

PHYSIOLOGICAL RECALCIFICATION OF CARIOUS DENTIN

BY

Hitoshi MIYAUCHI, Masaaki IWAKU and Takao FUSAYAMA*¹

ABSTRACT

In order to confirm the ability of physiological recalcification of the human carious dentin, the first layer of carious dentin was removed from the symmetric cavities of bilateral pairs of human teeth, disclosing it by 0.5% basic fuchsin-propylene glycol solution staining. One of the pair teeth was immediately extracted and the other was left in the mouth after filling the cavity with polycarboxylate cement. The Ca content and hardness of the remaining second layer immediately and three months after the operation were compared by an electron probe micro-analyzer and microhardness tester. They increased markedly after three months returning to the normal level from inside, proving physiological recalcification.

A similar experiment was performed by using bilateral pairs of dog teeth with cavities having artificially decalcified dentin floor. After removing the fuchsin-stainable first layer, one of the pair was immediately extracted and the other was left in the mouth for three months after exposing or filling the cavity with various cements. As the Ca content was compared, a marked recalcification of the second layer of softened dentin was observed after three months returning to the normal level from the inside. The effect of different cavity treatments was slight.

INTRODUCTION

Complete removal of carious dentin has been considered an essential requirement for restoration of carious teeth¹⁻³. Softening and discoloration were the criteria for the carious dentin. Terashima *et al.*⁴, however, found a considerable thickness of the pathologic dentin in the cavity floor after the clinical removal of the carious dentin using such criteria and appreciable deviation in the hardness of the remaining dentin. Fusayama *et al.*⁵ found that the discoloration front and bacterial invasion front of dentin were fairly hard and close to the softening front in chronic decay while they were very soft with a great distance among the three fronts in acute decay. These facts indicate that hardness is not a reliable guide for removal of in-

fectured softened dentin and that discoloration is also undependable in acute decay.

Kato and Fusayama⁶) found that artificially decalcified softened dentin of dog teeth was composed of two layers. The first (outer) layer was highly decalcified and was physiologically unrecalcifiable. The second (inner) layer was intermediately decalcified and was physiologically recalcifiable. Kurosaki and Fusayama⁷) found the outer and inner layers to be of different character also in the carious dentin of human teeth. Fusayama and Terashima⁸) found that a 0.5% basic fuchsin-propylene glycol solution clearly stained the outer layer of the softened dentin but not the inner layer at all.

Sato and Fusayama⁹) confirmed that bacterial infection occurs in the fuchsin-stainable first (outer) layer but never in the

*¹ 宮内 均・岩久正明・総山孝雄: Department of Operative Dentistry (Chief: Prof. T. FUSAYAMA), School of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).

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fuchsin-unstainable second (inner) layer. Ohgushi and Fusayama¹⁰⁾ found that in the artificially decalcified dentin as well as in the natural carious dentin of the extracted human teeth the first layer lost the characteristic structure of collagen fibers and contained irregularly scattered granular inorganic crystals. The second layer, although partially decalcified, had essentially normal collagen fibers, with apatite needle crystals regularly attached to them and had odontoblastic processes remaining. Kuboki, Ohgushi and Fusayama¹¹⁾ found that the intermolecular cross-links of the collagen fibers were irreversibly broken in the first layer while they only shifted reversibly to the precursor form in the second layer. These facts lead the authors to speculate that the first (outer) layer is physiologically unrecalcifiable but the second (inner) layer is recalcifiable in the natural carious dentin of human teeth, as had been shown in the artificially softened dentin of dog teeth by Kato and Fusayama⁹⁾.

This study was undertaken to confirm the ability of physiological recalcification of the second (inner) layer of the carious softened dentin of human teeth. The effect of cavity treatment on recalcification was also investigated.

MATERIALS AND METHODS

Human tooth specimens Three bilateral pairs of third molars having symmetric dentin caries not reaching the pulp and expected to be extracted were used. Under local anaesthesia with 2% Xylocaine, the pairs of carious cavities were opened with a round bur mounted on a low-speed electric engine. After washing and drying, a drop of 0.5% basic fuchsin-propylene glycol solution (hereafter abbreviated as fuchsin solution) was applied to each cavity and immediately flushed with water. Using a low-speed round bur, the remaining fuchsin-stained first layer was completely removed, leaving all of the unstained second layer (Fig. 1 and 2).

One of the bilateral pair teeth was immediately extracted and used for control. The other was filled with the polycarboxylate cement, extracted after 3 months and examined.

Dog tooth specimens Forty bilateral pairs were used including the upper and lower canines and first molars from ten adult dogs. Under general anaesthesia by intramuscular injection of 5.8% hydrochloric Ketamine solution at the rate of 20 mg/kg and by intravenous injection of

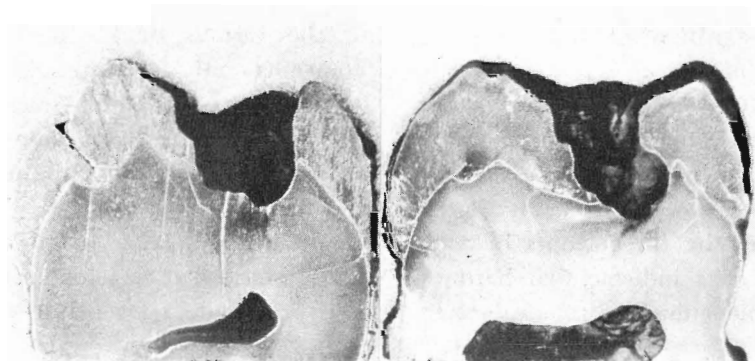


Fig. 1. Sections of bilateral human teeth having symmetric decay extracted immediately (left) and 3 months (right) after treatment.

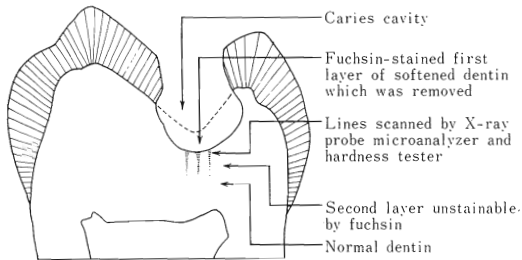


Fig. 2. Schema of human tooth section through the center of decay.

5% sodium pentobarbital solution at the rate of 10 mg/kg, round class V cavities were prepared on each facial surface by using a diamond point*² mounted on an air turbine with a spray coolant. The diamond point had a brim at 1.5 mm from the tip, which allowed cutting of the cavities to a uniform depth (approximately 1 mm in the dentin) and thereby an equal thick-

ness of floor dentin was obtained in the bilateral pair. The prepared pair of teeth was isolated with a rubber dam and a specially prepared frame⁶⁾. The cavity floor dentin was decalcified by dropping Plank & Rychlo's decalcifying solution¹²⁾ at the rate of 1 drop per second for 20 minutes and then neutralizing with 1% NaOH solution. After washing and drying, the fuchsin solution was applied and immediately flushed. Only the first layer of the softened dentin which was heavily stained red was carefully removed with a sharp long spoon excavator, leaving all of the unstained second layer (Fig. 3 and 4).

One of the bilateral pair teeth was immediately cut off with a diamond disk at the level of its cervix and used as control. The other was left in the mouth after treating in one of the following four ways:

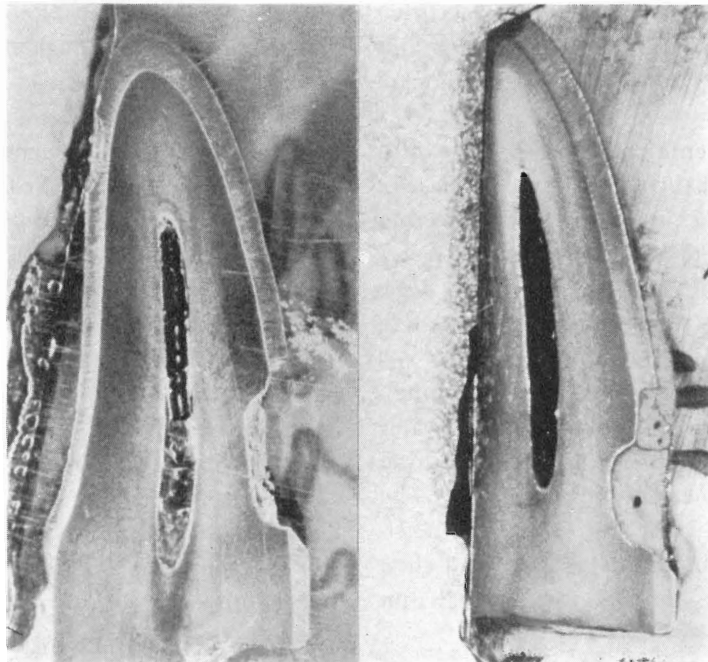


Fig. 3. Sections of bilateral dog teeth having cavities with artificially decalcified dentin floor taken out immediately (left) and 3 months (right) after treatment.

** FG456, Shofu Co., Ltd., Kyoto, Japan.

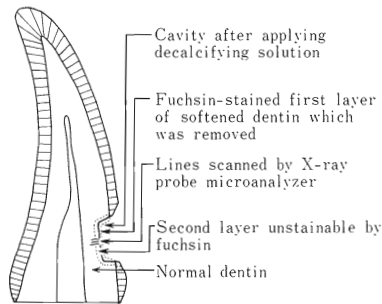


Fig. 4. Schema of dog tooth section through the center of artificially decalcified dentin floor.

(1) The cavity was left open without filling.

(2) The cavity floor was capped with the zinc oxide eugenol cement*³ and then covered with the polycarboxylate cement*⁴.

(3) The cavity was directly filled with the polycarboxylate cement.

(4) The cavity floor was capped with the zinc oxide eugenol cement containing calcium hydroxide as 50% of the powder and then covered with the polycarboxylate cement.

The experimental teeth were taken out, cutting them off at the level of the cervix after sacrificing the dog by injecting strychnine nitrate after 3 months.

Preparation of the tooth specimens The tooth specimens were immediately fixed in 10% neutral formalin solution.

The human tooth specimen was longitudinally sectioned under running water through the center of the carious lesion with a hard tissue-sectioning machine. The section surface was polished with a whetstone followed by diamond paste and then subjected to the hardness test and calcium content analysis.

Each dog tooth specimen was dehydrated

by using alcohol of increasing concentration, immersed in a styrene monomer for 3 days and embedded in a polyester resin. It was longitudinally sectioned under water through the center of the cavity with a hard tissue-sectioning machine. The section surface was polished as described above and subjected to calcium content analysis.

Hardness determination Hardness was determined only on the natural carious dentin since the thickness of the artificially decalcified dentin was too small to determine the hardness. After fixing the section horizontally on a modelling compound block, it was tested with the Knoop indenter under a 50 g load for 15 seconds. Contact was made every 50 μm from the cavity floor to the depth of 1,000 μm towards the pulp through the central part of the decay. This was repeated 3 to 4 times.

Calcium content analysis After vacuum plating with carbon vapor, the calcium content was determined by line analysis with an electron probe microanalyzer*⁵ with 3×10^3 cps/ 10^{-8} A and 20 kv accelerating voltage for $\text{CaK}\alpha$ characteristic X-ray, scanning 3 to 4 times through the central part of the lesion from the cavity floor to the depth of 1,000 μm , following the traces of hardness determination on the natural carious dentin and to the depth of 60 to 70 μm on the artificially decalcified dentin.

For the analysis of the calcium content or hardness change during the period of the experiment, a curve from each specimen 3 months after the operation was superimposed on a curve from its contralateral specimen immediately after the operation.

Eleven pairs of curves from the three pairs of human teeth immediately and 3

*³ Eugenol Cement, Showa Yakuhin Kako Co., Ltd., Tokyo, Japan.

*⁴ Carlon, Sankin Industrial Co., Ltd., Osaka, Japan.

*⁵ JSM-U3, Japan Electron Optics Laboratory, Tokyo, Japan.

months after operation were compared for the value of the Ca content or the hardness at the depth of 100 μm , which was roughly the middle of the second layer. Forty pairs of curves from the forty pairs of dog teeth immediately and 3 months after operation were also compared for the Ca content at the 20 μm depth which was roughly the middle of the second layer of artificially decalcified dentin.

RESULTS

Change in the second layer of natural

carious dentin The curves from the experimental cases superimposed on the correspondent curves from the control cases showed the following facts (Fig. 5):

Case 1: This case seemed to be chronic caries having only a slight decrease in the Ca content and hardness in the second layer. The immature dentin near the pulp appeared at the end of the curves, for the cavity floor was near the pulp. The initial curves showed the level of normal dentin at the depth of 500 to 800 μm and a slight decline of the second layer of the softened

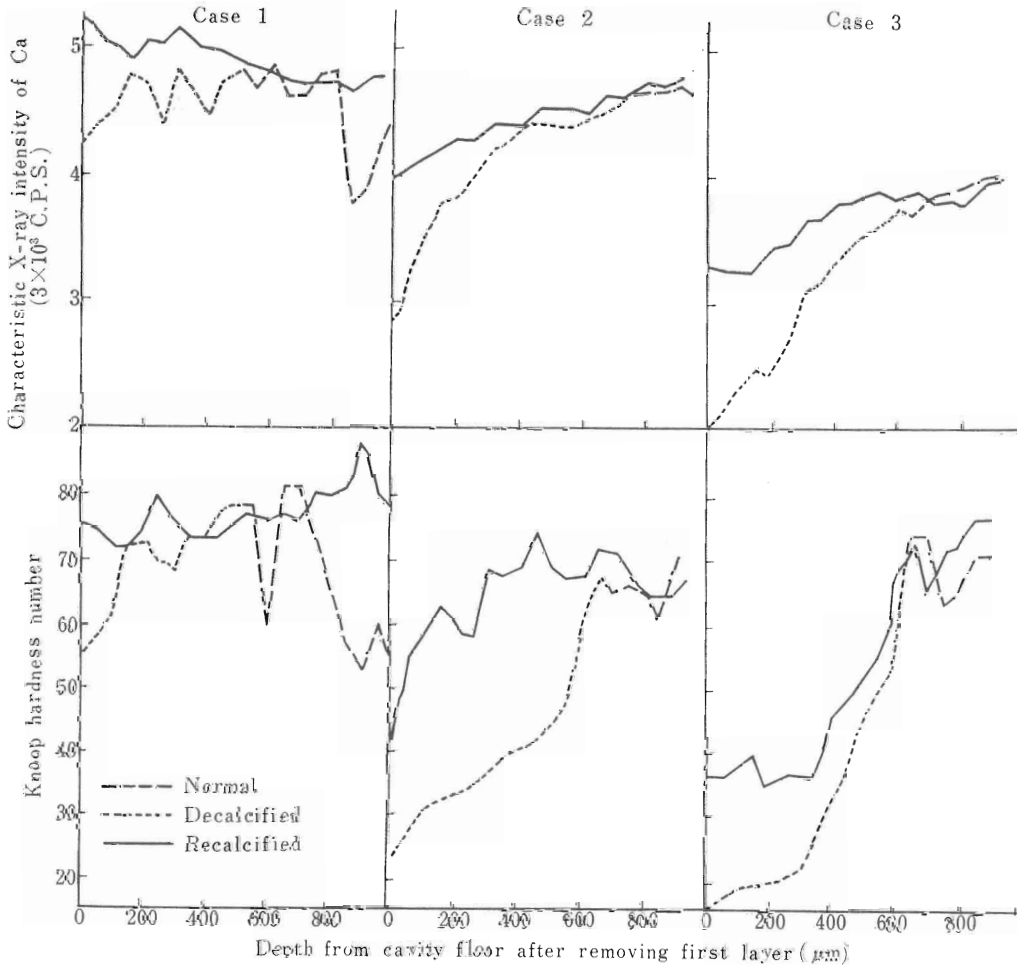


Fig. 5. Representative curves of Ca content and hardness according to depth of second layer of human carious softened dentin immediately and 3 months after removal of first layer.

dentin at the depth of less than 500 μm . In this layer the Ca content and hardness returned to the normal level after 3 months. The increase in the Ca content and hardness found in the layer close to the pulp seemed to be due to maturation.

Case 2: This case had a shallow cavity. The initial curves showed the level of normal dentin at the depth of more than 600 μm and a slight decline of the second layer at the depth of less than 600 μm . After three months, the Ca content increased remarkably at the depth of less than 400 μm and slightly at the depth of 400 to 600 μm . The hardness increase was more remarkable, returning to the normal level at the depth of 400 to 600 μm .

Case 3: This case was an acute caries of a young tooth soon after eruption, hav-

ing generally a low hardness. The initial curves showed the level of the normal dentin at the depth of more than 600 μm and a considerable decline of the second layer at the depth of less than 600 μm . Even with such a drop in the Ca content and hardness, the recovery was marked after three months.

The representative values of the Ca content and hardness at the depth of 100 μm in all of the individual series of measurements were also compared, obtaining the following facts (Table 1): After three months, the Ca content and hardness were found to be increased in all parts measured, without exception. The average increase in the Ca content was 0.50, 0.75 and 0.52 and that in the hardness was 10.7, 30.3 and 26.0, respectively, in Cases 1, 2 and 3, show-

Table 1. Ca content and hardness of second layer of human carious softened dentin of depth of 100 μm immediately and 3 months after removal of first layer

Case and series of measurements		Characteristic X-ray intensity (3×10^3 c.p.s.) of Ca			Knoop hardness number		
		Immed.	3 Mon.	Increase	Immed.	3 Mon.	Increase
Case 1	1	4.23	4.85		62.0	68.5	
	2	4.50	4.93		61.0	71.8	
	3	4.40	5.05		52.0	44.0	
	4	4.05	4.35		58.0	71.5	
	av.	4.30	4.80	0.50	53.3	64.0	10.7
Case 2	1	3.20	4.25		40.0	60.5	
	2	2.80	3.60		27.0	58.5	
	3	3.50	4.13		30.5	58.5	
	4	4.01	4.55		25.5	66.5	
	av.	3.38	4.13	0.75	30.8	61.0	30.3
Case 3	1	3.90	4.10		21.0	61.0	
	2	2.30	3.25		18.0	38.0	
	3	3.95	4.35		37.0	55.0	
	av.	3.38	3.90	0.52	25.3	51.3	26.0

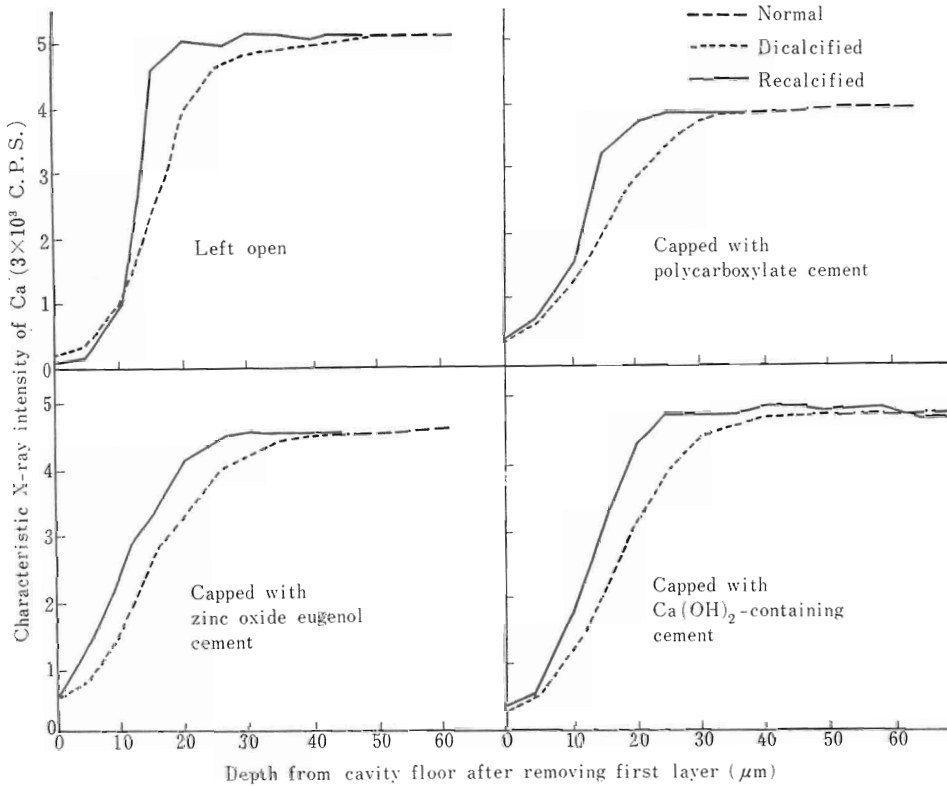


Fig. 6. Comparison of representative curves of Ca content according to depth of second layer of artificially decalcified softened dentin of dogs immediately and 3 months after removal of first layer.

ing that the more the Ca content increased, the more the hardness increased.

Change in the second layer of artificially decalcified dentin The effect of the four different treatments on recalcification was investigated by comparing the representative Ca content curves of the individual groups immediately and three months after the operation (Fig. 6).

(1) Group left open. After three months, the Ca content seemed to be slightly decreased at the depth of less than 10 μm in many cases, but it was found to be markedly increased at the depth of more than 10 μm , returning to the level of the normal dentin at the depth of more than 20 μm in all cases.

(2) Group capped with zinc oxide euge-

nol cement. The Ca content increase was found also in the superficial layer and was almost uniform at the depth of 5 to 25 μm . The Ca content returned to the level of the normal dentin at the depth of more than 25 μm without further increase.

(3) Group capped with polycarboxylate cement. The Ca content increase was slight at the superficial layer and increased markedly with depth. The Ca content returned to the level of the normal dentin at the depth of more than 25 μm .

(4) Group capped with $\text{Ca}(\text{OH})_2$ -containing cement. The Ca content increase was found even immediately under the cavity floor and gradually increased up to the depth of 20 μm . The Ca content returned to the level of the normal dentin

Table 2. Characteristic X-ray intensity (3×10^3 c.p.s.) of Ca of second layer of artificially decalcified softened dentin of dogs immediately and 3 months after removal of first layer

Treatment	Left open			Capped with ZOE cem.			Capped with PC cem.			Capped with Ca (OH) ₂ cem.		
	Immed.	3 Mon.	Increase	Immed.	3 Mon.	Increase	Immed.	3 Mon.	Increase	Immed.	3 Mon.	Increase
1	2.55	3.80	1.35	3.30	4.10	0.80	2.95	3.20	0.25	3.93	4.88	0.95
2	2.23	3.50	1.27	3.05	3.37	0.32	3.05	4.00	0.95	3.33	4.43	1.10
3	4.27	5.03	0.76	3.15	4.00	0.85	3.90	4.65	0.75	3.50	4.85	1.35
4	3.65	4.95	1.30	2.87	3.73	0.86	2.70	3.67	0.97	3.75	4.55	0.80
5	3.87	5.05	1.18	3.30	4.13	0.83	3.33	4.10	0.77	3.12	4.40	1.28
6	3.97	4.70	0.73	3.17	3.60	0.43	3.35	4.35	1.00	3.58	4.60	1.02
7	2.65	3.98	1.33	3.33	3.70	0.37	3.40	4.20	0.80	3.85	5.20	1.35
8	2.95	4.05	1.10	4.60	4.90	0.30	2.80	3.50	0.70	3.85	5.25	1.40
9	3.05	3.80	0.75	3.48	3.95	0.47	2.93	3.70	0.77	3.35	4.13	0.78
10	2.98	3.95	0.97	3.70	4.40	0.70	2.60	3.30	0.70	3.15	4.30	1.15
Average	3.22	4.28	1.07	3.40	3.99	0.59	3.10	3.87	0.77	3.54	4.66	1.12
St. dev.			0.26			0.22			0.20			0.22

at the depth of more than 25 μm .

The representative values of the Ca content at the depth of 20 μm in all individual series of measurements were also compared, obtaining the following facts (Table 2): After three months, the Ca content was found to be increased in all groups as well as in all cases compared. The average increase was 1.07, 0.59, 0.77 and 1.22, respectively, in the group left open (1), in the group capped with the zinc oxide eugenol cement (2), in the group capped with the polycarboxylate cement (3) and in the group capped with the $\text{Ca}(\text{OH})_2$ -containing cement (4). The values of (1) and (4) were greater than those of (2) and (3), the difference, though slight, being statistically significant at the level of 95% confidence limit. No significant difference was found between (1) and (4) and between (2) and (3).

DISCUSSION

Physiological recalcification of the second layer of natural carious dentin Many authors reported that extracted teeth were recalcified when immersed in calcifying solutions¹³⁻¹⁷. A clinically significant reinforcement by such a penetration of calcium from the outside may be expected in the enamel composed almost purely of inorganic material, but it is quite doubtful in the dentin which is a vital tissue having a high content of organic material. It was reported that the radioopacity¹⁸ or the phosphorus content¹⁹ in the carious dentin of vital teeth increased when capped with $\text{Ca}(\text{OH})_2$, but it was not clarified whether it was a mere penetration of calcium from the outside or a physiological recalcification by the vitality of teeth.

A definite physiological recalcification of the softened dentin was first observed experimentally by Kato and Fusayama using dog teeth⁶. They produced an artificially

decalcified dentin by using a decalcifying solution and capped the cavities for given periods. A slight calcium increase due to the penetration from the outside was observed in the first layer of the softened dentin of both vital and pulpless teeth, but a significant recalcification was observed only in the second layer of the softened dentin of vital teeth.

In this study, physiological recalcification of the natural carious dentin of human teeth was confirmed experimentally and a definite restoration of the calcium content and hardness was found in the fuchsin-unstainable second layer of softened dentin in all cases of the vital teeth after three months. The calcium content and hardness returned completely to the level of normal dentin in Case 1, and the curves of Cases 2 and 3 also seemed to be returning to the normal level after some time.

Effect of various cavity treatments on physiological recalcification Kato and Fusayama⁶ found physiological recalcification only in the second layer but not in the first layer in their experiment using the artificially decalcified dentin of dog teeth. In this study, in which the first layer was removed beforehand, the Ca content increased markedly in the remaining second layer, returning to the level of the normal dentin from the inside three months after all four treatments. The Ca increase was always more remarkable in the layer closer to the normal dentin forming an extension of the plateau of the normal dentin. It is, therefore, obvious that it was a physiological recalcification from the inside.

When the cavity was left open, recalcification at the depth of more than 10 μm seemed to be accelerated by the stimulation from the outside, while the layer of less than 10 μm deep seemed to be rather decalcified, though slightly. To leave a cavity

open is not clinically recommended since it is likely to produce such a weak superficial dentin layer. Moderate and uniform recalcification under the zinc oxide eugenol cement capping is considered to be due to the mild stimulation of the cement. The short-time initial acidity of the setting polycarboxylate cement seemed to slightly disturb the function of the odontoblastic processes, preventing recalcification in the very superficial layer, though the deeper layer was well recalcified under its protection.

When capped with the $\text{Ca}(\text{OH})_2$ -containing cement, the slight Ca increase in the very superficial layer may be a mere penetration of calcium from the outside, as observed in the first layer by Kato and Fusayama⁶⁾. Regarding the increased Ca increase in the deeper layer, however, it is difficult to know whether this increase was a mere penetration of calcium, an increased physiological recalcification or an incidental one. The $\text{Ca}(\text{OH})_2$, when applied directly to the exposed pulp, is believed to be caustic enough to rapidly produce a crust, under the protection of which the formation of a reparative hard tissue is promoted²⁰⁻²⁴⁾. The effect of the $\text{Ca}(\text{OH})_2$ applied on the dentin, however, has not yet been clarified. Mjör *et al.*²⁵⁾ reported that when $\text{Ca}(\text{OH})_2$ was applied to the cavity floor dentin the hardness of the underlying dentin increased. Eidelman *et al.*¹⁹⁾ reported that the application of $\text{Ca}(\text{OH})_2$ increased the phosphorus content in the cavity floor dentin, suggesting the formation of calcium phosphate. Although the slight increase in the Ca increase in this group might be due to the penetration from the outside, it is not yet known if such a penetrating calcium contributes to the apatite formation on the collagen fibers as physiological recalcification.

CONCLUSIONS

The ability of physiological recalcification of the second layer of carious softened dentin was confirmed, and the effect of cavity treatment on the recalcification was also investigated. Bilateral pairs of human teeth having symmetric dentin caries and dog teeth having symmetric cavities, the floor dentin of which was artificially decalcified were used. The first layer of the softened dentin was removed by disclosing with 0.5% basic fuchsin-propylene glycol solution staining. One of the bilateral pair teeth was immediately extracted. The other was left in the mouth after filling the cavity with polycarboxylate cement in the case of the human teeth or treating in various ways in the case of the dog teeth and extracted after three months. The Ca content and the hardness of the second layer immediately and three months after the operation were compared by an electron probe microanalyzer and microhardness tester. The findings were as follows:

1. The second layer of the human carious softened dentin showed a marked increase in the Ca content and hardness after three months in all cases. Physiological recalcification of the second layer returning to the normal level was thus confirmed in the natural carious dentin of the human teeth.

2. The second layer of the artificially decalcified dentin of dog teeth also showed an increase in the Ca content, which returned to the normal level after three months in all cases of the group left open as well as of the groups capped with the three cements.

3. The Ca content increase was greater in the groups left open and capped with the $\text{Ca}(\text{OH})_2$ -containing cement than in the groups capped with zinc oxide eugenol

cement and polycarboxylate cement. It was observed, however, that the recalcification was disturbed at the depth of less than 10 μm in the group left open.

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