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THE EFFECT OF VARIOUS DEMINERALIZING AGENTS ON DOG TOOTH ENAMEL

by

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Outer and inner spots have been produced *in vitro* on human enamel surfaces, using lactate buffers and sugar-saliva mixtures (1, 2). The appearance of the type of spot was dependent on the time of exposure, as well as on the pH and the concentration. In a previous paper the normal appearance and structure of dog enamel have been described (3). It was found that dog teeth may be well suited for the study of experimentally produced lesions in the enamel. The purpose of the present study was to determine how dog tooth enamel behaves when exposed to various demineralizing agents.

MATERIAL AND METHODS

The laboratory experiments carried out numbered 186. The teeth were obtained from dogs of different age. On each tooth an experimental area was encircled by means of wax and exposed to the agent in question.

The following agents were used

(1) Sugar-saliva mixture,
1 ml 10 % cane sugar was added to 10 ml saliva collected during tooth brushing. The exposure time varied from 6 to 32 hours.

(2) Sodium lactate buffer, 0.5 M, pH 2.9—5.44.
The variation of exposure time and pH is shown in Table 1.

(3) Sodium aspartate buffer, 0.5 M, pH 3.5—5.8.
The time of exposure and pH were varied systematically as shown in Table 1.

(4) *a.* Saturated solution of EDTA in distilled water, 0.001 M, pH 3.03,
b. Solution of trisodium salt of EDTA in distilled water, 0.117 M, pH 7.94,
c. Solution of tetrasodium salt of EDTA in distilled water, 0.07 M, pH 11.0,
The time of exposure was changed systematically. (Table 1)

The experiments with sugar-saliva mixtures took place in an incubator at 37°C. All other experiments were performed at room temperature.

Table 1

Substance	Number of experim.	Molar conc.	pH	Exposure time	Inner spot	Outer spot
Sugar-saliva	34	—	—	6— 24 hrs.	28	6
„	28	—	—	16 hrs.	2	26
Lactate buffer	9	0.5	4.0	25 min.	0	9
„	7	0.5	4.0	25— 60 min.	6	1
„	7	0.5	4.0	40—120 min.	1	6
„	6	0.5	5.0	4— 5 hrs.	5	1
„	9	0.5	5.0	6 hrs.	1	8
Aspartate buffer	6	0.5	3.6	18— 32 min.	0	6
„	7	0.5	4.15	4 days	0	7
„	5	0.5	5.8	60 days	No effect	
EDTA in dist. water	16	0.001	3.03	12— 24 hrs.	4	12
„	6	0.001	3.03	24 hrs.	0	6

Subsequent to the exposure, ground sections were made, the thickness being approximately 80 microns. The sections were examined in a Leitz polarizing microscope after imbibition in

distilled water. The sections were also exposed to soft X-rays. As to the equipment and procedure, see article on normal dog enamel(3).

OBSERVATIONS

The enamel lesions produced by the above described demineralizing agents showed characteristics and specific patterns which seemed to be dependent on the agents used. For this reason, the development of the lesions will be described separately.

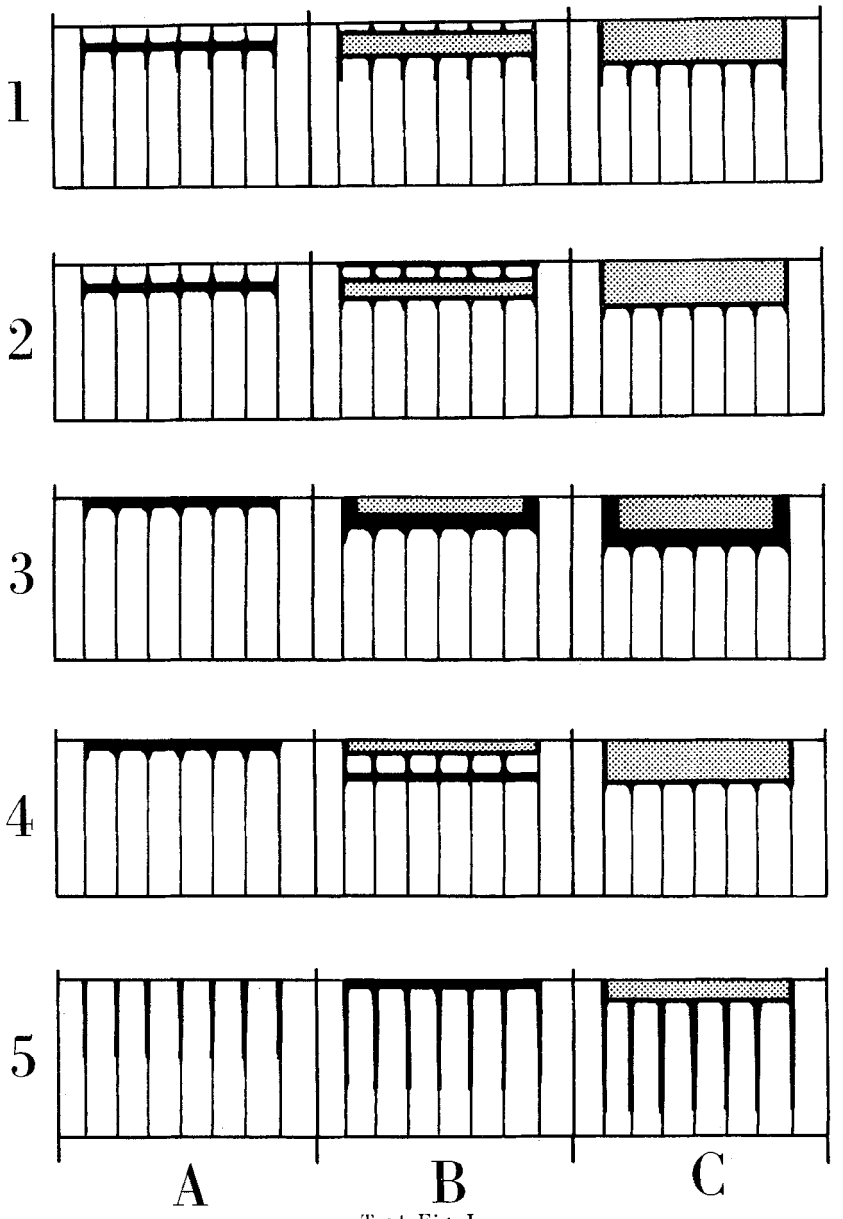
Sugar-saliva mixtures. 62 experiments

The drop in pH of the sugar-saliva mixture was roughly as follows:

Exposure time Start	pH neutral
after 2 hours	5.0
„ 6 „	4.5
„ 24 „	4.0
„ 32 „	3.8

Polarization microscopy

In Text Fig. I, the development of the enamel lesions is shown schematically. After an exposure period of 6—10 hours, an inner isotropic spot developed (1 A). (See also Table 1.) These initial spots tended to widen towards the surface as well as inwards. After 10 to 16 hours of exposure, positive birefringence occurred in the central part of the lesion (Text Fig. I, 1 B and Fig. 1 A). After 16 to 24 hours of exposure, the lesion had extended to the enamel surface (Text Fig. I, 1 C). The entire lesion exhibited positive birefringence, except for a narrow zone of isotropy separating the latter from normal enamel. Text Fig. II, 1 A is a diagram showing the relationship between the isotropic zone and the accentuated borders between the prisms, (ABP). Measurements revealed that the length of the ABP amounted to 20—30 microns, whereas the isotropic zone varied from 10 to 15 microns.



Text Fig. I.

Schematic drawings showing the characteristic developmental stages of enamel lesions as they appear in ground sections between crossed nicols after imbibition in distilled water.

1. sugar-saliva mixtures, 2. sodium lactate buffers, 3. sodium aspartate buffers, 4. watery solutions of EDTA, 5. tetrasodium salts of EDTA.

White: negative birefringence, Dotted: positive birefringence, Black: isotropy.

Microradiography

The microradiographic picture (Fig. 3 B) showed an inner spot consisting of a radiolucent material. The extension of this area corresponded to the isotropic and positively birefringent zones in Fig. 1 A. The surface layer of enamel which in polarized light was characterized by a negative birefringence appeared in the microradiogram as a radio-opaque zone, which was discontinued in certain areas.

Sodium lactate buffers. 38 experiments

0.5 M lactate buffers within the pH range 4—5 caused lesions as shown in Text Fig. I, 2. An inner isotropic spot (2 A) appeared after 25 minutes of exposure to a buffer of pH 4.0. At pH 5 the development of the same type of lesion required 4 hours. Increase of the exposure time resulted in an increase of the width of the inner spot, which at this stage exhibited positive birefringence. Simultaneously with this increase of the inner spot, the initiation of a superficial lesion took place (2 B). Between the latter and the inner spot the negative birefringence persisted, although a confluence of the outer and inner spots could be observed in limited areas (Fig. 1 B). A complete fusion of the inner and outer spots occurred after 1 hour at pH 4.0 and after 6 to 7 hours at pH 5.0 (Text Fig. I, 2 C). Buffer solutions with pH below 4.0 or above 5.0 produced outer spots only. The width of the isotropic zone measured from 5 to 10 microns. The length of ABP was 10—20 microns. (Text Fig. II, 1 B).

Microradiography

The microradiographic image of sections from teeth treated with lactate buffers at pH 5 revealed a relatively broad inner radiolucent zone with accentuated borders between the prisms. A narrow radio-opaque layer separated this inner spot from a thin and irregular radiolucent zone at the surface (Fig. 3 C).

Sodium aspartate buffers. 18 experiments

Aspartate buffer solutions used in the present series (Table 1) produced outer spots only (Text Fig. I, 3, and Fig. 1 C). At pH

3.6 a superficial defect appeared after 24 hours. At pH 4.15 the same type and size of lesion occurred after 4 days of exposure. Buffer solutions with pH 5.8 did not seem to produce lesions even after 60 days of exposure.

The isotropic zone adjacent to the outer spot was wide (30—40 microns), whereas the ABP could hardly be observed (Fig. 1 C).

Solutions of EDTA and EDTA sodium salts

The examination of the sections of this series revealed two different ways in which lesions developed. Text Fig. I, 4 A demonstrates the characteristic lesions produced after 18—24 hours by saturated EDTA solutions in distilled water, the pH being 3.03 and the concentration 0.001 M. The defect consists of an outer spot and an underlying narrow isotropic zone with the length of the ABP amounting to 5 microns (Fig. 2 A).

In Text Fig. I, 4 B is shown the development of a combined lesion produced with the same solution. After 12—18 hours a narrow isotropic outer spot appeared. After 24 hours this zone exhibited positive birefringence with an adjacent isotropic band. Simultaneously, an isotropic zone appeared in the deeper part of the enamel (inner spot) separated from the outer spot by a negatively birefringent layer (Fig. 1, D). With increased time of exposure the two spots coalesced into a broad outer spot. (Text Fig. I, 4 C).

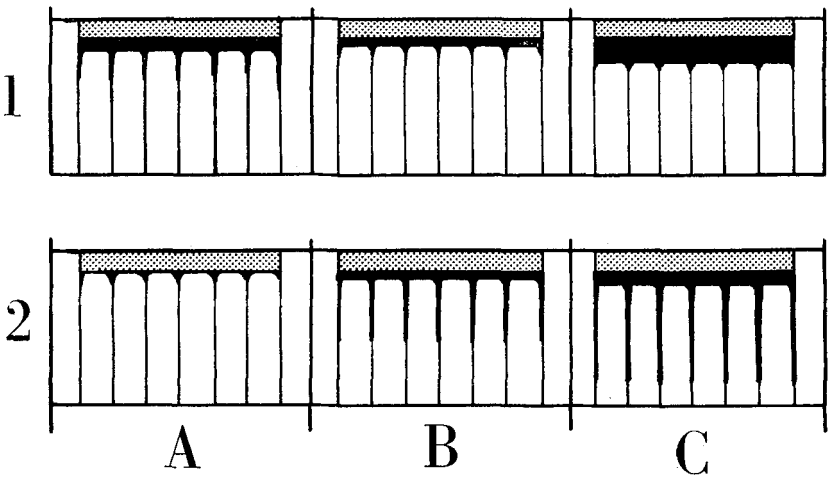
Microradiography

The microradiographic picture of the sections exposed to EDTA solutions of pH 3.0 showed a homogeneous radiolucent area, which corresponded in width to the positively birefringent outer spot and the adjacent isotropic layer (Fig. 3 D).

The combined lesions which appeared in polarized light in some cases subsequent to treatment with EDTA solutions (Fig. 1 D) showed a narrow inner radiolucent zone which was located at some distance from the surface. A relatively broad zone of radio-opacity separated this zone from a narrow and irregularly bordered superficial radiolucent layer.

Di-, Tri-, and Tetra- sodium salt solutions of EDTA

These solutions produced outer spots in all instances (Fig. 2, B and C). With an increase in pH a lengthening of ABP occurred (Text Fig. II, 2 B, C and Fig. 2 B, C). A decrease in concentration of the solutions caused a shortening of the ABP. The development of an outer spot with exposure to tetrasodium salt at pH 11.0 and 0.221 M is shown in Text Fig. I, 5. After 8 hours of exposure only ABP could be seen (A). After 14 hours a superficial isotropic layer with ABP of increased length could be observed (B). After 18—20 hours of exposure, a broad outer spot with an isotropic zone of 10—15 microns and ABP of 100 to 120 microns developed (C). Under the same conditions a solution of the same pH but with a concentration of 0.001 M produced an outer spot with a width of the isotropic zone of 2 microns, the length of the ABP being 2 microns.



Text Fig. II.

Schematic drawings showing the relationship between the isotropic zone (horizontal black) and the length of accentuated borders between prisms (ABP) (vertical black) as this appears in ground sections in polarized light. 1 A: sugar-saliva mixtures, 1 B: sodium lactate buffer, 1 C: sodium aspartate buffer,

2 A: saturated watery solutions of EDTA, 2 B: solution of trisodium salt of EDTA in distilled water, 2 C: solution of tetrasodium salt of EDTA in distilled water.

Microradiography

The microradiograms of the sections from the teeth treated with tetrasodium salt solutions of EDTA showed a radiolucent surface with an irregular border sending narrow protrusions into the adjacent radio-opaque enamel (Fig. 3 E).

DISCUSSION

It is apparent that the experimental lesions produced in the present series by exposing the enamel to sugar-saliva mixtures do not entirely correspond to those obtained by *Hals, Torell & Mörch* (2) on human enamel. According to these authors, the initial attack which can be seen in polarized light consists of an accentuation of the prisms sheaths in a narrow superficial zone of the enamel. In the present series the consistent finding made was that the first stage of the development of enamel lesions by sugar-saliva mixtures is the appearance of a subsurface isotropic zone (Fig. 1 A). This discrepancy may be due to structural difference in human and dog enamel (3). On the other hand, in the present series it was found that the pH of the sugar-saliva mixtures decreased from neutrality to pH 5 during 2½ hours of incubation. This period may be too short for the initiation of a lesion on the enamel surface, as the experiments with lactate buffers show that superficial defects can only be found after 6 hours when buffer solutions with a pH above 5.0 are used. After incubation periods longer than 2½ hours, the pH of the sugar-saliva mixtures was in the range of 4—5. This pH area has been found to be appropriate for the production of inner spots with lactate buffers (1). The development of pure inner spots in the sugar-saliva mixture series may under these conditions be caused by the presence of calcium and phosphate ions in the saliva. As the main part of the enamel consists of calcium phosphate, the rate of the solution of the surface layer may be lower in the sugar-saliva mixtures than in lactate buffers (2).

The same phenomenon may explain the appearance of combined lesions after exposure to lactate buffer solutions with pH 4—5 (Fig. 1 B, Text Fig. I, 2 B). As these buffers do not contain calcium and phosphate ions, the demineralization may take place at the surface, as well. In the pH areas below 4 and above 5, only

inner spots developed. This is in accordance with the results reported earlier for human teeth.

Aspartate buffer solutions in the pH area below 5.8 always produce outer spots. The finding that no defect could be produced within 60 days of exposure to an aspartate buffer solution of pH 5.8 is very interesting, but it is felt that more information is needed before it can be explained.

The effect of EDTA solutions in distilled water may be explained as an effect of a pure acid. Due to a low concentration of the solution, the binding of free hydrogen ions takes place. The lesion accordingly appears as narrow outer spots (Fig. 2 A). The fact that in some cases combined lesions were found may be explained by a simultaneous acid and chelating action (4). The initiation of the enamel lesion after exposure to sodium salts of EDTA is characterized exclusively by the accentuation of the borders between the prisms. This finding is in accordance with the results of the replica studies on human enamel by *Müller & Schait* (4) and by *Mühlemann* (5). By treating the enamel surface with sodium salts of EDTA solution of pH 8, they found that these agents caused a dissolution of the interprismatic substance. In this way the replica showed a very regular differentiation between prisms and interprismatic matter. On the other hand, such a regular pattern could not be obtained with other demineralizing agents. By making replicas of the enamel treated with other agents, outer spots or inner spots as well as combined lesions were shown. In case of an outer spot, both the prisms and the interprismatic material are partly destroyed. In case of inner spots, the superficial layer of the enamel is fairly intact. Consequently, in these instances replicas will either show a weather-beaten or/and an intact enamel surface. Theoretically, it would be possible to obtain replicas with regular patterns by the use of these agents if the replicas could be made at the very stage when only the ABP appears at the surface. When it is the question of treatment with lactate buffers, this stage lasts for a very short period of time. Together with the varying degree of mineralization of the surface enamel, this makes it next to impossible to obtain replicas with regular patterns. The fact that it is easy to get good replicas from enamel treated with alkaline solutions of EDTA sodium salts may be explained by the persistence of the

ABP picture for hours of exposure. It seems that the development of ABP is not entirely dependent on the pH of the sodium salt solutions of EDTA, but it is also influenced by their concentration (6). In this way we can get the polarization microscopic picture shown in Text Fig. II, 2 B by a high concentration of Na_3 EDTA or by a lower concentration of Na_4 EDTA. Both Na_3 EDTA and Na_4 EDTA have an alkaline pH, and the development of ABP may be directly related to this alkalinity, as the mode of penetration of an alkaline solution most likely is determined by the organic contents of the interprismatic material (6).

SUMMARY AND CONCLUSIONS

The experiments *in vitro* reported in the present paper were performed in order to study the defects produced when the enamel surface of dog teeth was exposed to sugar-saliva mixtures, lactate buffers, aspartate buffers, EDTA and solutions of EDTA sodium salts. The defects were examined in ground sections with polarizing microscope as well as by microradiography. It was observed that the defects produced by acid buffer solutions were different from those obtained by chelating agents at an alkaline level. A possible explanation of the appearance of the different types of defects is given. It was found that the defects produced to a great extent were similar to those obtained on human teeth in earlier investigations. This indicates that dog teeth may constitute a valuable supplementary test material in experimens of this kind.

RÉSUMÉ ET CONCLUSIONS

ACTION DE DIVERS AGENTS DÉCALCIFIANTS SUR L'ÉMAIL DENTAIRE DU CHIEN

Les expériences *in vitro* dont rend compte cet article ont été faites dans le but d'étudier les lésions produites lorsque la surface de l'émail dentaire du chien est exposée à des mélanges sucre-salive, des tampons au lactate, des tampons à l'aspartate, de l'EDTA et des solutions de sels sodiques d'EDTA. Les lésions ont été étudiées sur des coupes par usure au microscope de polarisation et par des microradiographies. Il a été observé que

les lésions produites par les solutions tampons acides étaient différentes de celles obtenues par les agents de chélation à un niveau alcalin. Les auteurs donnent une explication possible de l'aspect des différents types de lésions. Il est apparu que les lésions produites étaient dans une grande mesure analogues à celles obtenues sur les dents humaines lors des recherches antérieures. Cela indique que les dents de chien peuvent constituer un matériel expérimental supplémentaire de valeur pour les études de ce genre.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

DIE WIRKUNG VERSCHIEDENER ENTKALKUNGSMITTEL AUF DEN SCHMELZ BEI HUNDEN

In der vorliegenden Arbeit wird über Experimente, die *in vitro* an Hundezähnen ausgeführt wurden, berichtet.

Die Zähne wurden verschiedenen Lösungen ausgesetzt (Zucker-Speichel, Milchsäure-Puffer, Aspartat-Puffer, EDTA und EDTA-Natrium-Salz). Die Veränderungen am Schmelz wurden polarisationsmikroskopisch sowie mikroröntgenologisch untersucht. Dabei konnte festgestellt werden, dass sich die durch Säurepuffer hervorgerufenen Veränderungen von denen durch chelierende Agentien bei alkalischer Reaktion hervorgerufenen unterschieden. Eine Erklärung für das Auftreten dieser unterschiedlichen Veränderungen wird gegeben. Diese künstlich erzeugten Läsionen waren denen sehr ähnlich, die man in früheren Untersuchungen an Menschenzähnen erhielt. Dies ist ein Beweis dafür, dass man Hundezähne für Untersuchungen dieser Art sehr gut als Testmaterial verwenden kann.

RESUMEN Y CONCLUSIONES

EFECTOS DE VARIOS AGENTES DESMINERALIZANTES SOBRE EL ESMALTE DENTARIO DE PERRO

Los experimentos *in vitro* referidos en este trabajo, fueron llevados a cabo con el fin de estudiar los defectos producidos en la superficie del esmalte de dientes de perro, cuando eran expuestos a la acción de mezclas de saliva con azúcar, buffers de lactato, buffers de aspartato, EDTA y soluciones de sales sódicas de EDTA. Los defectos fueron examinados en cortes por desgaste

con microscopio de polarización y por microradiografía. Se observó que los defectos producidos por soluciones de buffers ácidos eran diferentes a los obtenidos por agentes quelantes en medio alcalino. Se da una posible explicación de la aparición de los diferentes tipos de defectos. Se encontró que los defectos producidos eran similares en gran parte a los obtenidos en dientes humanos en investigaciones anteriores. Esto indica que el diente de perro puede constituir un valioso material de prueba suplementarios en experimentos de esta clase.

REFERENCES

1. *Hals, E., T. Mörch and H. F. Sand,* 1955: Effect of lactate buffers on dental enamel *in vitro,* Acta odont. scand. *13:* 85—122.
2. *Hals, E., P. Torell and T. Mörch,* 1959: Enamel lesions produced *in vitro* by sugar-saliva mixtures. Acta odont. scand. *17:* 299—309.
3. *Löe, H. and C. Ravnik,* 1961: A morphological study of the surface layer of dog tooth enamel. Acta odont. scand. *19:* 483—493.
4. *Müller, Gerd and Angela Schait,* 1957: Morphologic differences in replicas of intact enamel decalcified in acid or EDTA. Helv. odont. Acta *1:* 5—8.
5. *Mühlemann, H. R.,* 1960: Experimental modifications of the enamel surface. Helv. odont. Acta *4:* 5—24.
6. *Sand, H. F., C. Ravnik and T. Mörch:* Enamel lesions produced *in vitro* with solutions of EDTA and EDTA-sodium salts. To be published in Acta odont. scand.

PLATES

Plate 1.

Fig. 1. Photomicrographs of transverse ground sections of treated dog enamel in polarized light after imbibition in distilled water. 360 ×.

A. Sugar-saliva mixtures for 16 hrs.
Broad inner spot of positive birefringence bordering centrally on a narrow zone of isotropy towards the normal enamel. Narrow zone of negative birefringence at the surface.

B. Sodium lactate buffer, (0.5 M, pH 5) for 4 hrs.
Positively birefringent inner spot extending to the negatively birefringent surface in limited areas.

C. Sodium aspartate buffer pH 4.15 for 4 days presenting positively birefringent outer spot.

D. Saturated EDTA solution in distilled water for 24 hrs.
Combined lesion consisting of an outer spot of positive birefringence with adjacent isotropic band bordering on a zone of negative birefringence. Further, centrally an inner spot of isotropy with accentuated borders between the prisms (ABP).

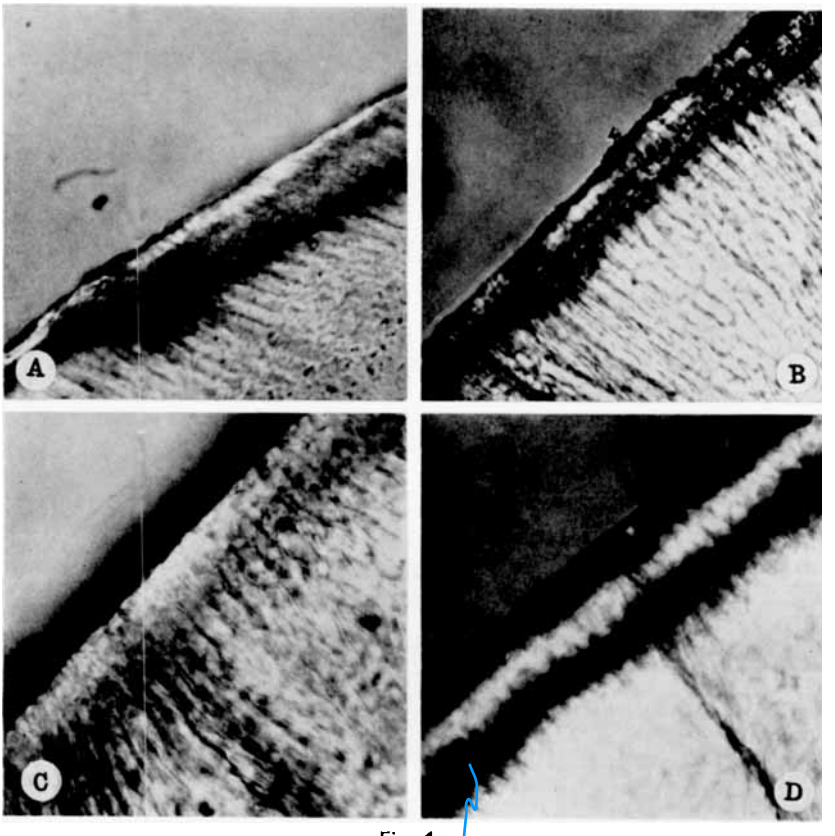


Fig. 1.

Plate 2.

Fig. 2. Photomicrographs of transverse ground sections of treated dog enamel in polarized light after imbibition in distilled water. 360 ×.

A. Saturated EDTA solution in distilled water, 0.001 M, pH 3.03 for 24 hrs.
Outer spot presenting positive birefringence and an adjacent narrow isotropic zone with short ABP.

B. Trisodium salt solution of EDTA, 0.117 M, pH 7.94.
Outer spot of positive birefringence and adjacent isotropic layer.
Note the length of ABP.

C. Tetrasodium salt solution of EDTA, 0.0702 M, pH 11.01.
Outer spot of positive birefringence and adjacent zone of isotropy.
Increased length of ABP.

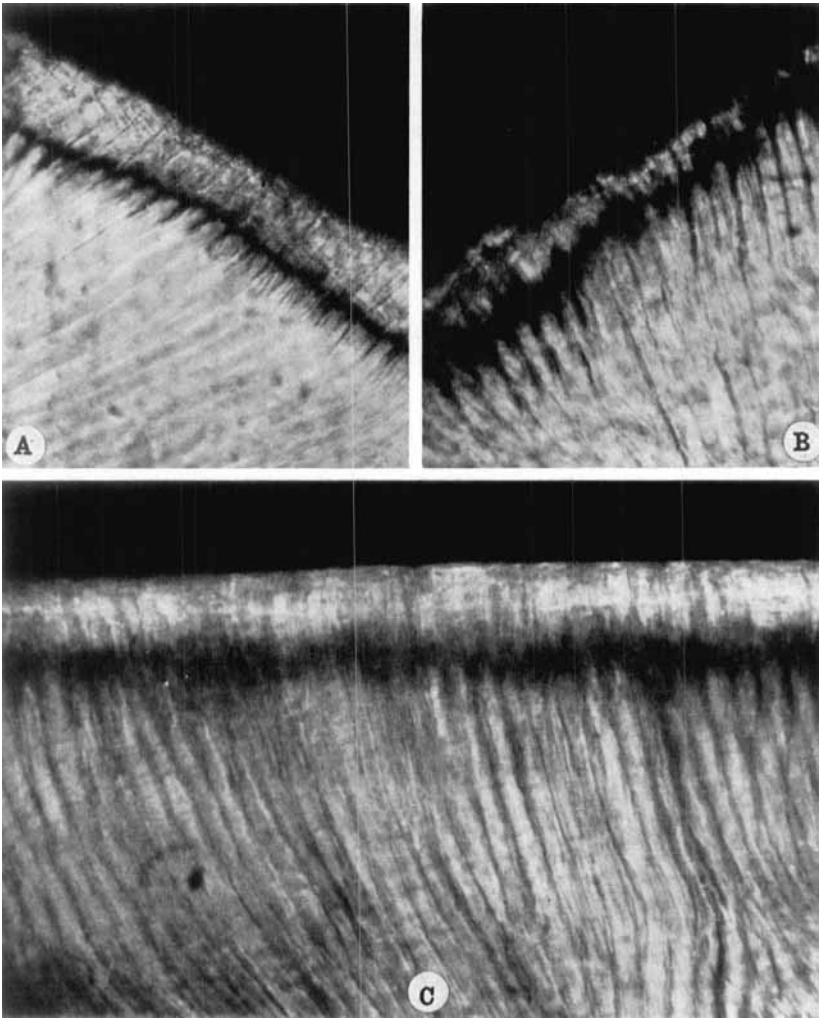


Fig. 2.

Plate 3.

Fig. 3. Microradiograms.

A. Normal enamel.

B. Enamel treated with sugar-saliva mixtures.
(Same section in polarized light shown in Fig. 1A).

C. Enamel treated with sodium lactate buffer.
(Same section in polarized light shown in Fig. 1B).

D. Enamel treated with saturated EDTA solution in distilled water.
(Same section in polarized light shown in Fig. 2A).

E. Enamel treated with tetrasodium salt solutions of EDTA, pH 11.0.

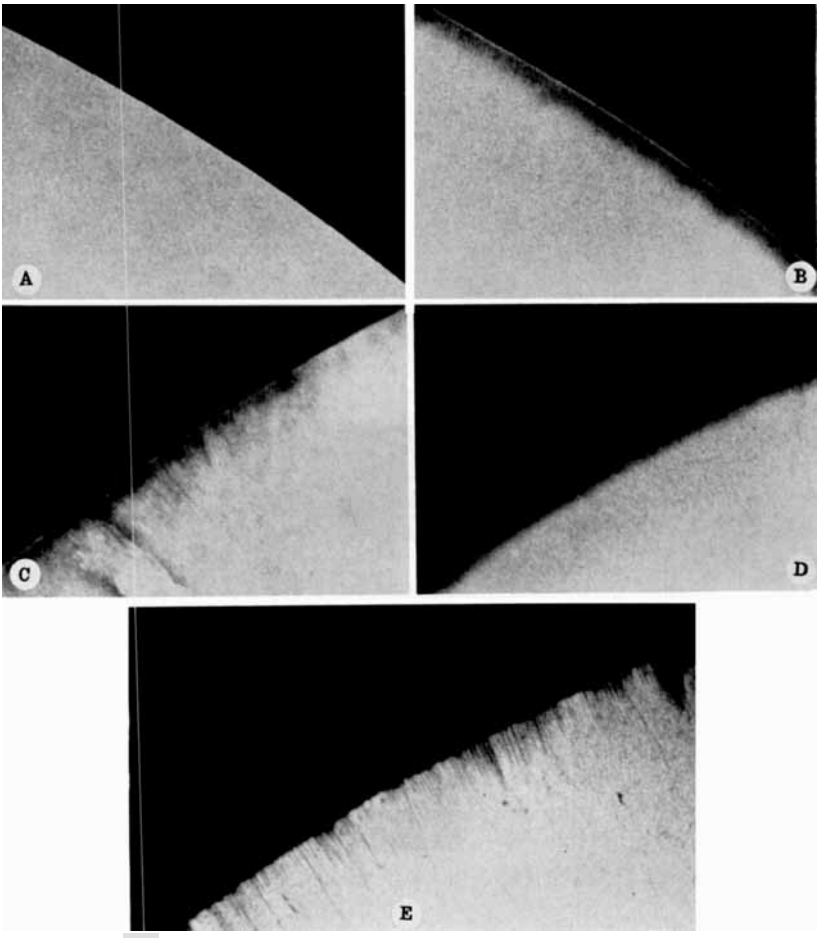


Fig. 3.