From: The Royal Dental School, Stockholm, Sweden

EFFECT OF CHLORIDE IONS ON THE FLUORIDE UPTAKE BY DENTAL ENAMEL

by

YNGVE ERICSSON

During a pilot study of the possible influence of different inorganic ions on the uptake of F^{18}-labelled fluoride ions in powdered dental enamel it was found that this uptake was strongly reduced in the presence of chloride ions. This gave rise to a closer study of the phenomenon, with a view especially to the following clinically important circumstances:

1) the positive correlation between salivary chloride content and caries incidence, which has repeatedly been observed (*Anders* 1956, 1957; *Shannon* 1958; *Carter & al.* 1958); this appears difficult to explain;

2) the theoretically attractive method of caries-preventive fluoride administration with kitchen salt; if chloride ions reduce the fluoride uptake by the enamel this would be a serious drawback of the method.

EXPERIMENTAL

General methods

Two concentrations of sodium chloride were used for the tests: 35-mM, corresponding to the upper range of salivary chloride content, and 350-mM, corresponding to a salt-rich foodstuff.

Different fluoride concentrations were applied; however, in most of the tests 0.1-mM NaF was used. This corresponds to 1.9 ppm F and may be said to represent the content of a fluorine-rich drinking-water; it may also represent the fluorine concentration of a dish prepared with fluoridated kitchen-salt. F^{18}-labelling was done with diluted distillates of neutron-irradiated lithium hydroxide (*Ericsson & Ullberg* 1958).

Enamel powder was produced in the following way. The crowns of extracted teeth were separated from their roots by means of burs, the crown dentine was drilled away and the enamel pulverized in a steel mill. Powdered remains of the crown dentine were removed according to the Manly-Hodge flotation technique (1939), and the enamel powder was dried and sifted into fractions of different particle size. Two fractions were used for the experiments, one passing sieve no. 24 (mesh width 0.25 mm) but not no. 40 (mesh width 0.15 mm), and the other one passing no. 40 but not no. 100 (mesh width 0.06 mm).

In the tests the solutions containing labelled fluoride with or without chloride ions were shaken with the enamel powder in plastic tubes for 30 min. periods. 50 mg powder was used for every ml of solution. After shaking, the powder was centrifuged down, and either the label remaining in the supernatant or the fraction taken up by the enamel was determined, in the latter case after washing the powder repeatedly with volumes of distilled water in a standardized manner.

The radiometric analyses were performed in a well-type scintillation detector. The counting error was generally below 1 %, but in a few instances amounted to 1—2 %.

For tests with intact enamel surfaces homologous extracted tooth pairs, usually upper or lower first premolars, were used according to the technique described in two previous papers (*Ericsson* 1958, 1961).

Chloride effect on the fluoride uptake by powdered enamel

Sodium chloride in 35-mM and 350-mM concentrations consistently reduced the fluoride uptake by powdered enamel, although to a varying degree in different experiments. A typical example is given in Fig. 1.

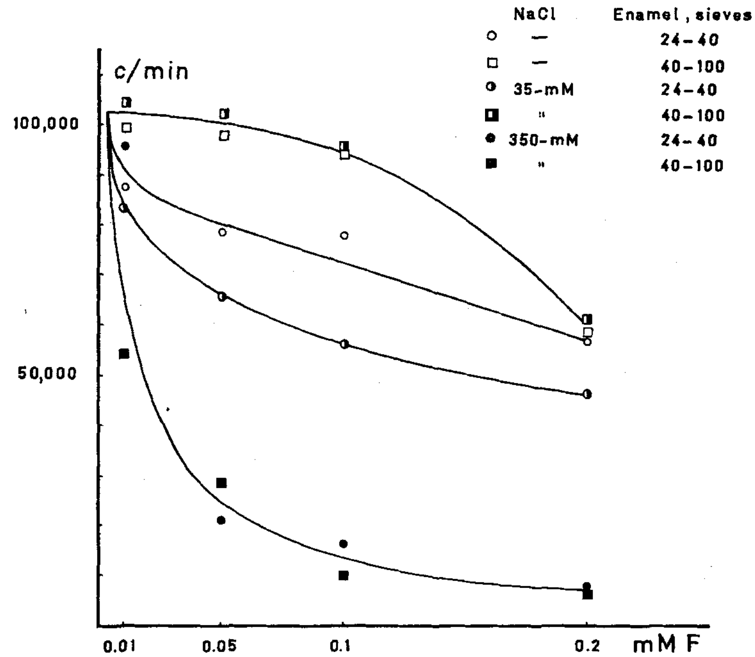

Fig. 1.

Reduction through chloride of F^{18} uptake by powdered dental enamel.

A control experiment using 350-mM $NaNO_3$ instead of NaCl, 0.1-mM NaF labelled with F^{18}, and enamel powder of the fraction 40—100 demonstrated that the sodium ion had no reducing influence on the fluoride uptake, which was thus to be ascribed to the chloride ions.

Chloride effect on the fluoride uptake by synthetic tricalcium phosphate

The striking reduction of the fluoride uptake by powdered dental enamel, which was brought about by the applied chloride concentrations, made it desirable to investigate whether similar reductions would be obtained in the uptake in salts and salt mixtures of the same kind as make up the main part of the enamel. For this purpose the F^{18} uptake from F^{18}-labelled 0.1-mM sodium fluoride, with and without added chloride, was tested with the following salts and salt mixtures:

1) synthetic tricalcium phosphate (which is crystallized as $Ca_5(PO_4)_3OH$),

2) the same phosphate after shaking for 24 hours with 50 parts by weight of 50-mM NaF solution, followed by washing and drying,

3) the same phosphate after corresponding treatment with 350-mM NaCl solution,

4) 95 % of the same phosphate + 5 % calcium carbonate,

5) 93 % of the same phosphate + 7 % magnesium triphosphate.

The salts employed were analytically pure. The added carbonate and magnesium, respectively, correspond roughly to the CO_2 and Mg contents of the enamel. The pre-treatment with NaCl or NaF, respectively, aimed at testing the possible effect on the fluoride uptake of saturating the crystal surfaces with the ions in question.

The solid: solution proportions used were the same as in the tests with enamel powder, as were also the chloride concentrations and the shaking time. Each test was performed *in duplo*. The pH values were adjusted with small quantities of nitric acid.

The results of two typical series of experiments are given in Tables 1 and 2.

It appears that increased chloride concentrations have *increased* the fluoride uptake by the calcium phosphate, whether this latter was pre-treated with fluoride or chloride ions or not and whether carbonate or magnesium was present or not.

When similar experiments were performed at pH values below 6.5 the fluoride uptake was consistently over 99 per cent of the total content of the liquid phase, with no apparent differences between the different solids or between liquids with varying chloride content. Freshly precipitated calcium phosphate quantitatively absorbed the fluoride even at higher pH values and without any influence of the chloride concentration up to 350-mM.

The results of these experiments thus did not parallel, and still less explain, the results obtained with the enamel powders.

Table 1.

Chloride effect on fluoride uptake by synthetic tricalcium phosphate, with or without previous treatment with NaF or NaCl.

Percentage figures refer to the total content of the liquid phase.

Chloride concentration	Untreated phosphate		NaF-treated phosphate		NaCl-treated phosphate	
	% uptake	final pH	% uptake	final pH	% upptake	final pH
350-mM NaCl	26.6	7.50, 7.60	11.2	7.75, 7.60	18.2	7.25, 7.30
35-mM NaCl	19.6	7.40	10.4	7.60, 7.50	16.8	7.45, 7.50
—	18.8	7.45	10.6	7.85, 7.70	15.4	7.55, 7.60
350-mM NaCl	40.6	7.35, 7.40	22.6	7.05	29.7	7.00, 7.05
35-mM NaCl	35.1	7.35, 7.40	20.6	7.10	28.4	7.20, 7.25
—	33.9	7.35, 7.40	19.8	7.20	28.1	7.30, 7.25

Table 2.

Chloride effect on fluoride uptake by calcium phosphate with calcium carbonate or magnesium phosphate added.

Percentage figures refer to the total content of the liquid phase.

Chloride concentration	95 % $Ca_3(PO_4)_2$ + 5 % $CaCO_3$		93 % $Ca_3(PO_4)_2$ + 7 % $Mg_3(PO_4)_2 \cdot 5H_2O$	
	% uptake	final pH	% uptake	final pH
350-mM NaCl	28.1	8.7	21.9	7.10, 7.15
—	25.9	8.8	19.1	7.15, 7.40
350-mM NaCl	18.0	8.8, 9.0	11.0	7.45, 7.80
—	16.4	9.0, 9.1	9.8	7.50, 7.65

Chloride effect on the fluoride uptake of intact tooth surfaces

However interesting the effect of chloride on the fluoride uptake by enamel powder may be, decisive importance has to be attached to its possible effect on the corresponding uptake by the enamel surfaces. This was tested using intact homologous premolars that had been extracted for orthodontic reasons.

The teeth had been stored in physiologic saline saturated with thymol since the time of extraction. They were rinsed with tap water followed by distilled water for several hours before the experiments.

Of each tooth pair, one was exposed to 0.1-mM sodium fluoride solution, which was labelled with F^{18}, of neutral reaction and containing at the same time either 350-mM or 35-mM sodium chloride. The homologous tooth was exposed to the same labelled fluoride solution without any chloride content. Care was taken that only the enamel surface was exposed to the labelled fluoride solution (*Ericsson* 1958, 1961).

After 60 min. exposure the teeth were rinsed with running tap water for 1 min. and the wax covering the root was removed. Each tooth was placed in a standard position with the crown in the bottom of the well type scintillation crystal, and the F^{18} uptake by the crown was analyzed.

In the first series, 10 tooth pairs were tested with 350-mM and 5 pairs with 35-mM sodium chloride. In the second series, which was performed after the complete decay of the F^{18} taken up in the first experiment, the homologous teeth changed place so that the previously NaCl-exposed tooth was the control and the previous control was exposed to the NaCl-containing solution.

The results of these experiments appear in Table 3.

It is seen in the table that the enamel surface uptake of labelled fluoride has in most cases been *greater* from the NaCl-containing solution than from the control solution. As regards the inevitable individual differences between the two teeth of a homologous pair, it is seen that where one tooth shows an especially strong relative uptake from the chloride-containing solution, the uptake of the other one from the same solution is comparatively weak and often below 100 % of the control. In the 350-mM chloride

Table 3.

F^{18} uptake by enamel surface from NaCl-containing neutral solution of labelled 0.1-mM NaF, as percentage of uptake by homologous tooth surface from the corresponding NaCl-free solution

350-mM NaCl					35-mM NaCl				
Tooth No.	Up-take %	Tooth No.	Up-take %	Average Uptake %	Tooth No.	Up-take %	Tooth No.	Up-take %	Average Uptake %
1 a	161	1 b	169	165	11 a	129	11 b	119	124
2 a	220	2 b	110	165	12 a	127	12 b	130	128.5
3 a*	751	3 b*	40	395.5	13 a	77	13 b	189	133
4 a	111	4 b	172	141.5	14 a	113	14 b	92	102.5
5 a	242	5 b	65	153.5	15 a	75	15 b	147	111
6 a	79	6 b	274	176.5					
7 a	127	7 b	181	154					
8 a	221	8 b	94	157.5					
9 a	110	9 b	85	97.5					
10 a	74	10 b	213	143.5					
M	209.6		140.3	174.95		104.2		135.4	119.8

* 3a and b had extensive opaque but smooth patches, apparently due either to defective mineralization or incipient enamel dissolution.

tests of Table 3 the uptake of the tooth series a and b showed a correlation coefficient $r = -0.662$, $P = 0.02$—0.05.

The average F^{18} uptake by each pair of tooth crowns from chloride-containing solution is in every case but one greater than the average uptake of the same crowns from chloride-free solution.

Possible chloride effect on fluoride uptake under conditions of dissolution and precipitation

The unexpected finding that chloride ions apparently enhance the fluoride uptake by enamel surfaces, while the same ions decrease the uptake by enamel powder, called for an explanation. One possible cause was thought to be the specific changes of the surface enamel by the dissolution and precipitation processes that have been found to follow each other. The following tests were designed to throw some light, if possible, on this question.

Dissolution

The possibility that superficial dissolution of the enamel surface might influence the chloride effect on fluoride uptake was tested in the following way.

Ten pairs of intact extracted homologous teeth were shaken for one hour in 4 ml 0.2-M acetate buffer pH 5.0 for each tooth. They were then rinsed thoroughly with tap and distilled water, and the roots were covered with wax as described above. One of each pair was placed in neutral 0.1-mM sodium fluoride solution labelled with F^{18}, at the same time containing a 350-mM concentration of sodium chloride; the other tooth was placed in the same solution without chloride.

After one hour's exposure time the teeth were thoroughly rinsed with water, the wax was removed and the activities taken up analyzed.

Table 4.

F^{18} uptake by acid-etched enamel surface from NaCl-containing neutral solution of labelled 0.1-mM NaF, as percentage of uptake by homologous acid-etched ooth surface from the corresponding NaCl-free solution.

350-mM NaCl				
Tooth No.	Uptake %	Tooth No.	Uptake %	Average Uptake %
1 a	142	1 b	128	135
2 a	206	2 b	113	159.5
3 a	283	3 b	70	176.5
4 a	239	4 b	58	148.5
5 a	192	5 b	107	149.5
6 a	113	6 b	130	121.5
7 a	153	7 b	129	141
8 a	198	8 b	98	148
9 a	200	9 b	93	146.5
10 a	92	10 b	245	168.5
M	181.8		117.1	149.45

In a second experiment, which was carried out after the decay of the F^{18} activity taken up in the first experiment, the homologous teeth changed places, but were otherwise treated and exposed in the same way.

The results appear in Table 4. The chloride has enhanced the F^{18} uptake by the acid-etched enamel surfaces in the majority of cases, and in every case when the average of two homologous teeth is calculated. As expected, there is again a negative correlation between the tooth series a and b, evidently due to the individual differences within each tooth pair ($r = -0.837$, $P = 0.001$—0.01).

Precipitation

Precipitation of calcium phosphate in the presence of F^{18}-labelled fluoride, with or without chloride ions present, was carried out *in vitro* using 0.075-M calcium nitrate and 0.225-M sodium phosphate buffer pH 7.0. These proportions gave pH values of 6.5—7.45 after half an hour's precipitation time. The fluoride concentration was 0.0125-mM, roughly corresponding to the salivary fluoride content and at the same time, together with the above-mentioned calcium concentration, to the solubility product of calcium fluoride. Since the calcium-containing solution was added last to the fluoride-phosphate system and immediately caused heavy precipitation of calcium phosphate, no calcium fluoride precipitation should have occurred.

Precipitation was carried out in triplicate centrifuge tubes containing 350-mM NaCl, 35-mM NaCl and no NaCl, respectively.

After 30 min. precipitation time the tubes were centrifuged and decanted, and the remaining activities and the pH values of the supernatants determined.

The pH values of the supernatants were 6.50—6.75 in the different tubes. The activities remaining in solution were about 0.5 per cent of the original activities in all the tubes, without differences between varying chloride concentrations.

In another series calcium phosphate was precipitated in a similar way on the day before the production of F^{18}. The precipitates were washed and dried at 100° overnight and were then dispersed again in 0.1-mM neutral NaF solution labelled with F^{18} and containing 350-mM NaCl, 35-mM NaCl or no NaCl, all *in duplo.* After 30 min. shaking the tubes were centrifuged and the activities and the pH values of the supernatants determined.

The results appear in Table 5.

Table 5.

Chloride effect on fluoride uptake by precipitated and washed calcium phosphate

Chloride conc.	pH of supernatant	Activity of supernatant, % of original
—	6.6	1.66
35-mM	6.2	1.82
350-mM	6.0	2.33

It appears that in this test chloride somewhat reduced the fluoride uptake by the phosphate precipitate, in spite of the fact that the pH values were lower in the chloride tubes. Little significance should, however, be attached to this difference since in any case the uptake by the phosphate precipitate was close to 100 %.

DISCUSSION

The experiments described have demonstrated that chloride ions, while reducing the fluoride uptake by powdered enamel, enhance the uptake by intact or acid-etched enamel surfaces. This is not the first time that a difference has been found between the chemical reaction of enamel surfaces and enamel powder, which emphasizes the limitations of laboratory tests with powdered enamel in the studies of caries-preventive mechanisms.

The tests carried out so far have not revealed the cause of the difference between enamel surfaces and enamel powder as regards the chloride effect on fluoride uptake. Since even the fluoride uptake by salt mixtures resembling the inorganic composition of enamel was increased by chloride ions, it is possible that either the organic component of the enamel powder or some phase of the preparation process of this powder may be responsible for its decreased fluoride uptake in the presence of chloride ions. The large crystal size of the bulk of the enamel powder might also be a differentiating factor.

The results of this investigation may be said to support the use of kitchen salt as a vehicle for caries-preventive administration of fluoride from one point of view; several other aspects naturally have to be considered.

SUMMARY

The uptake of F^{18}-labelled fluoride ions by powdered dental enamel was strongly reduced in the presence of chloride ions.

Tricalcium phosphate took up more F^{18}-labelled fluoride from chloride-containing solutions whether this salt was pretreated with fluoride or chloride ions or not and whether carbonate or magnesium, corresponding to the enamel content of these substances, was added or not.

The F^{18} uptake by intact enamel surfaces, as studied with homologous extracted premolars, was increased in the presence of 350 or 35 millimolar chloride concentrations.

Tests on the possible effect of dissolution or precipitation processes, such as may occur on the enamel surfaces, gave no clue to the differences between powdered and intact enamel.

The results support kitchen salt fluoridation from the point of view of enamel fluoride uptake and illustrate the limitations of experiments with powdered dental enamel.

RÉSUMÉ

EFFET D'IONS CHLORURE SUR L'INCORPORATION DE FLUOR DANS L'ÉMAIL DENTAIRE

L'incorporation des ions fluorure marqués de F^{18} dans l'émail dentaire pulvérisé était fortement réduite en présence d'ions chlorure.

Le phosphate tricalcique absorbait plus de fluorure marqué de F^{18} de solutions contenant du chlorure, que le phosphate ait été prétraité avec des ions fluorure ou chlorure ou non, et que du carbonate ou du magnésium, correspondant à la quantité de ces substances contenue dans l'émail, aient été additionés au phosphate ou non.

L'incorporation de F^{18} par des surfaces d'émail intactes, étudiée sur des prémolaires homologues extraites, était augmentée en présence de concentrations de chlorure 350 ou 35 millimolaires.

Des épreuves de l'effet éventuel de dissolution ou de précipitation pouvant se produire sur les surfaces d'émail n'ont pas donné l'explication de la différence entre l'émail intact et l'émail pulvérisé.

Les résultats sont en faveur de la fluorisation du sel de cuisine du point de vue de l'incorporation du fluor par l'émail, et démontrent les limitations des expériences avec de l'émail dentaire pulvérisé.

ZUSAMMENFASSUNG

EINWIRKUNG VON CHLORIDIONEN AUF DIE FLUORAUFNAHME IM ZAHNSCHMELZ

Die Aufnahme F^{18}-gemerkter Fluoridionen durch pulverisierten Zahnschmelz wurde in Gegenwart von Chloridionen stark reduziert.

Tricalciumphosphat nahm aus chloridhaltigen Lösungen mehr F^{18}-gemerktes Fluorid auf, sei es, dass das Phosphat mit Fluorid- oder Chloridionen vorbehandelt war oder nicht, oder dass Carbonat oder Magnesium, dem Schmelzgehalt dieser Stoffe entsprechend, dem Phosphat zugesetzt war oder nicht.

Die F^{18}-Aufnahme intakter Schmelzflächen, an homologen extrahierten Prämolaren studiert, wurde in Gegenwart von 350- oder 35-millimolären Chloridkonzentrationen erhöht.

Teste auf die etwaige Einwirkung von Auflösungs- oder Ausfällungsprozessen an der Schmelzoberfläche gaben keinen Schlüssel zum Unterschied zwischen pulverisiertem und intaktem Schmelz.

Die Ergebnisse unterstützen die Tafelsalzfluoridierung vom Gesichtspunkt der Fluoraufnahme des Schmelzes und illustrieren die Begrenzung der Versuche mit pulverisiertem Zahnschmelz.

REFERENCES

Anders, J. T., 1956: Estimation of the chloride level of saliva. J. dent. Res. *35:* 753.

—»— 1957: The chloride level of saliva. II. J. dent. Res. *36:* 230.

Carter, W. J. et al., 1958: Chloride levels in parotid secretion. J. dent. Res. *37:* 902.

Ericsson, Y., 1958: The distribution and reactions of fluoride ions in enamel-saliva environment, investigated with the radioactive fluorine. Acta odont. scand. *16:* 127.

—»— 1961: Fluorides in dentifrices. Investigations using radioactive fluorine. Acta odont. scand. *19:* 41.

Ericsson, Y. & S. Ullberg, 1958: Autoradiographic investigations of the distribution of F^{18} in mice and rats. Acta odont. scand. *16:* 363.

Manly, R. S. & H. C. Hodge, 1939: Density and refractive index studies of dental hard tissues. J. dent. Res. *18:* 133.

Shannon, I. L., 1958: Salivary sodium, potassium, and chloride levels in subjects classified as to dental caries experience. J. dent. Res. *37:* 401.

Address: *The Royal Dental School*
Stockholm
Sweden