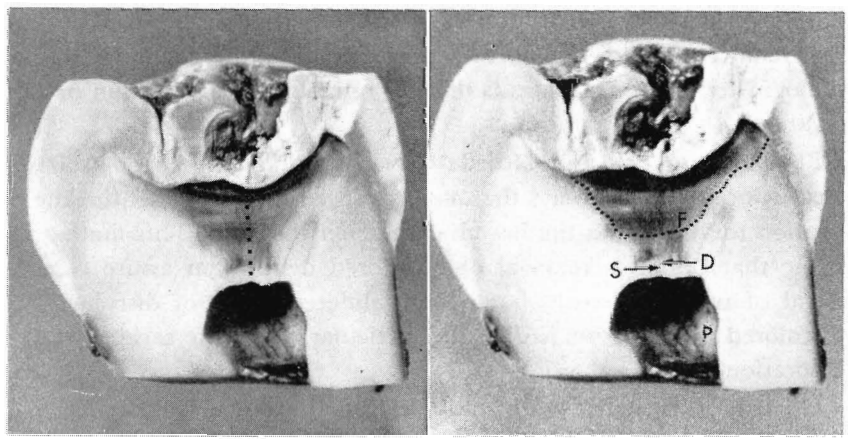

Fig. 1. *Left–A* tooth was vertically sectioned and the Knoop hardness of dentin surface was determined through the center of decay as shown by a dotted line. *Right*–The section surface was then stained with 0.5% basic fuchsin-propylene glycol solution for 5 seconds. The boundaries of the stained area was marked by dotted line since the violet-red color did not develop clearly in the black-and-white photograph.

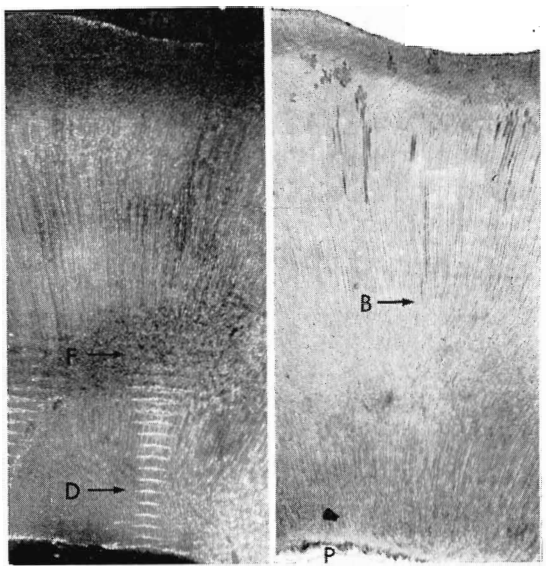

Fig. 2. Comparison of the Knoop indentation series observed on the dentin surface (*left*) and the bacterial invasion observed on the decalcified section (*right*) of the same area. B=bacterial invasion front, F=fuchsin-staining front, D=natural discoloration front, and P=pulp chamber wall.

preventing the hardness change by drying, the specimen was stored in water and the hardness determination was performed in minimum time. The hardness-depth curve was thus recorded and the spot of the very beginning of softening adjacent to the normal dentin layer was termed the softening front and marked as S on the curve.

The discoloration was observed on the same surface and its front was marked by a small cut with a razor blade. The location of the mark on the indentation series was recorded as D on the hardness-depth curve.

Fuchsin staining. After trying a variety of dyes (iodine tincture, Mercurochrome, Eosin, Safranine, Alcian Blue, Methyl, Violet, Methylene Blue, Rose Bengal, Vermail, Sudan-III, acid Fuchsin, and Nile Blue) of various concentrations in various solvents, 0.5% basic Fuchsin solution in propylene glycol was selected for the staining. The section surface was covered with this dye for 5 seconds and then washed with water. Only the superficial half of the decayed dentin was clearly stained violet-red (Fig. 1-right). The fuchsin-staining front was recorded as F on the hardness curve.

The stainability of dentin was not affected by the formalin fixation of the specimens. This fact was confirmed in a preliminary experiments and also supported by previous reports by Miller[16] and Wirthlin[17].

Bacterial detection. The specimen was then fixed in a 10% neutral formalin solution, decalcified in 10% formic acid for 30 days, and embedded in paraffin. A 6 μ section was cut from the layer nearest the bisected surface and stained by the Gram-Weigert technique for detecting gram-positive bacteria (Fig. 2). The gram-negative staining was also tried but there were found no gram-negative bacteria. The level of the deepest penetration of bacteria in a 500 μ wide zone along the hardness determination line was defined as the bacterial invasion front and recorded as B on the hardness curve.

Table 1. Depth and hardness of decayed dentin of comparatively acute type at the fronts of bacterial invasion, fuchsin-staining, and natural discoloration

	Bacterial invasion front		Fuchsin staining front		Discoloration front	
No.	Distance from normal dentin (μ)	Dentin hardness (K.H.N.)	Distanse from normal dentin (μ)	Dentin hardness (K.H.N.)	Distance from normal dentin (μ)	Dentin hardness (K.H.N.)
1	1220	<5.6	1200	<5.6	200	28.9
2	1570	〃	1270	〃	50	68.4
3	1390	〃	1000	〃	0	65.8
4	1110	〃	1150	〃	50	60.0
5	810	〃	650	〃	180	43.0
6	1150	〃	850	〃	600	20.8
7	1020	〃	650	〃	50	37.9
8	1300	〃	950	5.9	900	7.7
9	1900	〃	1300	6.3	200	67.1
10	1480	〃	900	7.2	900	7.2
11	1060	〃	750	7.5	750	7.5
12	850	〃	750	7.7	50	59.9
13	1830	〃	900	10.6	0	41.5
14	1200	〃	450	11.0	80	34.0
15	750	〃	630	11.1	250	44.1
16	1250	〃	750	11.9	470	51.0
17	2190	〃	1210	13.5	0	53.8
18	1130	〃	950	14.7	400	63.3
19	1650	〃	1100	18.5	0	74.1
20	560	〃	130	27.5	130	27.5
21	1070	〃	300	33.8	300	33.8
22	1700	〃	200	54.8	200	54.8
Avr.	1281	<5.6	820	<12.8	262	43.3

Table 2. Depth and hardness of decayed dentin of comparatively chronic type at the fronts of bacterial invasion, fuchsin-staining, and natural discoloration

	Bacterial invasion front		Fuchsin staining front		Discoloration front	
No.	Distance from normal dentin (μ)	Dentin hardness (K.H.N.)	Distance from normal dentin (μ)	Dentin hardness (K.H.N.)	Distance from normal dentin (μ)	Dentin hardness (K.H.N.)
1	600	6.4	680	< 5.6	0	63.3
2	1330	6.5	1200	9.8	250	46.3
3	480	7.0	450	8.6	200	50.3
4	730	9.0	680	10.4	150	44.8
5	450	9.1	200	28.1	200	28.1
6	1160	9.3	1350	5.6	0	43.4
7	350	11.8	350	11.8	100	52.9
8	440	12.5	700	6.3	100	71.2
9	500	12.7	550	9.5	250	55.7
10	570	14.0	600	12.8	450	21.0
11	780	15.0	680	19.5	130	65.0
12	370	15.0	550	7.4	50	51.1
13	610	15.3	950	< 5.6	50	63.3
14	830	16.5	950	18.3	250	58.8
15	1030	17.5	1100	14.3	450	57.8
16	1000	18.1	1480	12.4	200	56.7
17	890	18.7	1540	6.4	0	54.8
18	790	22.7	1250	< 5.6	0	55.7
19	640	28.0	850	11.7	280	47.6
20	340	42.5	980	< 5.6	180	67.5
21	310	43.0	1070	11.5	150	54.8
22	910	44.8	1620	< 5.6	110	56.7
23	420	45.5	1100	24.6	350	55.7
24	200	59.9	650	19.3	0	75.6
Avr.	655	20.9	897	<11.5	163	54.1

Results

Results were obtained from 46 decays of 41 teeth. The softening front (S) was always the deepest, the discoloration front (D) the next, and the bacterial front (B) the most superficial. By analysis of the relationship of the fuchsin-staining front (F) to the other fronts, the cases were classified into comparatively acute group of 22 cases having unmeasurably low hardness (lower than KHN 5.6) at the bacterial front and comparatively chronic group of 24 cases having measurable hardness higher than KHN 5.6 (Tables

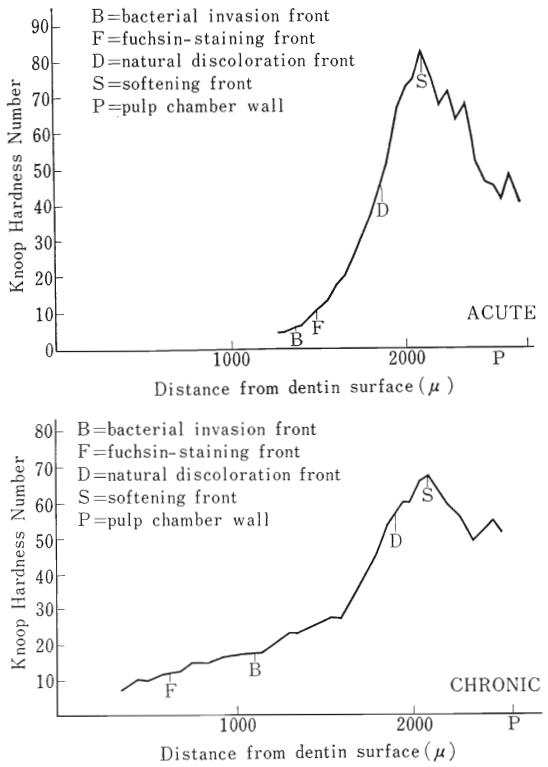

Fig. 3. Examples of hardness-depth curves of acute (*top*) and chronic (*bottom*) type decayed dentins with the marks of fronts of softening (S), natural discoloration (D), fuchsin-staining (F), and bacterial invasion (B).

1 and 2, Fig. 3).

Fuchsin-staining and hardness. Although the violet-red staining by fuchsin was not clearly visible in the black-and-white photograph, it was quite remarkable when observed by naked eyes or on color pictures, with definite boundaries between stained and unstained layers. In the acute decays, the staining generally inclined to be heavy with low dentin hardness at the staining front, whereas in the chronic decays the staining generally inclined to be light with comparatively higher dentin hardness at the front. The presence of heavier natural discoloration seemed to prevent the fuchsin staining to some extent, completely when very heavy.

Location of fuchsin-staining front. The fuchsin-staining front (F) never exceeded or reached the softening front (S) and was usually far behind. It was also generally behind the natural discoloration front (D) though it almost reached the discoloration front in some comparatively

soft and acute type cases. There were also more than 10 cases of staining fronts more than 1000 μ behind the discoloration front among both acute and chronic type cases.

The staining front very roughly corresponded to the bacterial invasion front (B), that is, the staining front was mostly ahead in acute and soft type cases and behind in chronic and hard type cases, with maximum distances of respectively 1500 μ from and 780 μ to the bacterial front.

Discussion

Fuchsin-propylene glycol staining. A number of researchers[16–22] have so far tried various staining techniques for the purpose of detecting permeability, acidity, or presence of various proteins, fats, and lipids in carious dentin. The present study was planned to find a staining technique to differentiate the first decalcified layer, heavily decalcified and unrecalcifiable, and the second decalcified layer, intermediately decalcified and physiologically recalcifiable, which were previously observed by Kato and Fusayama in dog teeth[6].

The 5-second staining with a 0.5% basic fuchsin-propylene glycol solution was found to stain the superficial layer of decalcified dentin distinctly and not the deep layer at all. Propylene glycol seemed to promote the dye penetration. Two reasons can be taken into consideration for the decayed dentin to become stainable to fuchsin. The first was that the organic substance was exposed to the dye by removing inorganic cover of it by decalcification[23–25]. The second was that the organic substance itself deteriorated by the effect of acid, enzyme or others[26]. If the first were the only reason, all layers of softened dentin should be stained to some degree, but stained and non-stained layers were clearly distinguished in this study. This fact indicated that the second was the main reason and the stained layer seemed to correspond to the necrotic layer so-called by Miller and Massler[18].

The physiological recalcification by vital reaction is considered to occur on the base of sound collagen fibers[27,28]. If the stained layer was the layer of deteriorated collagen, it could possibly correspond to the first decalcified layer which was not physiologically recalcifiable[6], though this fact should be confirmed by future study.

Natural discoloration and fuchsin staining. The fuchsin staining front was always behind the natural discoloration front and the staining was generally light or none in the chronic decays with heavier natural discoloration. Participation of the melanoidin pigment[29,30], seemed to pre-

vent the staining by covering the collagen fibers which had been partly exposed by intermediate decalcification[23-25].

The fact that the staining was quite remarkable in acute decays with lighter natural discoloration and so difficult to be clinically distinguished may suggest the usefulness of this staining in possible clinical application.

Bacterial invasion and fuchsin staining. The depth of the fuchsin-staining front (F), though very roughly, corresponded to that of the bacterial front (B). It is quite certain that, in acute decays, complete removal of the fuchsin-stainable layer results in a complete removal of infected dentin. On the other hand, the bacterial front was sometimes deeper than the staining front in chronic decays. The vitality of bacteria confined in such deep hard tissues of chronic decay is, however, considered to be low and insignificant[31-35]. A complete removal of the fuchsin-stainable layer might be sufficient to prevent the recurrence of decay on cavity floor. The staining in this study was, however, tried only on the section surfaces of extracted teeth. Clinical usefulness of this staining will be known by further investigation.

Conclusion

In order to differentiate the physiologically recalcifiable and unrecalcifiable layers of carious dentin, a variety of dyes in various solvents were tried and a 0.5% basic fuchsin-propylene glycol solution was found to stain the superficial layer of carious dentin distinctly and not to stain the deep layer at all. The relationship of the fuchsin-staining front to the softening, natural discoloration and bacterial invasion fronts was investigated on the section of 41 freshly extracted human teeth. The following findings were obtained:

(1) The softening front was always the deepest, the discoloration front the next, and the bacterial front the most superficial. The fuchsin-staining front roughly corresponded to the bacterial front and the former was usually ahead in acute decays and behind in chronic decays.

(2) The fuchsin-stained layer might possibly correspond to the first decalcified dentin layer with deteriorated collagen, but it must be confirmed by future study.

(3) The acute softer type of decayed dentin with lighter natural discoloration was heavily stained by fuchsin while the chronic harder type of decayed dentin was lightly stained.

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