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EVALUATION OF THE INHIBITING EFFECT OF VARIOUS FLUORIDES ON DOG ENAMEL *IN VITRO*

by

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In an earlier investigation it was found that dog teeth react in the same way as human teeth to various agents (1). The present study was undertaken in order to obtain information regarding the suitability of dog teeth for testing the inhibiting effect of topically applied fluorides in laboratory experiments.

MATERIAL AND METHODS

A series of 80 laboratory experiments was performed with dog teeth, using a method previously described (2). The buccal surface of each tooth was covered with wax except for a circular area which was left uncovered. A vertical groove was cut in the uncovered area with a diamond disc, thus dividing it into two halves. One half was covered with a different type of wax and served as a control, while the fluoride solution in question was applied to the other half for 10 minutes. After rinsing with tap water, the wax on the control half was removed, and both halves were allowed to "maturate" in neutral saliva for one hour. Then the experimental area was exposed to the agents used for the production of lesions in the enamel surface. The exposure time

for each agent was adjusted in accordance with the results obtained earlier (1).

The agents used were,

(1) Sugar-saliva mixtures, the pH of which gradually dropped from neutral to 4.0—4.5 during the time of exposure, (60 experiments).

(2) 0.5 molar lactate buffer, pH 2.9, (5 experiments).

(3) 0.5 molar lactate buffer, pH 5.44, (5 experiments).

(4) 0.001 molar EDTA in distilled water (saturated), pH 3.03, (5 experiments).

(5) 0.07 molar Na_4EDTA, pH 11.0, (5 experiments).

Topical applications were made with,

(A) $SnCl_4 + NaF$, ratio 1:3, 0.14 molar F, pH 1.55 (Stannic fluoride*).

(B) $SnCl_4 + NaF$, ratio 1:3, 0.14 molar F, pH 2.4 (Stannic fluoride*).

(C) A mixture of (B) and leucine hydrofluoride (283)**), 0.2 %, pH 2.65.

The inhibiting effect of the fluoride solution in question was evaluated by comparing the test half with the control half of the experimental area. This was first done macroscopically. Then ground sections were prepared and examined with a polarizing microscope. Microradiographs of some representative sections were also made.

The defects were divided into three groups according to the severity of attack. Each group was assigned a certain number of points as follows:

Macroscopic

Intact surface	0 points
Shiny surface, homogeneous or non-homogeneous white spot	1 "
Dull surface, homogeneous white spot	2 "

*) Subsequent analyses of the solutions employed have disclosed that the tin and fluorine may be present in other forms than SnF_4.

**) The leucine hydrofluoride (283) was kindly submitted by Professor H. R. Mühlemann, Zürich.

Microscopic

Intact surface ..... 0 points
Inner spot ..... 1 "
Outer spot ..... 2 "

The microscopic evaluation was based on a scheme previously described (2).

The difference between test and control areas was called *positive* when the test area showed less attack than did the control area. A difference of 2 points was designated as being "marked", while 1 point of difference was given the designation "slight".

RESULTS

The results obtained in the experiments with sugar-saliva mixtures are given in Tables 1 and 2, and in Figs. 1 and 2. Representative photomicrographs from this material are shown in Fig. 3, A—E.

Table 1 shows that a positive difference between test and control areas was more frequently observed with the stannic fluoride

Table 1. Difference in points between test areas and control areas. Sugar-saliva mixtures.

Substance	Number of exp.	Positive diff.				O		Negative diff., slight	
		Marked		Slight					
		MA	MI	MA	MI	MA	MI	MA	MI
Stannic fluoride, 0.14 M F, pH 1.55	20	3	3	8	9	8	4	1	4
Stannic fluoride, 0.14 M F, pH 2.4	20	4	5	9	11	7	4	—	—
Stannic fluoride, + leucine hydrofluoride, 0.2 % F, pH 2.65	20	3	11	8	5	9	4	—	—

complex solution of pH 2.4 and with the mixture of stannic fluoride and leucine hydrofluoride (283) than was the case with the stannic fluoride complex solution of pH 1.55.

Table 2. Percentage of inhibition calculated from the difference in severity of attack between test and control areas. Sugar-saliva mixtures.

Substance	Per cent inhibition	
	MA	MI
Stannic fluoride, 0.14 M F, pH 1.55	35	31
Stannic fluoride, 0.14 M F, pH 2.4	47	60
Stannic fluoride + leucine hydrofluoride, 0.2 % F, pH 2.65	42	69

Some statistical data concerning the values given in Table 2 are shown in Figs. 1 and 2.

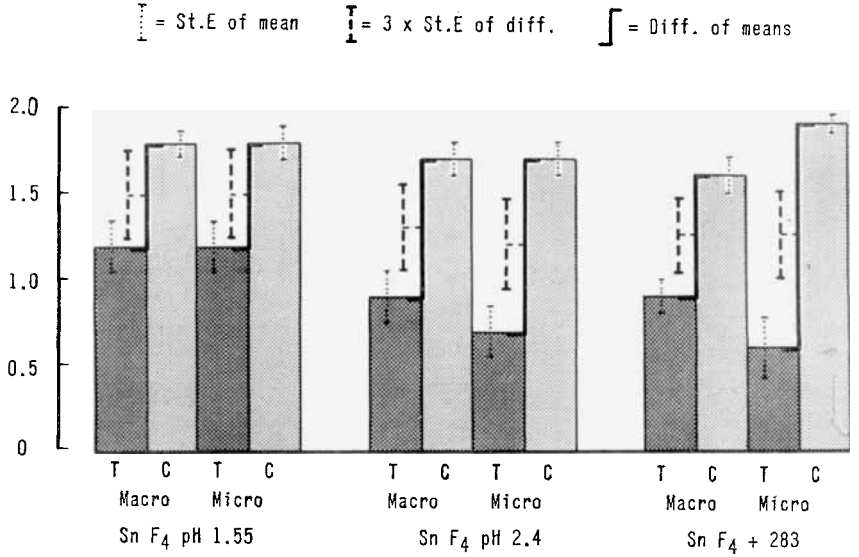


Fig. 1. Ordinate: Mean of severity of attack on test areas and control areas.

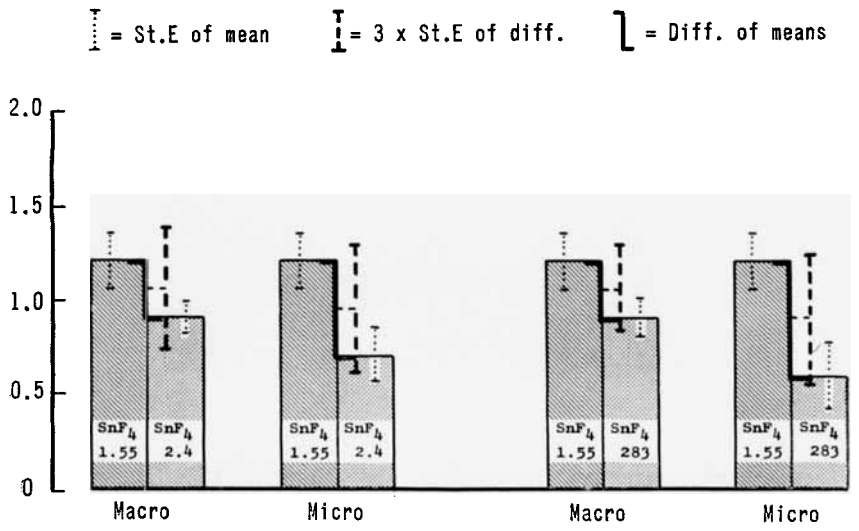
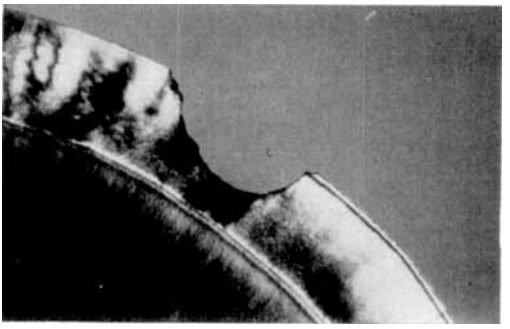


Fig. 2. Comparison of the inhibiting effect of the fluoride solutions employed. Ordinate: Mean of severity of attack on test areas and control areas.

Fig. 1 shows that a statistically significant inhibiting effect was obtained with all the three fluoride solutions employed. Both the macroscopic and the microscopic evaluation show that the inhibiting effect of the stannic fluoride solution of pH 1.55 was a little less than that obtained with the other two solutions. However, from Fig. 2 it is seen that no statistically significant difference existed between the different fluoride solutions used. On comparing macroscopic and microscopic evaluations, no statistically significant difference can be found between the two methods.

In Fig. 3 are shown some photomicrographs of representative ground sections. The differences between test areas and control areas in these cases are obvious both in polarized light and with microradiography.

The results obtained in the preliminary experiments with lactate buffers and solutions of EDTA and EDTA-tetrasodium salt showed that the fluoride solutions employed increased the surface resistance of dog enamel against all these buffers.



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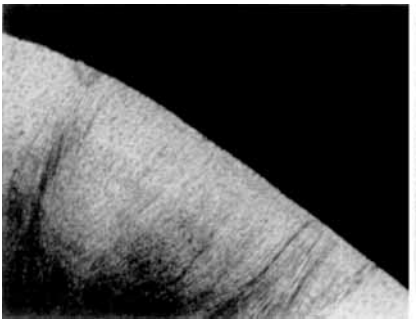

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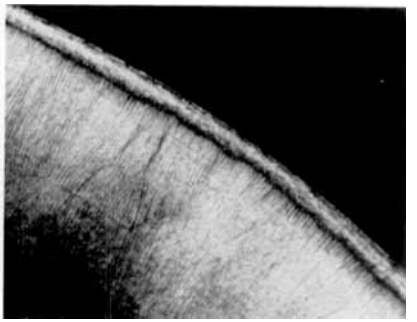

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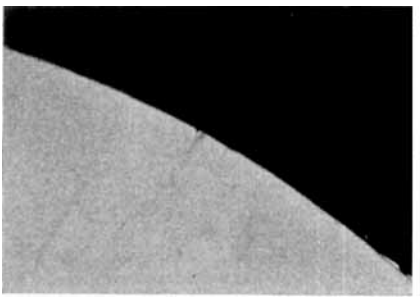

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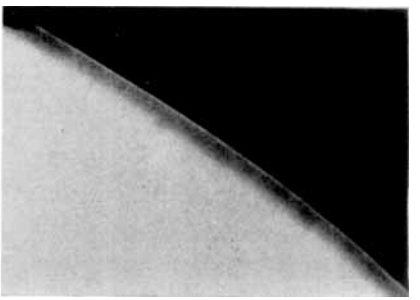

E.

Fig. 3. A, Dog tooth exposed to sugar-saliva mixture for 24 hours. Test area treated with SnF_4, pH 2.4, is intact. Control area shows inner spot. B and C, Higher magnification of the areas shown in A. D, Microradiograph from an intact test area. E, Microradiograph from a control area showing inner spot.

DISCUSSION

The finding that the fluorides used increased significantly the resistance of dog enamel surface against the attack of sugar-saliva mixtures is in accordance with the results obtained earlier when the same fluoride solutions were tested both *in vitro* (3) and *in vivo* (4) on human teeth. This indicates that dog enamel reacts to fluoride solutions in the same way as does human enamel. It may, therefore, be concluded that dog teeth can be used as a substitute for human teeth in experiments of this kind.

The results of macroscopic and microscopic evaluation indicate that both methods are of the same value. The results with acid lactate buffers and acid solution of EDTA are in accordance with those described above using sugar-saliva mixtures. This was to be expected, as all these agents probably cause an acid dissolution of the enamel. The results with the EDTA-tetrasodium salt solution of pH 11.0 are of a special interest, as the fluoride solutions seem to increase the resistance of enamel against chelating agents. Further experiments with alkaline EDTA-sodium salt solutions may give information about the mechanism underlying the inhibiting effect of topically applied fluoride solutions.

SUMMARY

The ability of fluoride solutions to increase the resistance of dog enamel was investigated in 80 laboratory experiments, using a modification of the window technique. Stannic fluoride solutions of pH 1.55 and 2.4, as well as a mixture of stannic fluoride and leucine hydrofluoride, pH 2.65, were employed. The protective action of the fluorides was tested with sugar-saliva mixtures, lactate buffers, EDTA solution, and a solution of Na_4EDTA.

A statistically significant increase of the resistance against sugar-saliva mixtures was found for all the fluoride solutions employed. The best results were obtained with the mixture of stannic fluoride and leucine hydrofluoride. A little less effect was noted for the pH 2.4 stannic fluoride solution, while the least

effect was found for the pH 1.55 stannic fluoride solution. However, the differences between the fluoride solutions used were not statistically significant.

It was also observed that the fluoride solutions employed increased the resistance of dog enamel against all the different agents used.

It may be concluded that dog teeth can be used as a substitute for human teeth in experiments of this kind.

RÉSUMÉ

ESTIMATION DE L'ACTION INHIBITRICE DE DIFFÉRENTS FLUORURES SUR L'ÉMAIL DU CHIEN *IN VITRO*

80 expériences de laboratoire suivant une modification de la "technique de la fenêtre" ont été effectuées dans le but d'étudier le pouvoir d'augmenter la résistance de l'émail du chien que présentent les solutions de fluorures. Des solutions de fluorure stannique de pH 1,55 et 2,4, et un mélange de fluorure stannique et d'hydrofluorure de leucine d'un pH de 2,65 ont été utilisés. L'action protectrice des fluorides a été éprouvée avec des mélanges sucre-salive, des tampons au lactate, une solution d'EDTA et une solution de Na_4EDTA.

Une augmentation significative du point de vue statistique de la résistance aux mélanges sucre-salive a été mise en évidence pour toutes les solutions de fluorures utilisées. Les résultats les meilleurs ont été obtenus avec le mélange de fluorure stannique et d'hydrofluorure de leucine. Un effet un peu moins bon a été noté pour les solutions de fluorure stannique de pH 2,4, et l'effet le moins grand a été trouvé pour la solution de fluorure stannique de pH 1,55. Cependant, les différences entre les solutions de fluorures employées n'étaient pas significatives du point de vue statistique.

On a aussi observé que les solutions de fluorures employées augmentaient la résistance des dents de chien à tous les différents agents utilisés.

On peut conclure que les dents de chien peuvent servir à la place des dents humaines pour les expériences de ce genre.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER DIE WIRKUNG VON VERSCHIEDENEN FLUORIDEN AUF DEN HUNDESCHMELZ *IN VITRO*

Unter Benutzung einer modifizierten Fenstertechnik wurde in 80 Laboratoriumsversuchen die Fähigkeit der Fluorlösungen untersucht, die Widerstandsfähigkeit des Zahnschmelzes beim Hund zu steigern. Zinnfluoridlösungen mit einem pH von 1,55 und 2,4 sowie eine Mischung von Zinnfluorid und Leuzinhydrofluorid, pH 2,65, kamen zur Anwendung. Die Schutzwirkung der Fluoride wurde mit Zucker-Speichelmischungen, Lactatpuffern, EDTA-Lösung und einer Na_4EDTA-Lösung geprüft.

Eine statistisch-signifikant höhere Widerstandsfähigkeit gegen Zucker-Speichelmischungen wurde für alle verwendeten Fluoridlösungen gefunden. Die besten Ergebnisse wurden mit der Zinnfluorid-Leuzinhydrofluorid-Mischung erzielt. Ein um etwas geringerer Effekt wurde für die Zinnfluoridlösung mit dem pH 2,4 gefunden, während die Zinnfluoridlösung mit pH 1,55 den geringsten Effekt hatte. Dennoch waren die Unterschiede zwischen den einzelnen zur Anwendung gebrachten Fluoridlösungen nicht statistisch signifikant.

Weiterhin wurde beobachtet, dass die verwendeten Fluoridlösungen die Widerstandsfähigkeit gegenüber den verschiedenen ausprobierten Medien erhöhten.

Es darf angenommen werden, dass Hundezähne anstelle von Menschenzähnen für Versuche dieser Art geeignet sind.

RESUMEN

EVALUACIÓN DE LA ACCIÓN INHIBIDORA DE LOS DISTINTOS FLUORUROS SOBRE EL ESMALTE DE PERRO *IN VITRO*

Se investigó la propiedad de las soluciones de fluoruros de aumentar la resistencia del esmalte en dientes de perro, en 80 experimentos de laboratorio, usando una modificación de la técnica de la ventana.

Se emplearon soluciones de fluoruro estánico, de pH 1.55 y 2.4, asi como también una mezcla de fluoruro estánico y fluorhidrato de leucina, de pH 2.65. La acción protectora de los fluoruros fué

probada con mezclas de saliva y azúcar, buffers de lactatos, solución de EDTA y una solución de Na_4 EDTA.

Se notó un aumento estadísticamente importante de la resistencia ante las mezclas de saliva y azúcar, con todas las soluciones de fluoruros empleadas. Los mejores resultados se obtubieron con la mezcla de fluoruro estánico y fluorhidrato de leucina. Se notó un efecto algo menor con la solución de fluoruro estánico de pH 2.4, mientras que el efecto menor fué el obtenido con la solución de fluoruro estánico de pH 1.55. Sin embargo, la diferencia entre las soluciones de fluoruro empleadas no fué estadísticamente importante. Asimismo se pudo observar que las soluciones de fluoruro utilizadas aumentaron la resistencia de los dientes de perro, ante los distintos agentes usados.

Puede concluirse que los dientes de perro pueden emplearse en reemplazo de dientes humanos, en experiencias de este tipo.

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