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LASER-INDUCED EFFECTS ON TOOTH STRUCTURE IV A STUDY OF CHANGES IN THE CALCIUM AND PHOSPHORUS CONTENTS IN DENTINE BY ELECTRON PROBE MICROANALYSIS

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The changes in the mineral contents of lased dentine were studied by microradiography and electron probe microanalysis. Irradiation with a CO₂-laser caused the formation of a crater in the wall of which were two radiopaque layers. The calcium and phosphorus contents of these layers were clearly higher than those of normal dentine. The contents were higher in the layer closer to the crater. The changes in mineral contents were considerably greater at the mouth of the crater, close to the surface of the tooth, than in the lower depths in the dentine.

The crater formation produced in the hard dental tissues by intense and continuous CO₂-laser radiation was studied microscopically and has been described in a previous study (*Scheinin & Kantola, 1969a*). Examination of the walls of this crater by microradiography and polarized light microscopy of sections revealed that certain layers had been formed which differed from normal dental enamel and dentine in their properties of X-ray absorption and of polarized light transmission (*Scheinin & Kantola, 1969b*). A radiopaque zone had been formed at the surface of the enamel, and, beneath the surface, a radiolucent layer, while in the peripheral dentine two distinct hypermineralized layers were observed, the outer of which was more radiopaque than the inner. This inner layer also absorbed X-rays more readily than normal dentine.

Since the absorption of X-rays in the hard dental tissues depends chiefly on the heavier element content of these tissues, it is likely that the calcium and phosphorus contents produced in the walls of the crater in the process of its formation would differ from those of normal dental enamel and dentine.

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The thickness of the layers produced by the laser effect is so small — about 50–100 μm — that the electron probe microanalysis procedure would appear to be most appropriate for analysing the elemental content of these zones.

The principles of this method were described in detail in certain earlier studies which recommended the microprobe method for analysis of dental tissues (Boyde, Switsur & Fearnhead, 1961; Boyde, Switsur & Stewart, 1963). In these studies the difficulties encountered in preparing biological specimens for examination were pointed out. Further difficulties in the precise interpretation of results are caused by the density variations in the specimen. Among the first studies of dental tissues by microanalyzer were descriptions of the changes in the calcium content of dentine surrounding a caries focus (Wykoff *et al.*, 1963). A study from the same laboratory (Frazier, 1966) stressed, as did the above-mentioned studies (Boyde *et al.*), the great importance of preparing the sample properly when biological specimens are to be examined with a microanalyzer, and also the necessity of accounting, when the results are interpreted, for potential effects of density variations and surface irregularities in the preparation.

The microanalyzer has also clarified the changes in the calcium content of developing enamel (Rosser, Boyde & Stewart, 1967). In this study a specimen made of natural apatite was employed as standard. Its calcium content was taken as 100, and the intensity of the CaK α line from the specimen under investigation was compared with the corresponding intensity obtained for the apatite specimen. The calcium content of the specimen was thus obtained as a percentage of the calcium content of the apatite. When a voltage of 15 kV was applied, it was found that the preparation remained unchanged with a 5×10^{-8} A specimen current. The electron beam had a diameter of 2 μm and the slow-scan speed was 11 $\mu\text{m}/\text{minute}$. The calcium concentration in developing enamel was found to increase with distance from the surface of the enamel in the direction of the amelo-dentinal junction. No increase in mineral concentration was observed on the surface of the enamel.

In dental studies, the microanalyzer has been employed mainly for investigations of calcium and phosphorus contents. Söremark and Grøn (1966) noted that the concentration of chlorine appeared to decrease continuously from the surface of the enamel to the amelo-dentinal junction.

A study carried out by Frank *et al.* (1966) of the distribution of calcium and phosphorus in unerupted, caries-resistant and caries-susceptible bicuspids demonstrated the applicability of the microanalyzer in determining the mineral contents of enamel and dentine. Natural apatite was used as standard,

and, after ethylene diamine extraction, and potassium iodide impregnation, highly polished, ground tooth sections were used for qualitative and quantitative electron probe analysis.

The microanalyzer can also be effectively employed in determining the different elements contained in dental tissues, and the quantitative results obtained with it are identical with those obtained by chemical means. Increases and decreases in the calcium concentration are accompanied by similar changes in the phosphorus concentration (*Wörner*, 1968).

Among studies carried out with the microanalyzer that of *Besic et al.* (1969) should be mentioned also. The specimens examined were taken from the teeth of people who lived in areas where the water had a fluorine content in the range of 2–8 ppm. The standard preparation employed in the study consisted of chemically analysed Ca-F apatite and of another containing sodium. All the samples were analysed to discover their calcium and phosphorus contents, and characteristic curves were obtained for the concentrations of the trace elements Mg, Na, and Cl in non-carious enamel and dentine in mottled enamel, going through the amelo-dentinal junction and the dentine of the teeth up to the pulp-dentine junction (PDJ).

The value of the electron microprobe in dental research is becoming more apparent as is shown, for example, by the study of *Wei* (1970), in which the changes in mineral concentrations produced by acid etching and by remineralizing with calcifying solutions were studied from the surface of the enamel into the deeper layers.

The aim of the present study was to determine by means of the X-ray microanalyzer what changes, if any, take place in the calcium and phosphorus contents of the layers, which absorb X-rays to different degrees, found in the walls of a crater which was produced by laser irradiation of the tooth, by comparison with normal dentine which was not irradiated in this way.

MATERIAL AND METHODS

Intact premolars which had been extracted for orthodontic reasons and from part of which the enamel had been removed by grinding were subjected to continuous CO₂-laser irradiation on the labial surface and as nearly perpendicular to that surface as was possible. The source of the radiation was a Philips CO₂-laser apparatus. The wavelength of the radiation from the laser was 10.6 μm . The laser beam employed had a diameter of 100 μm , and the output of the apparatus was 47 W, so that the effect of the irradiation per unit of the surface was 0.6 MW/cm².

With a effect of this magnitude crater-like formations could be produced in the teeth, the depth of the formation depending upon the irradiation time. The lased teeth were embedded in methylmetacrylate and sagittally sliced with a diamond disc. The sections were ground by hand with Corund 500 and 700 abrasive to a thickness of 40–60 μm .

Microradiographs of the sections were made in the same way as in the preceding study (*Scheinin & Kantola, 1969b*), using Ni-filtered Cu-radiation.

The calcium and phosphorus contents of the crater wall in the section were determined with a Cameca MS 46 microanalyzer at the metallurgy laboratory of the State Institute of Technology, Otaniemi. In the microanalyzer a focussed electron beam is directed on to the specimen which acts as an anode, the surface of which has been made electrically conducive by the application of a metal coating by means of vacuum evaporation. The X-ray spectrometers in the apparatus are used to analyse the characteristic X-rays of the elements under investigation as they are emitted by the specimen. The intensity of this radiation is proportional to the content, in per cent by weight, of the particular element in the specimen. The minimum diameter of the electron beam is 0.2 μm , therefore, the determination can be made for a very small area. Other factors, in addition to the beam diameter, have considerable influence on the size of the area examined. Usually, however, it is not expedient to examine the specimen point by point; it is more practical to perform a lineal analysis. This method involved the specimen being moved at the desired rate in accordance with the punctiform electron beam, and the characteristic X-ray intensity of the element under analysis being measured at the same time. It is thus possible to trace with a recorder the X-ray intensity distribution for the particular element, and this can be transformed into equivalent variations in content if the X-ray intensities from a suitable standard sample are measured under identical conditions.

RESULTS

A crater going through the enamel and dentine into the pulp and thence further into the dentine was produced when an area of $8 \times 10^{-5} \text{ cm}^2$ of the tooth was subjected to laser irradiation of 94 joules for two seconds. A microradiogram was made of a sagittal cut 50 μm in thickness from the centre of the crater. The radiograph in Fig. 1 shows that two different layers were formed in the dentine of the crater wall: the outer radiopaque layer, or I hypermineralized zone, and beyond this the inner radiopaque layer, or II hyper-

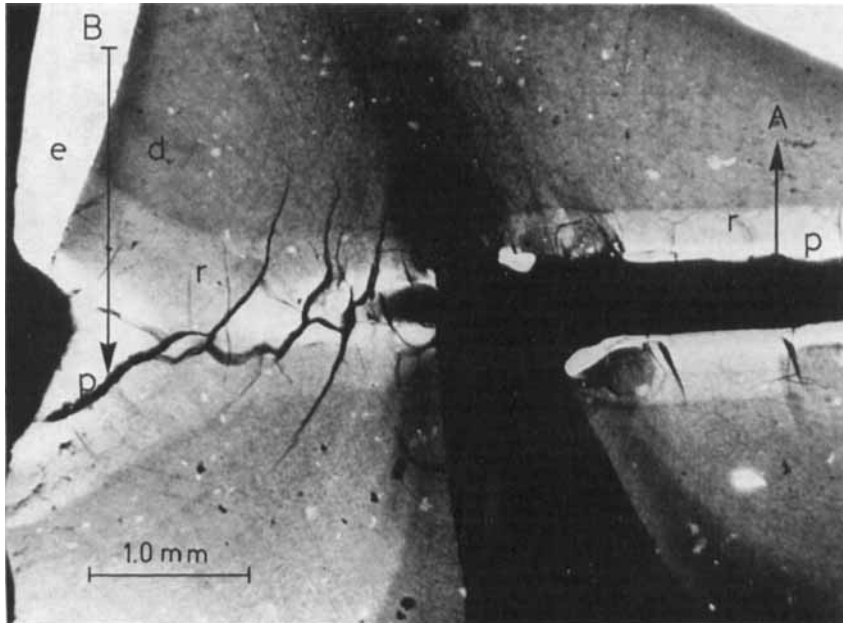


Fig. 1. Microradiograph of laser-induced crater. The radiation effect was $0,6 \text{ MW/cm}^2$.
 p = outer radiopaque zone in dentine.
 r = inner radiopaque zone in dentine.
 d = dentine.
 e = enamel.
 A = arrow indicating the scan line in dentine.
 B = arrow indicating the scan line from enamel to crater.

mineralized zone. This repeats the findings of the earlier study (*Scheinin & Kantola, 1969b*).

In order to clarify the origin of these radiopaque deposits, that is, to discover whether, during the formation of the crater, material of a higher density had «run off» from the enamel and the layers had been formed of this material — a crater was also produced in a tooth from which the enamel had been removed. This experiment resulted in the microradiogram reproduced in Fig. 2. The same layers are clearly apparent here too. Therefore, it can be said that the layers are produced from dentine under laser effect and that the enamel does not contribute to the formation of these deposits.

For the determination of the calcium and phosphorus contents of the various layers with the microanalyzer, the section was thinly coated with Cu by vacuum evaporation to make it electrically conductive. The microanalyzer was used with an accelerating voltage of 15 kV; the electron beam

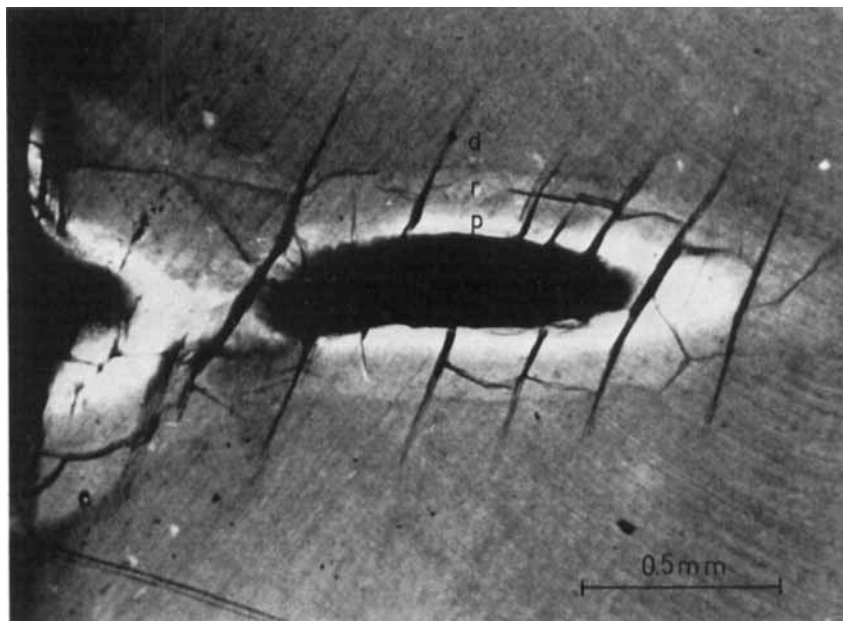


Fig. 2. Microradiograph of crater in dentine. Enamel has been removed before laser radiation.
 p = outer radiopaque zone in dentine.
 r = inner radiopaque zone in dentine.
 d = dentine.

diameter was about $2\ \mu\text{m}$; and the specimen current was $i = 30\ \text{nA}$. This voltage and current have to be used in order that there should be no damage to the specimen. The point of impact of the electron beam was moved from the crater edge deep in the dentine close to the bottom of the crater (Fig. 1) a distance of about $0.65\ \text{mm}$ in the direction indicated by the arrow A. From this the curves in Fig. 3 were obtained, and these represent the variations in the calcium and phosphorus $K\alpha$ X-ray intensities over the distance traversed. With the microanalyzer the calcium and phosphorus curves were obtained simultaneously for the same spots in the preparation. The X-ray intensity is proportional to the content of the element in question at the point under examination.

For this measurement the recorder speed was such as to make $1\ \text{cm}$ on the abscissa of the curve correspond to $0.026\ \text{mm}$ traversed on the preparation. It can be seen from the curves that, starting from the edge of the crater, both the calcium and the phosphorus content show their peak values over a distance of about $1.6 \times 0.026\ \text{mm} = 0.042\ \text{mm}$. This corresponds to

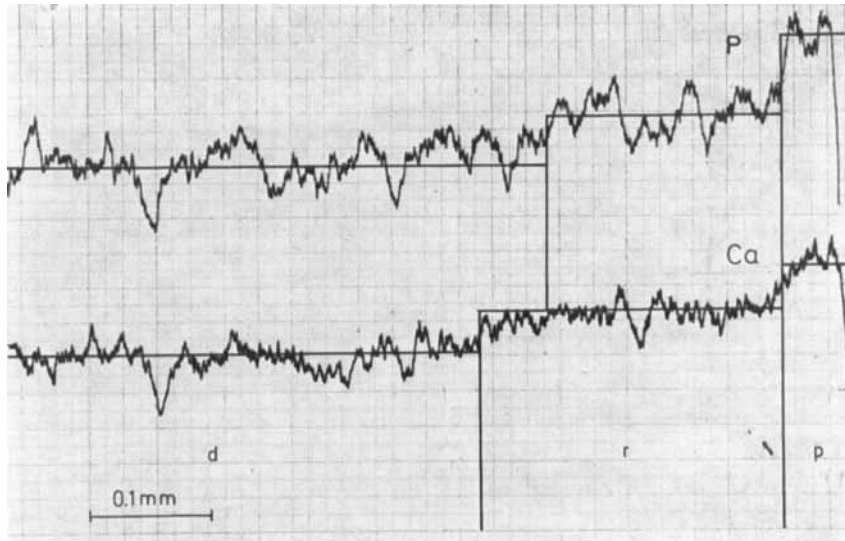


Fig. 3. Pen recorder tracers of P $K\alpha$ and Ca $K\alpha$ emission. Arrow A in Fig. 1 indicates the scan line.

p = outer radiopaque zone in dentine.
 r = inner radiopaque zone in dentine.
 d = dentine.
 P = phosphorus.
 Ca = calcium.

the I hypermineralized zone mentioned above. There follows a distinct decrease in the contents when the II hypermineralized zone is reached. Finally, at about $11 \times 0.026 \text{ mm} = 0.29 \text{ mm}$ from the edge of the crater, the dentine is reached, and here the calcium and phosphorus contents remain constant and lower than the preceding values.

To calculate the mean X-ray intensity values in the different zones, the areas subtended by the intensity curve between the ordinates of the boundaries of the layers were planimetrated, and the height of the rectangle with an equivalent area was found. This gave the values of the first run which are shown in Table I below.

Under laser effect two clearly distinguishable layers with calcium and phosphorus contents higher than those in the dentine are formed in the dentine itself.

Since the X-ray intensities given by the microanalyzer are proportional to the content of the particular element in per cent by weight of the specimen, the increase in the calcium and phosphorus contents here can be attributed mainly to the burning off of organic matter from the tissue under the laser

Table I.
Determinations made at scan line A (Fig. 1).

| | Ca Intensity curve | | | P Intensity curve | | |
|--------------------------|---|--------------------------------------|------|---|--------------------------------------|------|
| | Planimetrically calculated height | Increase compared with dentine | | Planimetrically calculated height | Increase compared with dentine | |
| | cm | cm | % | cm | cm | % |
| <i>First run</i> | | | | | | |
| I Hypermineralized zone | 16.54 | 2.51 | 17.9 | 23.17 | 3.49 | 17.7 |
| II Hypermineralized zone | 15.23 | 1.20 | 8.6 | 20.94 | 1.26 | 6.4 |
| Dentine | 14.03 | | | 19.68 | | |
| <i>Second run</i> | | | | | | |
| I Hypermineralized zone | 16.98 | 2.18 | 14.7 | 23.44 | 2.74 | 13.2 |
| II Hypermineralized zone | 15.70 | 0.90 | 6.1 | 21.32 | 0.62 | 3.0 |
| Dentine | 14.80 | | | 20.70 | | |
| <i>Third run</i> | | | | | | |
| I Hypermineralized zone | 16.50 | 2.30 | 16.2 | 22.72 | 3.18 | 16.3 |
| II Hypermineralized zone | 15.17 | 0.97 | 6.8 | 20.81 | 1.27 | 6.5 |
| Dentine | 14.20 | | | 19.54 | | |

effect. As a result of this, the density and relative mineral content of the material increased.

Two further runs were made in the same way at point A (Fig. 1) from almost exactly the same place in the preparation in both cases. The values under the headings second run and third run in Table I were obtained, and these are in agreement with those of the first run.

It must be pointed out when the results from the various runs are compared that these runs were not made with an accuracy consistent with the accuracy of measurement: they were directed only to cover the same area approximately.

Thus, it is shown that in the I hypermineralized layer at the surface of the crater the calcium and phosphorus contents were 13 to 18 per cent higher than in normal dentine, and in the II hypermineralized layer they were 3 to 8 per cent higher.

These runs provide information about the effect of laser irradiation in dentine to a relatively great depth in the wall of a crater extending past the pulp.

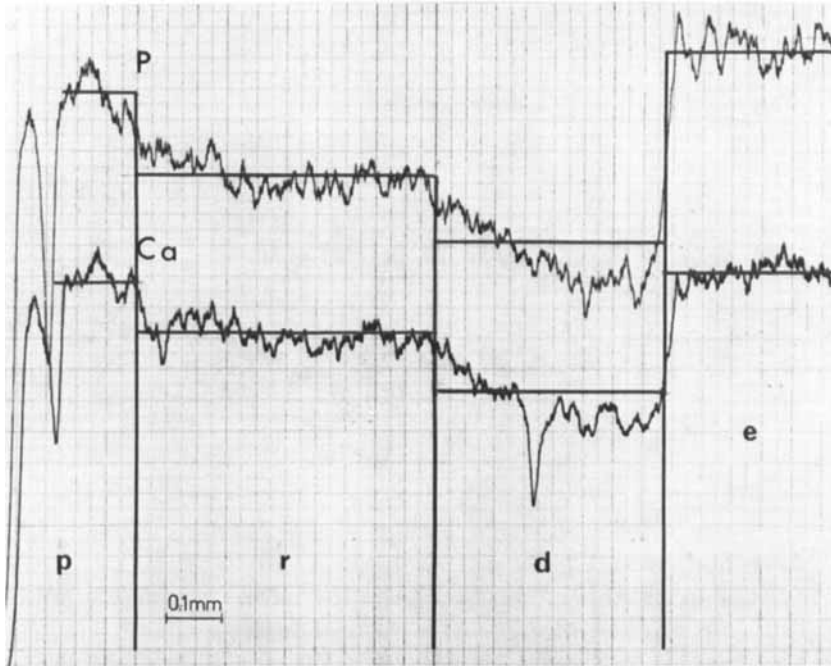


Fig. 4. Pen recorder tracers of P $K\alpha$ and Ca $K\alpha$ emission. Arrow B in Fig. 1 indicates the scan line.

e = enamel.
 d = dentine.
 r = inner radiopaque zone in dentine
 p = outer radiopaque zone in dentine.
 P = phosphorous.
 Ca = calcium.

At point B close to the surface of the tooth a run was carried out in the direction indicated in the diagram, from the enamel (e) over the normal dentine (d) and over the areas (r) and (p). The curves shown in Fig. 4 were obtained. The scanning speed of this run was such that 1 cm on the chart represented 0.052 mm in the preparation. The curves reveal very clearly that in this direction the breadth of the outer zone (p) is $2.8 \times 0.052 \text{ mm} = 0.14 \text{ mm}$, the breadth of the inner zone (r) is $9.8 \times 0.052 \text{ mm} = 0.51 \text{ mm}$, and the breadth of the dentine layer (d) is $7.3 \times 0.052 \text{ mm} = 0.38 \text{ mm}$. These measurements derived from the intensity curves are in complete agreement with those derived from the microradiograph. The curves indicate very clearly the amelo-dentinal junction at which a marked decrease in mineral content occurs.

Table II.
Determinations made at scan line B (Fig. 1).

| | Ca Intensity curve | | | P Intensity curve | | |
|--------------------------|---|--------------------------------------|------|---|--------------------------------------|------|
| | Planimetrically calculated height | Increase compared with dentine | | Planimetrically calculated height | Increase compared with dentine | |
| | cm | cm | % | cm | cm | % |
| I Hypermineralized zone | 13.80 | 3.66 | 36.0 | 19.80 | 4.70 | 31.2 |
| II Hypermineralized zone | 12.17 | 2.03 | 20.0 | 17.30 | 2.20 | 14.6 |
| Dentine | 10.14 | | | 15.10 | | |
| Enamel | 14.11 | 3.97 | 39.1 | 21.30 | 6.20 | 41.1 |

Planimetric calculation of the mean ordinates for the different layers gave the results shown in Table II.

It should be noted here that the laser effect on the dentine was greater at the top of the crater: the changes in the calcium and phosphorus contents were greater here than they were deeper in the tissue, which is only natural.

DISCUSSION

After continuous irradiation of teeth with a powerful CO₂-laser, it was discovered by macroscopic, microradiographical, and polarized light examination that changes had occurred in the dentine, producing various deposits in the tissue surrounding the crater which was formed. It was clearly evident from the microradiograms that changes in the mineral contents had taken place. The increased mineral contents of these layers, each with a different capacity for absorbing X-rays, were established with an X-ray microanalyzer. The calcium and phosphorus contents increased most at a point closest to the crater wall, so that in the outer hypermineralized layer the calcium content was 15 to 18 per cent higher than in the normal dentine, and the phosphorus content was 13 to 16 per cent higher.

In the next layer the calcium content was 6 to 9 per cent higher, and the phosphorus content 3 to 6 per cent higher, than in the normal dentine.

Macroscopically also, fusing and recrystallization of material were seen to have occurred in the crater wall. The crater wall is surrounded by a carbonized layer. It appeared possible that the vitreous, glassy layer, fused

and resolidified, might be derived from the enamel. However, this proved not to be so because when a tooth which had been stripped of its enamel by grinding was subjected to irradiation, deposits were produced in the dentine similar to those produced when the enamel was intact. The crater is formed as a result of the pressure from gases produced at the fusing of the dentine, the evaporation of water, and the combustion of organic substances caused by the absorption of laser energy.

In the second hypermineralized layer this effect was not so complete, and part of the organic matrix, which had been carbonized, remained in the tissue. The formation of the different layers might be attributed to the nature of the laser beam. The heat effect of it is extremely powerful because it is produced over a very small area in a short time, so that the effect is not attenuated by the dissipation of the heat into a larger area by conduction. The burning of the organic matrix, which is complete in the outer hypermineralized layer and incomplete in the inner hypermineralized layer, is thought to contribute to the increases in the mineral contents of these layers. Another probable cause is the formation of a new compound with a different composition and structure in the process of recrystallization.

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