From: The Norwegian Institute of Dental Research, Oslo
(Director: Dr. Philos. *B. Nygaard Östby*).

ENAMEL LESIONS PRODUCED IN VITRO BY SOLUTIONS OF EDTA AND EDTA-SODIUM SALTS*

by

C. RAVNIK

H. F. SAND

T. MÖRCH

Only a few laboratory investigations have been made with the purpose of studying the lesions produced when enamel surfaces are exposed to solutions of EDTA and EDTA-sodium salts. Thus, the effect of a sodium salt solution of EDTA, pH 8.0, was studied with the replica method (1, 2). Different sodium salt solutions of EDTA were employed in a study with the so-called "Lichtschnitt" method (3).

The present investigation was undertaken in order to study the lesions produced when teeth are exposed to various solutions of EDTA and EDTA-sodium salts. Further, it was of interest to compare the lesions produced by these solutions with those produced earlier by means of other agents.

MATERIAL AND METHODS

The material comprises 46 experiments *in vitro* on newly extracted young human premolars and 96 experiments *in vitro* on dog teeth. An experimental area was isolated on each tooth by

*) This investigation was supported by PHS research grant No. D-901 from the National Institute of Dental Research, U.S. Public Health Service.

means of blue wax, according to the modified window technique described earlier (4). The experimental areas were exposed at room temperature to solutions of EDTA or EDTA-sodium salts. The solutions were prepared according to the results of physico-chemical studies (5). The exposure time was for each solution adjusted in accordance with their ability to decalcify enamel as determined in preliminary studies. The range was from 40 minutes to 60 hours.

After exposure, the lesions produced were first examined macroscopically. Then followed a microscopic examination of ground sections with polarized light. Supplementary microradiographs of representative sections were also made.

The solutions were made in the following manner:—

I. A saturated solution of EDTA (the pure acid) in distilled water. The concentration was about 0.001 molar.

II. The disodium salt was prepared by dissolving the pure acid in 0.474 normal NaOH. Since two moles of NaOH are required to convert the acid to disodium salt, 0.474 moles of NaOH will convert 0.237 moles of EDTA, *i.e.* $292.24 \times 0.237 = 69.261$ g of EDTA to 1 litre of 0.474 normal NaOH. The concentration of the solution was determined by titration with NaOH. The total concentration was found to be 0.234 molar, the ionic strength 0.702. (The maximum concentration 0.237 could not be reached because of increase in volume of the solution when adding EDTA to the NaOH solution).

III and IV. The trisodium salt as well as the tetrasodium salt was prepared from solution II. Since most chemical properties are related to the ionic strength rather than to the concentration, it was decided to make solutions of the same ionic strength. The ionic strength of a solution can be calculated from the formula

$$\tfrac{1}{2} \Sigma \, (c_1 \cdot z_1^2 + c_2 \cdot z_2^2 \ldots\ldots\ldots\ldots c_n \cdot z_n^2).$$

If c is the concentration of the EDTA, then the concentration of sodium will be: For disodium salt 2c, for trisodium salt 3c, and for tetrasodium salt 4c.

The ionic strength will be:

For disodium salt $\frac{1}{2} \Sigma (c \cdot 2^2 + 2c \cdot 1^2) = 3c$.

For trisodium salt $\frac{1}{2} \Sigma (c \cdot 3^2 + 3c \cdot 1^2) = 6c$.

For tetrasodium salt $\frac{1}{2} \Sigma (c \cdot 4^2 + 4c \cdot 1^2) = 10c$.

Thus, to get the same ionic strength the disodium solution had to be diluted 3:6 for trisodium salt and 3:10 for tetrasodium salt. Also to convert the disodium to trisodium and tetrasodium salts NaOH had to be added.

According to earlier findings (5) the pH of the trisodium solution should be about 7.90. Therefore, 125 ml of the disodium salt solution were titrated with 0.474 normal NaOH. After addition of 67 ml the pH was 7.86. The solution was then transferred to a 250 ml flask, and water was added to the mark. The final pH was 7.94, the ionic strength 0.702, and the concentration 0.117 molar.

To make the tetrasodium salt, 75 ml of the disodium salt solution were also titrated with 0.474 normal NaOH. After addition of 70 ml the pH was 10.94. The solution was then transferred to a 250 ml flask, and water was added to the mark. The final pH was 11.01, the ionic strength 0.702, and the concentration 0.0702 molar.

In some cases solutions of 0.001 molar concentration were prepared by diluting the solutions mentioned above with water.

RESULTS

Representative pictures are shown in the Figs. 1—9.

As a rule an outer spot appeared with all solutions used (Figs. 1 and 3—8). In four out of 22 experiments with EDTA, 0.001 molar, pH 3.03, on dog teeth an inner spot developed (Fig. 2). In two out of 13 experiments on human teeth with Na_2EDTA, 0.234 molar, pH 4.09, the same type of defect appeared.

In all cases accentuated borders between the prisms (ABP) were observed (Figs. 3—8). As a rule this zone appeared in connection with an advanced defect (Fig. 3). However, in a few cases this zone was the only recognizable effect (Fig. 4).

The width of the zone of ABP varied considerably with the pH

and concentration of the solutions used (Figs. 5—8). With EDTA, 0.001 molar, pH 3.03, this zone was narrow (Figs. 5 and 9 A), while a broader zone was noticed with Na_2EDTA (Fig. 6) and Na_3EDTA (Figs. 7 and 9 B). An extra broad zone appeared with

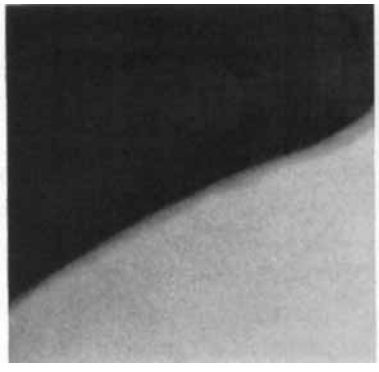


Fig. 1.

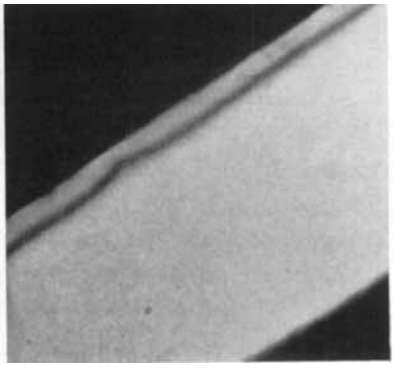


Fig. 2.

Fig. 1. Microradiograph of dog tooth, exposed for 36 hours to 0.001 molar EDTA, pH 3.03. At bottom of cavity a zone of homogeneously radiolucent enamel.

Fig. 2. Microradiograph of dog tooth, exposed for 24 hours to 0.001 molar EDTA, pH 3.03. Inner spot.

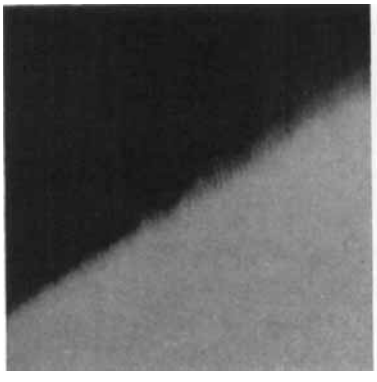


Fig. 3.

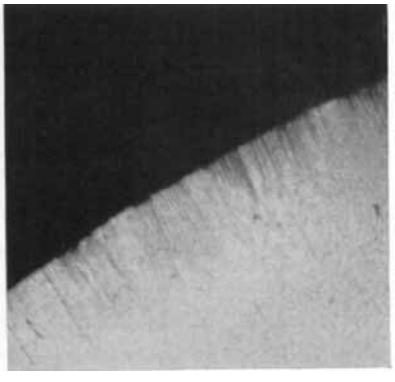


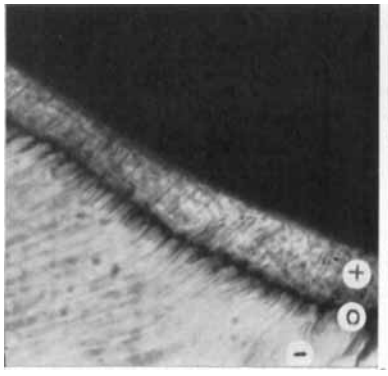
Fig. 4.

Fig. 3. Microradiograph of human tooth, exposed for 48 hours to 0.224 molar Na_4EDTA, pH 11.8. Outer spot. Outer part of enamel partly radiolucent, with stripes of ABP. Advanced defect.

Fig. 4. Microradiograph of dog tooth, exposed for 48 hours to 0.224 molar Na_4EDTA, pH 11.8. Zone of ABP the only recognizable effect.

Na_4EDTA (Figs. 8 and 9 C). With the same sodium salt of EDTA the width of the ABP zone increased with the concentration.

Each separate solution produced the same type of defect on human teeth and dog teeth. As a rule the ABP zone was more pronounced on dog teeth than was the case on human teeth.



Eig. 5.

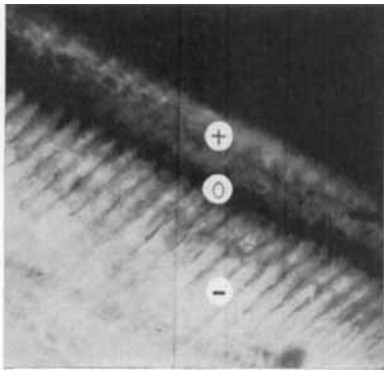


Fig. 6.

Fig. 5. Dog tooth, exposed for 16 hours to 0.001 molar EDTA, pH 3.03. Polarized light. Outer spot. Narrow zone of ABP.

Fig. 6. Human tooth, exposed for 120 minutes to 0.231 molar Na_2 EDTA, pH 4.09. Polarized light. Outer spot with ABP.

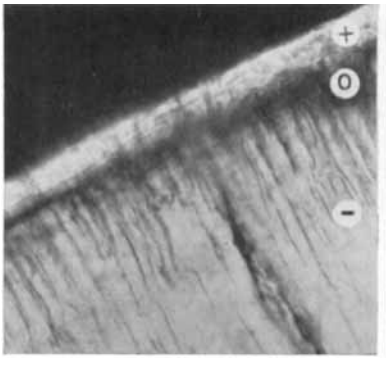


Fig. 7.

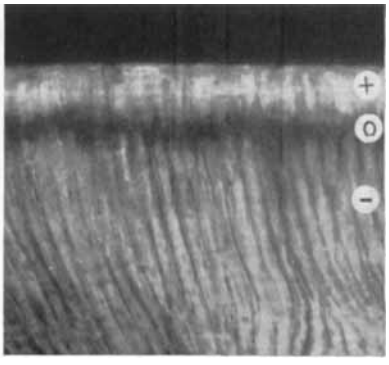


Fig. 8.

Fig. 7. Human tooth, exposed for 180 minutes to 0.117 molar Na_3 EDTA, pH 7.94. Polarized light. Outer spot. Broader zone of ABP.

Fig. 8. Dog tooth, exposed for 20 hours to 0.224 molar Na_4EDTA, pH 11.8. Polarized light. Outer spot. Extra broad zone of ABP.

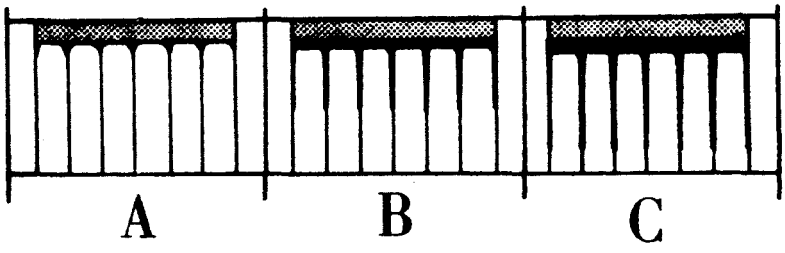


Fig. 9. Schematic drawings showing the characteristic developmental stages of ABP as they appear in ground sections between crossed nicols after imbibition in distilled water. Polarized light.

White: negative birefringence.

Dotted: positive birefringence.

Black: isotropy.

A: saturated watery solutions of EDTA.

B: solution of trisodium salt of EDTA in distilled water.

C: solution of tetrasodium salt of EDTA in distilled water.

DISCUSSION

EDTA is known to be a chelating agent. (Chelation is a special form of complex formation). The chelating ability of EDTA depends on the pH (6). When equivalent amounts of EDTA and calcium are present, nearly 100 per cent of the calcium is complex bound at pH above 9.0, at pH 5.0 only 50 per cent. At pH 3.0 no chelation takes place.

In our experiments the solution of EDTA (as the free acid) had a pH of 3.03. This solution, therefore, acts as any other acid and not as a chelating agent. The disodium solution had a pH of 4.09. At this pH the chelating power is about 20 per cent, and this solution may, consequently, act on the enamel both as an acid and as a chelating agent. It is, therefore, of special interest to compare the defects obtained by means of this solution with those obtained in earlier experiments with lactate buffers within the same pH-range.

Two of the solutions were alkaline. The action was, therefore, only due to chelation. As to the type of defects obtained with

these solutions, no material from earlier investigations is available for comparison.

With a few exceptions, outer spots appeared in all experiments. An inner spot occurred in 4 out of 22 experiments on dog teeth with EDTA, pH 3.03, and in 2 out of 13 cases on human teeth at pH 4.09. In earlier experiments on human teeth, no inner spot has been observed at a pH below 4.0 with 0.5 molar lactate buffers. It might well be that inner spots can be obtained even at lower pH-values with less concentrated lactate buffers. It is also possible that the occurrence of an inner spot at the low pH-value in the present experiment may be due to a difference in composition between enamel of human and dog teeth.

The inner spots in human teeth occurred within the pH-range in which inner spots were obtained in earlier experiments with lactate buffers.

In all experiments a zone of accentuated borders between the prisms (ABP) were seen. This zone was narrow with acid solutions (Fig. 5), intermediate at pH 7.8 and very broad at pH 11.0 (Fig. 8). This same zone was also observed in earlier experiments with lactate buffers, but even with EDTA, pH 3.03, the zone was broader than with lactate. However, since no chelation takes place at this low pH-value, the difference cannot be due to chelation. It may be explained by assuming a difference in rates of penetration. The penetration of the decalcifying solution seems primarily to take place (in the border) between the prisms. From here the solutions penetrate into the prisms from the sides.

Thus, the first recognizable effect of the decalcification is a widening of the border between the prisms. Now, if the EDTA solutions penetrate more rapidly in the direction along the prisms than does lactate, this would account for the observed difference.

It is assumed that the penetration into the enamel occurs in the organic material between the crystallites. This material is known to be attacked by alkaline solutions. The alkaline solutions of EDTA may, therefore, act upon the mineral as well as on the organic constituent of the enamel, and the changes may favour the penetration. It may also be mentioned that the surface tension is considered important for the penetrability of liquids. In separate experiments it was found that the surface tension of the alkaline

solutions was lower than that of the disodium salt solutions. The values found were:

71.2 dyn/cm for Na_2EDTA, 0.1 molar, pH 4.56.
58.0 „ „ Na_3EDTA, 0.1 „ „ 8.22.
50.0 „ „ Na_4EDTA, 0.1 „ „ 11.32.

Thus, the broader zone of ABP with EDTA solutions as compared to lactate buffers is probably not due to the chelating effect of EDTA, but rather to a difference in the rate of penetration.

From the experiments the conclusion may be drawn that the defects produced in the enamel by the action of EDTA are not different in principle from those obtained with lactate buffers.

The data obtained do not allow any definite conclusion with respect to the prevailing caries theories.

SUMMARY

Human and dog teeth were exposed to solutions of EDTA and EDTA-sodium salts.

The effect on the enamel surface was studied macroscopically, as well as microscopically in ground sections with polarized light. In some cases microradiographs were also made.

As a rule an outer spot was produced. Only in a few cases with acid solutions an inner spot was observed.

In all cases a zone of accentuated borders between the prisms was seen. As a rule this zone appeared in connection with an advanced defect. However, in a few cases this zone was the only recognizable effect. The width of the zone increased with increasing pH of the solution.

The defects produced were not different in principle from those obtained earlier with lactate buffers.

RÉSUMÉ

LÉSIONS DE L'ÉMAIL PRODUITES IN VITRO PAR DES SOLUTIONS D'EDTA ET DES SELS SODIQUES D'EDTA

Des dents humaines et des dents de chien ont été exposées à des solutions d'EDTA et des sels sodiques d'EDTA.

L'action sur les surfaces d'émail a été étudiée d'une part mac-

roscopiquement, et d'autre part microscopiquement sur des coupes par usure en lumière polarisée. Dans quelques cas, des microradiographies ont aussi été faites.

En général, une tache externe a été produite. Une tache interne n'a été observée que dans quelques cas seulement avec des solutions acides.

Dans tous les cas, on a observé une zone d'accentuation des limites interprismatiques. En général, cette zone se présentait en même temps qu'une lésion marquée. Dans certains cas cependant, cette zone était le seul effet visible. La largeur de la zone augmentait avec l'élévation du pH de la solution.

Les lésions produites ne différaient pas particulièrement de celles obtenues antérieurement au moyen de tampons au lactate.

ZUSAMMENFASSUNG

SCHMELZLÄSIONEN ERZEUGT IN VITRO DURCH EDTA UND EDTA-NATRIUMSALZE

Menschen- und Hundezähne wurden einer Lösung von EDTA und EDTA-Natriumsalze ausgesetzt. Die Wirkung auf die Schmelzoberfläche wurde makroskopisch und mikroskopisch in Schleifschnitten mit polariziertem Licht untersucht. In einigen Fällen wurden auch Mikro-Röntgenaufnahmen gemacht.

In der Regel wurden oberflächliche Veränderungen allein festgestellt. Nur in wenigen Fällen mit sauren Lösungen wurden auch Veränderungen im Innern beobachtet. In allen Fällen wurde eine Zone mit einem stärkeren Hervortreten der interprismatischen Substanz beobachtet. In der Regel erschien diese Zone in Verbindung mit einer fortgeschrittenen Veränderung. In wenigen Fällen war aber diese Zone die einzige Veränderung, die beobachtet werden konnte. Die Breite dieser Zone nahm mit steigendem pH der Lösung zu.

Die hervorgerufenen Läsionen unterschieden sich nicht grundsätzlich von denen, die in früheren Untersuchungen mit Laktatpuffern erzeugt wurden.

SUMARIO

LESIONES IN VITRO EN EL ESMALTE PRODUCIDAS POR LAS SOLUCIONES DE EDTA Y LAS SALES SÓDICAS DE EDTA

Se trataron dientes humanos con soluciones de EDTA y de sales sódicas de EDTA. Se investigó macroscópicamente el efecto sobre la superficie del esmalte; el estudio microscópico se realizó en cortes por desgaste, con luz polarizada. En algunos casos también se hicieron microradiografías.

Por lo general se produjo una mancha externa; sólo en pocos casos, empleando soluciones ácidas, se observó una mancha interna.

En todos los casos pudo verse una zona donde los límites interprismáticos aparecían acentuados. Generalmente esta zona aparecía junto con perturbaciones serias; no obstante, en unos pocos casos esta zona fué el único efecto apreciable. El ancho de la zona era mayor con la elevación del pH de la solución.

Los defectos producidos no mostraron ser particularmente diferentes de los obtenidos en trabajos anteriores con buffers de lactatos.

REFERENCES

1. *Müller, Gerd & Angela Schait,* 1957: Morphologic differences in replicas of intact enamel decalcified in acid or EDTA. *Helv. odont. acta 1:* 5.
2. *Mühlemann, H. R.,* 1960: Experimental modifications of the enamel surface. *Helv. odont. acta 4*: 5.
3. *Wandelt, S.,* 1959: Über die Wirkung von Chelatoren auf die Zahnhartsubstanz. *Dtsch. zahnärztl. Z. 14*: 1255.
4. *Hals, E., P. Torell & T. Mörch,* 1959: Enamel lesions produced in vitro by sugar-saliva mixtures. *Acta odont. scand. 17*: 299.
5. *Sand, H. F.,* 1961: The dissociation of EDTA and EDTA-sodium salts. *Acta odont. scand. 19:* 469.
6. *Alrose Chem. Co., Providence, R. I., U. S. A.,* 1955: Sequestrene. Cit. *H. Flaschka:* Über die Verwendung von Komplexon in der Massanalyse. *Fortschr. chem. Forsch.* 3: 253.

Address: *Josefinegate 32*
Oslo, Norway