Acta odont scand 1972 *30:* 475—484

Keywords:
Lasers
Enamel
Microanalysis

From:
The Department of Cariology,
Institute of Dentistry,
University of Turku, Finland

LASER-INDUCED EFFECTS ON TOOTH STRUCTURE
V ELECTRON PROBE MICROANALYSIS AND POLARIZED LIGHT MICROSCOPY OF DENTAL ENAMEL

SIRKKA KANTOLA

The changes in the calcium and phosphorus contents of enamel which had been irradiated with a laser were studied by X-ray microanalysis and polarized light microscopy. Zonal changes were observed in the mineral concentration of the enamel. The changes were seen to take the form of a pressure-wave moving in the direction of the propagation of the laser beam. A similar zone formation was apparent in polarized light micrographs of the sections which were examined. A comparison was made between the results obtained by these two methods.

In a previous study (*Kantola,* 1972) the effect of intense CO_2-laser radiation on dentine was studied with the aid of an X-ray microanalyzer. Two layers with a markedly increased capacity for absorbing X-rays were formed in the walls of the crater, indicating calcium and phosphorus contents higher than those of untreated dentine. The calcium and phosphorus contents were highest in the surface layer of the crater, this being the layer which absorbed X-rays most readily. The mineral contents in the next layer were lower, but were still considerably higher than those of normal dentine. These results suggested that changes in mineral distribution might also take place in enamel when it is subjected to CO_2-laser irradiation. The aim of this work, therefore, was to study this possibility by means of X-ray microanalysis and polarized light microscopy.

MATERIAL AND METHODS

Tooth material as described in a previous study (*Scheinin & Kantola,* 1969b) was used in this study also. A CO_2-laser (Siemens, Munich) was used with

Received for publication, June 9, 1972.

a wavelength of 10.6 μm; the maximum output of the laser was 50 W. In studies of on the effects of lasers upon enamel it has been found that a energy of 20 joules is most suitable. At this level an effect is produced which is both distinct and confined to the enamel itself. Higher laser power causes cracks in the enamel which hinder the use of the microanalyzer.

The preparation of the sections and the microradiographic technique used in this study were described in detail in an earlier paper (*Scheinin & Kantola,* 1969b). The polarized light microscopy was performed with an Orthoplan-Pol microscope (Leitz, Wetzlar). The sections were examined between crossed polarizing filters and with the aid of a gypsum compensator, first order. For the retardation measurements a Berek compensator was used.

The X-ray microanalyzer was the same as that used in the previous study (*Kantola,* 1972), where it was described in detail. The operating data in the microanalytical work was as follows: acceleration voltage 10.3 kV; electron beam diameter 2 μm; specimen current 27 nA (TC = 2s). 1 cm on the recorder chart was equivalent to 15 μm in the preparation. The standard employed was natural apatite containing, according to chemical analysis, 37.5 % calcium, 18.2 % phosphorus, and 2.6 % fluorine. The microanalyses for the calcium and phosphorus were carried out simultaneously.

RESULTS

Under the effect of the laser a pit-like crater with a depth of about 0.22 mm was produced in the enamel and structural changes resulted. These structural changes resembled pressure waves at the edge of the crater, and they can be clearly seen in a polarized light micrograph of the section (Fig. 1). The changes are less clearly visible in the microradiograph (Fig. 2).

The preparations were examined with a microanalyzer, beginning at the bottom of the crater and proceeding in a direction parallel to that of the laser beam as far as the amelo-dentinal junction. Changes in the concentrations of calcium and phosphorus take place in the enamel of the lased tooth and follow a similar pattern, so that those areas in the preparation which show an increased calcium content also show an increased phosphorus content, and vice versa. The changes in mineral content appear to have resulted in distinct, arc-shaped zones with different individual mineral contents (Fig. 1).

Analysis of the X-ray intensity curves obtained with the microanalyzer clearly revealed six layers of differing mineral contents (Fig. 3). These layers

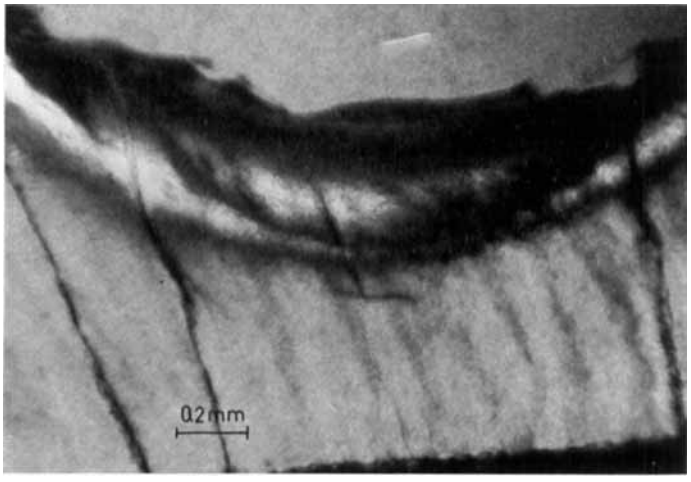

Fig. 1. Microphotograph in polarized light of laser-induced crater in enamel, imbibed in water.

are numbered I to VI in the direction taken by the electron beam. The mean heights of the X-ray intensity curves over the zero line were determined for each zone. A comparison was then made between these values and the corresponding calcium and phosphorus intensities in natural apatite so that the calcium and phosphorus contents in the said zones could be calculated in per cent by weight. The results are given in Table I.

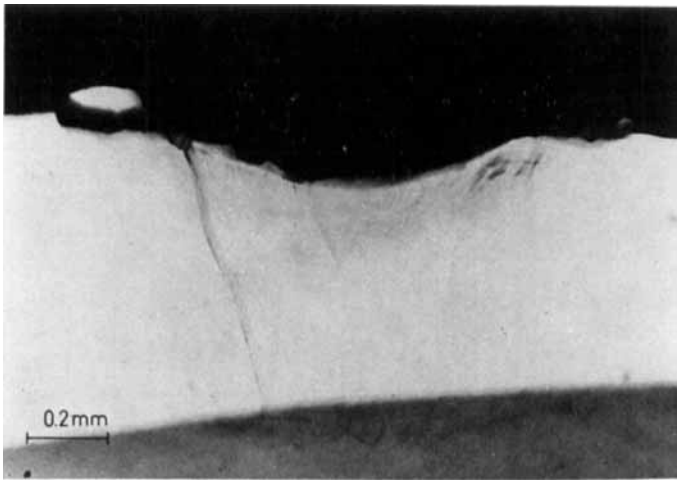

Fig. 2. Microradiograph of laser-induced crater in enamel. Lasing energy 20 joules, time 1 second.

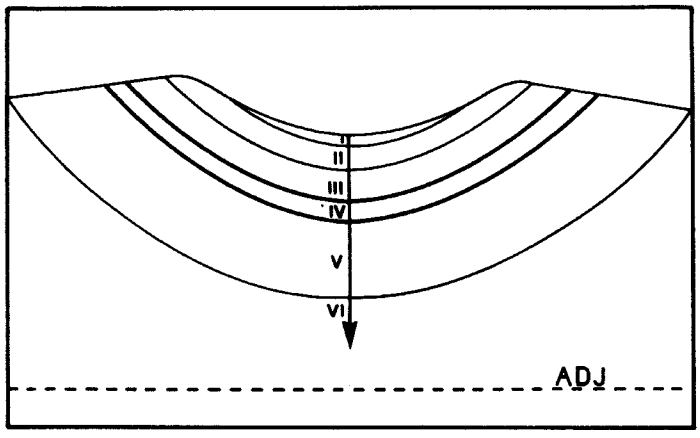

Fig. 3. Schematic illustration of laser-induced layers in enamel. ADJ = amelo-dentinal junction.

The results of the microanalysis as given in Table I were compared with those obtained for the same specimen by polarized light microscopy (Fig. 1). In these investigations a zonal formation was observed. A correspondence between both sets of zones was revealed. The thin first layer (0.04 mm) at the bottom of the crater had the lowest calcium and phosphorus contents (17.5 % and 9.8 % respectively), and showed no polarization effect owing to carbonization. There had also been carbonization in the next layer, for

Table I.

Electron micro-probe analysis of calcium and phosphorus contents in apatite standard and in zones I—VI in lased enamel

Zone	Width	Calcium record		Phosphorus record		Ca/P
	mm	Height cm	Ca content %	Height cm	P content %	
I	0.04	9.5	17.5	8.8	9.8	1.79
II	0.10	16.0	29.6	14.0	15.6	1.90
III	0.14	18.0	33.3	15.5	17.3	1.92
IV	0.08	16.0	29.6	13.5	15.1	1.96
V	0.32	19.0	35.1	16.5	18.4	1.91
VI	0.40	17.0	31.4	14.0	15.6	2.01
Apatite standard		20.3	37.5*	16.3	18.2*	2.06

* result by chemical analysis.

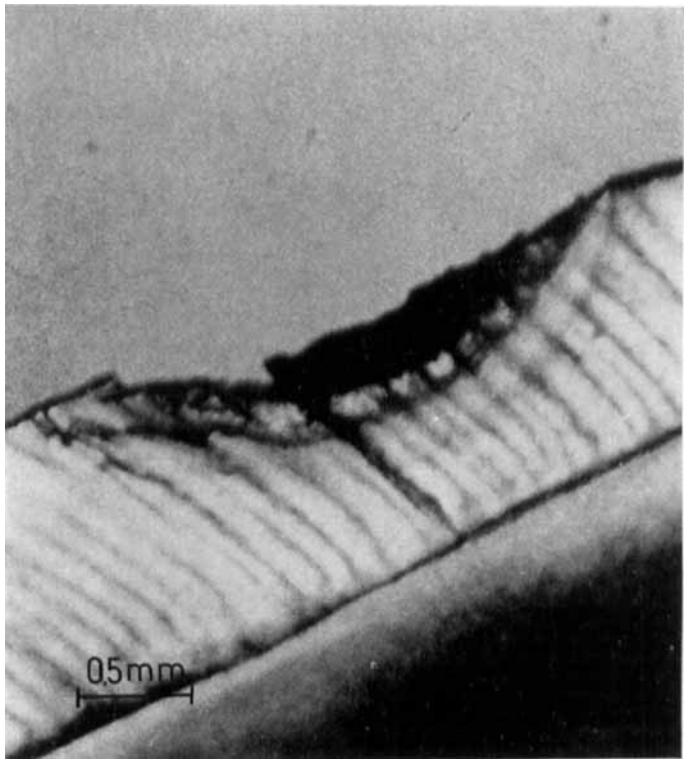

Fig. 4. Microphotograph in polarized light of laser-induced enamel. Imbibition in water.

which the microanalyzer gave a calcium content of 29.6 % and a phosphorus content of 15.6 %. The third layer, which the polarized light micrograph showed to have been partly carbonized, had a width of 0.14 mm according to both the polarized microphotograph and the microanalyzer curve. It had calcium and phosphorus contents of 33.3 % and 17.3 % respectively. The fourth layer was narrow, 0.08 mm, and, on the basis of its optical appearance, positively birefringent. It had lower calcium and phosphorus contents than the preceding layer. In the polarized light micrograph narrow isotropic areas can be seen on either side of this layer. No distinct changes in mineral content were revealed in these areas by the microanalyzer, although the X-ray intensity curve shows a slight rise in passing from the layer IV with positive refraction to either of the isotropic areas. The fifth layer, which was 0.32 mm wide, shows strong negative birefringence and had the highest mineral contents: 35.1 % of calcium and 18.4 % of phosphorus. The sixth layer was 0.40 mm wide, and by microanalysis was found to have a calcium content

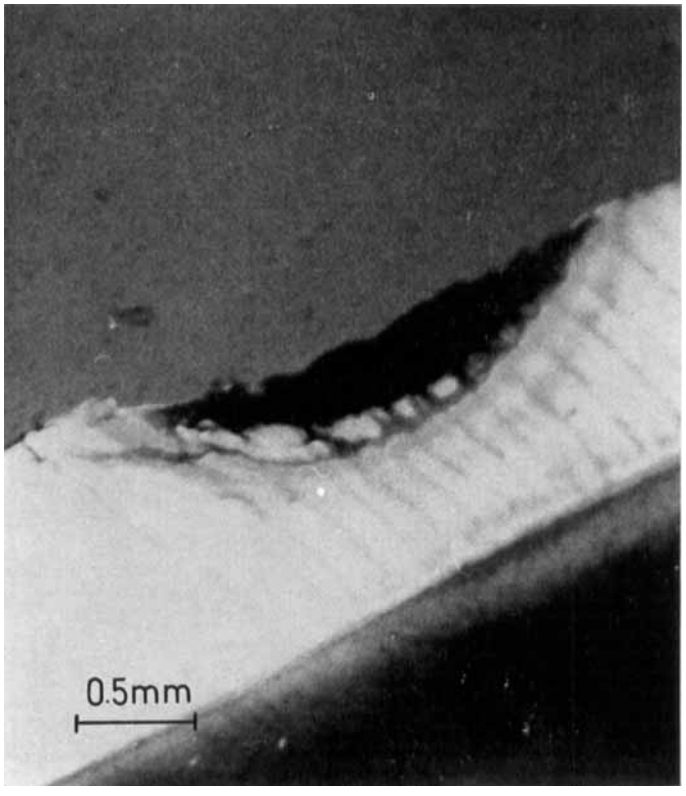

Fig. 5. The same specimen as in Fig. 4. imbibed in Clerici-solution. Microphotograph in polarized light.

of 31.4 % and a phosphorus content of 15.6 %. Under polarized light this layer was found to be negatively birefringent.

The Ca/P wt% ratios varied from 1.79 (in the first layer) to 2.01 (in the sixth layer). Compared to border areas of the layer the positively birefringent fourth layer had a relatively high Ca/P ratio of 1.96, which was caused by the decreased phosphorus content.

In order to clarify the nature of the zones' birefringence, not only samples which had been imbibed in water (Fig. 4), but also samples which had been imbibed in ethyl alcohol and in Clerici solution (n = 1.615) (Fig. 5) were examined. Examination by polarized light revealed that imbibing in alcohol caused no changes in the positive layer IV or in the zero zones on either side of it. It was also discovered that the polarized light micrographs which were obtained for preparations imbibed in water and for those imbibed in Clerici solution were identical, thus demonstrating that the birefringence of

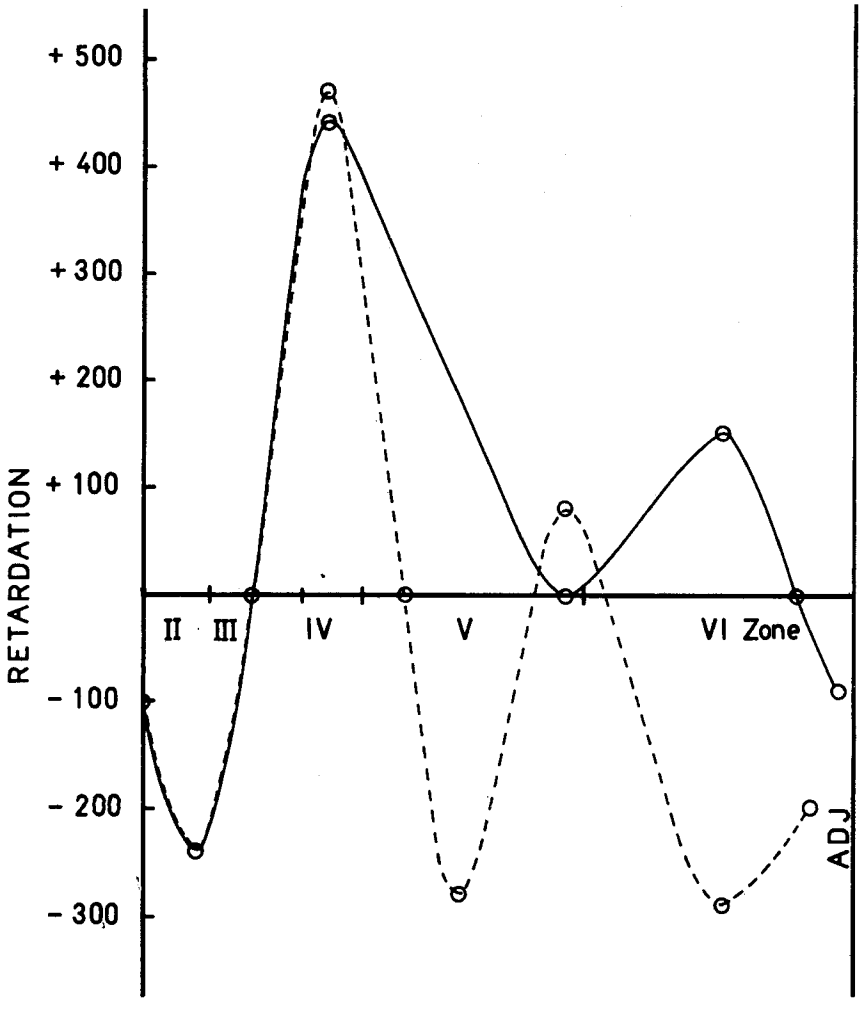

Fig. 6. Retardation curves of laser-induced enamel measured by Berek compensator. - - - - retardation in water, ———— retardation in alcohol. II—VI indicates the different zones of calcium and phosphorus contents as obtained by microanalysis. Schematic illustration of the position of zones is shown in Fig. 3.

the layers was not »form-birefringence». Changes were observed in layers V and VI after they had been imbibed in alcohol in that the colour altered in places to a red zero region and a yellow positive region. This was caused by the organic matrix which was present in these layers but which was totally burnt out closer to the opening of the crater. This observation is corroborated by retardation measurements the results of which are given in Fig. 6. This

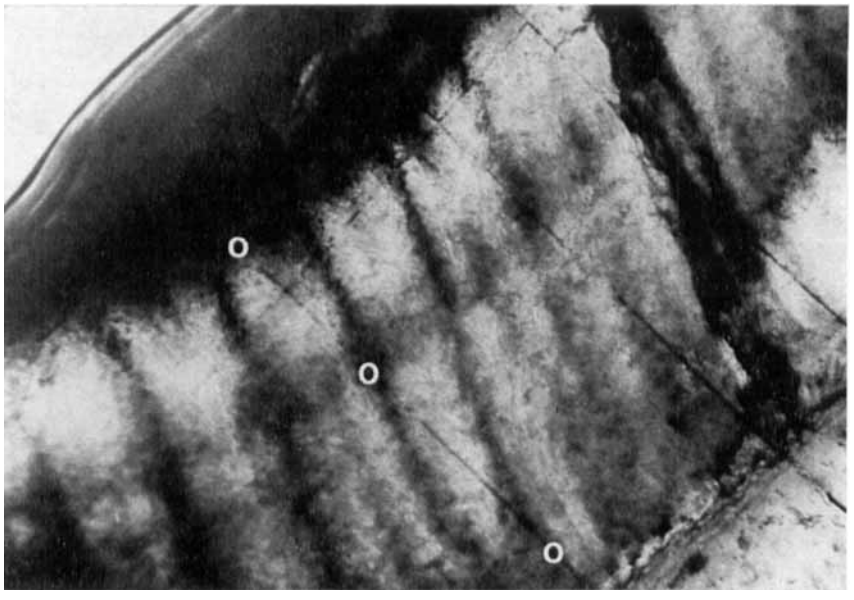

Fig. 7. The survey line o—o—o shows the path of the electron probe microanalyzer. The retardation was measured also along this line. The specimen was imbibed in alcohol and observed in polarized light. At points o the retardation was = 0. Compare to corresponding graph in Fig. 6.

figure illustrates that no great changes occurred in the retardation which was observed in the positive area of the specimen imbibed in water when that specimen was imbibed in alcohol. By contrast, the negative retardation observed for layers V and VI when imbibed in water was replaced by positive retardation under the effect of alcohol.

DISCUSSION

In the present work a study was made of the changes in the calcium and phosphorus contents caused by intense CO_2-laser irradiation of the enamel region. The X-ray microanalyzer was used to determine these mineral contents, and it proved very suitable for this purpose. It was also possible to analyze the carbonized region at the edge of the crater. The mineral distribution was not uniform; rather, a zonal pattern was observed in the mineral contents of the various zones. The same zonal structure was clearly evident when the areas which had not been affected by carbonization were examined with polarized light.

Opinions about the use of polarized light microscopy for determining mineral contents differ widely. The technique has been applied in connection with another method by numerous investigators (*Darling*, 1956; *G. Gustafson*, 1945, 1957; *Gustafson & Gustafson*, 1961; *Soni & Brudevold*, 1960; *Allan*, 1959a; *Suga & Gustafson*, 1962). In studies performed in imbibition fluids (*Soni & Brudevold*, 1959) the elimination of form-birefringence was not as successful with oily immersion substances as with water-soluble ones (*Soni, Silberkweit & Parrish*, 1965).

In the application of polarization micrography in studies of enamel it should be remembered that the result is influenced not only by the quantity of the minerals but also by the organic composition and the ultra-structural organisation in general.

In the present study imbibition of the preparations in water, in alcohol, and in water-soluble Clerici solution (n = 1.615) did not cause any transformation of the positively birefringent layers into negative ones. This demonstrates that the birefringence of these layers cannot be due to »form-birefringence». The greatest change was observed as a result of imbibing in alcohol when in layers V and VI the negatively birefringent area became positive in some places. A corresponding change was also apparent in the retardation curves, although the number of points measured was comparatively small. Thus, similar interpretations can be made of the changes observed here on the basis of the strength of the change both in polarization colours and in retardation.

Acknowledgements. I want to express my thanks to professor K. J. Neuvonen, Ph.D., for good advice in retardation measurements, to Pertti Tuominen, M.Sc., for the electron probe analyzes, to Mr. Martin Butcher for checking the language of the manuscript, and Mr. Jarmo Koskinen for microphotography.

REFERENCES

Allan, J. H., 1959a: Investigations into the mineralization pattern of human dental enamel. J. dent. Res. *38:* 1096.

Darling, A. I., 1956: Studies of the early lesion of enamel caries with transmitted light, polarised light and radiography. Britt. dent. J. *101:* 289.

Gustafson, G., 1945: The structure of human dental enamel. A histological study by means of incident light, phase contrast microscopy, fluorescence microscopy and microhardness tests. Odont. Tidskr. *53:* Suppl.

Gustafson, G., 1957: The histopathology of caries of human dental enamel. With special reference to the division of the carious lesion into zones. Acta odont. scand. *15:* 13.

Gustafson, G. & A-G. Gustafson, 1961: Human dental enamel in polarized light and contact micro-radiography. Acta odont. scand. *19:* 259.

Kantola, S., 1972: Laser-induced effects on tooth structure. IV A study of changes in the calcium and phosphorus contents in dentine by electron probe microanalysis. Acta odont. scand. *30:* 463.

Scheinin, A. & S. Kantola, 1969b: Laser-induced effects on tooth structure. II Microradiography and polarized light microscopy of dental enamel and dentine. Acta odont. scand. *27:* 181.

Soni, N. N. & F. Brudevold, 1959: Microradiographic and polarizing microscopy study of sound enamel. J. dent. Res. *38:* 1181.

Soni, N. N. & F. Brudevold, 1960: Microradiographic and polarized light studies of artifically produced lesion. J. dent. Res. *39:* 2.

Soni, N. N., Silberkweit, M. & B. A. Parrish, 1965: A microradiographic-microphotometric and polarized-light study of the demineralization pattern in carious lesions. Oral. Surg. *20:* 321.

Suga, S. & G. Gustafson, 1962: Studies on the development of rat enamel by means of histochemistry, microradiography and polarized light microscopy. Arch. oral. Biol. Suppl. 223.

Address:
Institute of Dentistry,
University of Turku,
Lemminkäisenkatu 2,
SF-20520 Turku 52, Finland