The effect of formalin fixation upon fluoride uptake in enamel and dentin of extracted human teeth

KJELL BJORVATN & ANNE BJØRG TVEIT

Department of Dental Research, University of Bergen, School of Dentistry, Bergen, Norway

Bjorvatn, K. & Tveit, A. B. The effect of formalin fixation upon fluoride uptake in enamel and dentin of extracted human teeth. *Acta Odontol. Scand.* 1981, 39, 125 – 131

The fluoride uptake in enamel and dentin surfaces, as evidenced by the reduction in [F^-] in fluoride containing solutions, was studied in formalin fixed and unfixed specimens. Compared to untreated specimens, pretreatment with formalin solutions increased the fluoride uptake in enamel and reduced the uptake in dentin. Only minor differences were found between specimens treated with unbuffered acid formalin solution and with buffered neutral formalin solution. Pretreatment with sodium lactate buffers increased F uptake in both enamel and dentin. Lowering of the pH in the lactate buffer resulted in a higher fluoride uptake in both tissues. A specific formalin effect seems to be responsible for the paradoxical reduction in fluoride uptake in formalin-treated dentin.

Key-words: F^- ion in selective electrode; chemistry

Kjell Bjorvatn, Department of Dental Research, School of Dentistry, Årstadveien 17, N-5000 Bergen, Norway

Extracted teeth are routinely used for research purposes. To avoid putrefaction of pulps and desiccation of hard tissues, the teeth are frequently stored in formalin solutions prior to use. It has been indicated, however, that a formalin solution may cause a deterioriation in tooth specimens, thereby changing experimental conditions (3, 4). To avoid, or minimize, this problem the use of a buffered neutral formalin solution has been recommended (3).

Information concerning the possible adverse effects of formalin on the chemistry of enamel and dentin is very scarce. A series of studies was therefore carried out in order to elucidate the problem. The present paper examines the effect of formalin storage upon the subsequent fluoride uptake in dental hard tissues.

MATERIAL AND METHODS

The fluoride uptake in enamel and dentin previously stored in buffered (neutral) or unbuffered (acid) formalin solution is compared with that of previously untreated specimens. To study the effect of pH changes per se, a parallel analysis of F^- uptake in enamel and dentin pretreated with neutral and acid sodium lactate buffers is included.

A formalin solution was prepared according to the following formula:

Received for publication, April 28, 1980

40 % formaldehyde	500 ml
$NaH_2PO_4 \cdot 2H_2O$	20 g
$Na_2HPO_4 \cdot 2H_2O$	40.75 g
Distilled water to	5000 ml.

Before final addition of water, pH was adjusted to 7.15 with NaOH.

When the formalin solution was prepared without the phosphate buffer system and with no adjustment of pH, the pH was found to be 3.40.

Lactate buffers were prepared by diluting stock solution of 50 % sodium lactate (Merck) and adjusting pH to 7.00 or 5.00 by the use of NaOH or HCl.

Thirty-five young premolars, extracted for orthodontic reasons, were used in this study. Before use the teeth were kept moist, at 10°C, in capped polystyrene bottles. Small crystals of thymol were added to prevent bacterial growth. After a storage period of one week, the teeth were rinsed in distilled water. Each tooth was then sectioned in four parts, two «enamel» and two «dentin» specimens (Fig. 1). Plane dentin surfaces were prepared by grinding the outer surface of the root specimens with carbide paper no. 400. The enamel surfaces were left untouched.

The enamel and dentin samples were made up of five specimens, one from each of five teeth. The total material thus consisted of 14 enamel and 14 dentin samples.

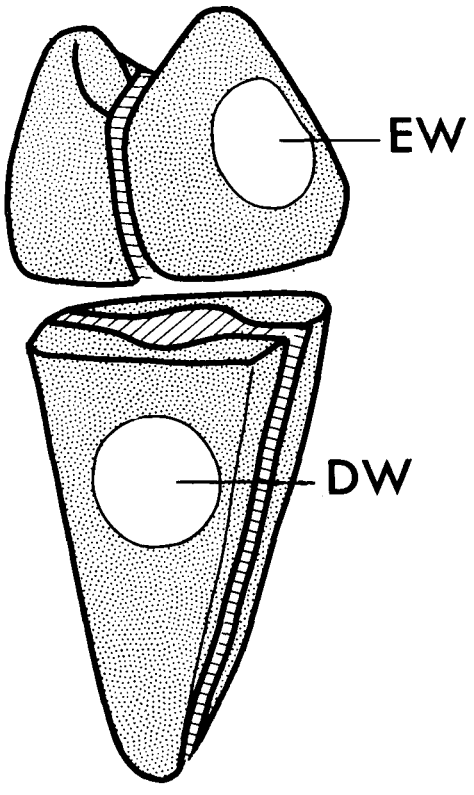

Fig. 1. Schematic drawing of extracted premolar, split in four test specimens. Surface of the specimens, apart from circular enamel (EW) or dentin (DW) windows (Ø 5 mm), covered by nail varnish & sticky wax.

Treatment of samples

Before exposing the samples to fluoride solutions, the specimens were pretreated as shown in Table 1.

After 10 days storage at room temperature, the pH of the solutions was measured. The specimens were then removed from the bottles, rinsed in distilled water and dried with an absorbant tissue. A circular piece of adhesive tape, Ø 5 mm, was then attached to the surface of each specimen. The remaining surface area of the specimens was covered by nail varnish and sticky wax. The tape was then removed, exposing circular enamel or dentin windows of approximately 20 mm^2 (Fig. 1).

The specimens of each sample were immersed in a 2 ml aliquot of fluoride solution. One enamel and one dentin sample pretreated with buffered formalin, as well as one control sample from each tissue, were placed in solutions containing 100 ppm F (Orion fluoride standard 94.09.07), pH 4.25. The remaining 24 samples were all exposed to solutions containing 905 ppm F (0.2 % NaF), pH 6.30.

Table 1. *Distribution of the experimental material*

Pretreatment	Number of samples Enamel	Dentin
10 % buffered formalin, pH 7.15	3	3
10 % unbuffered formalin, pH 3.40	2	2
5 % Na lactate, pH 7.00	2	2
5 % Na lactate, pH 5.00	2	2
No pretreatment (controls)	5	5

Finally, five additional tooth specimens, totally covered by nail varnish and sticky wax, were exposed to 2 ml of 905 ppm F solution.

Fluoride analysis

The fluoride concentration of the test solutions was analyzed at regular intervals using an F^- ion selective electrode (Combination fluoride, 96.09, Orion), connected to an Orion Model 901 Microprocessor Ionalyzer. The Ionalyzer was calibrated to give F^- concentrations in parts per million (mg F/l). No ionic strength adjustment buffer was added for analyses taken during the ongoing experiment. One week after the termination of the regular experiment, however, the [F^-] was remeasured: first without buffer adjustment, then 1.0 ml of the fluoride solution was extracted, 0.1 ml TISAB III (Orion) was added, the Ionalyzer was recalibrated, and a final analysis was performed.

RESULTS

As shown in Fig. 2, a rather slow, somewhat erratic, reduction in fluoride concentration was observed in all test solutions during the first hours after the immersion of the tooth specimens. The reduction in [F^-] continued during the five-day period as shown in Figs. 3, 4 and 5. The curves presented in Figs. 3 and 5 represent the average value of fluoride reduction in the relevant samples. The individual curves for identical samples showed very little variation.

In solutions containing enamel specimens pretreated with formalin,

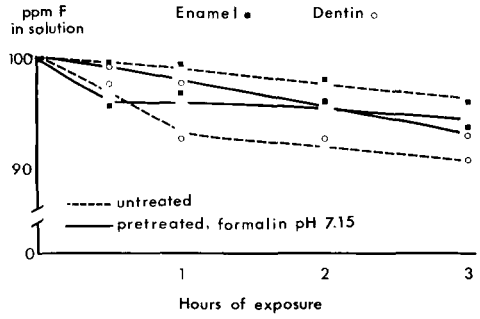

Fig. 2. Short term reduction in [F^-] in solutions subsequent to the immersion of enamel or dentin specimens. Initial fluoride concentration 100 ppm. Total area of exposed enamel or dentin 100 mm². No mechanical stirring.

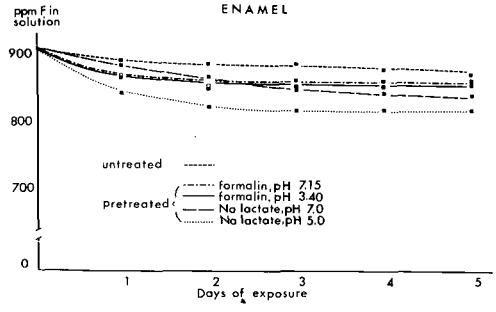

Fig. 3. Fluoride concentration in solutions containing 5 enamel specimens (100 mm² exposed surface area). Daily analyses. Initial [F^-] 905 ppm. No stirring.

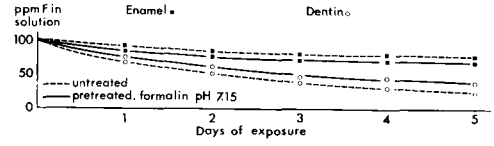

Fig. 4. Fluoride concentration in solutions containing enamel or dentin specimens (100 mm² exposed area). Daily analyses. Original [F^-] 100 ppm. No stirring.

the reduction in fluoride concentration was more pronounced than in the solutions containing untreated specimens. The effect of formalin fixation was most clearly seen during the earlier phases of the experiment: more than 100 % increase in the fluoride loss in solutions containing formalin treated enamel specimens at one day observation versus approximately 25 % at five days, in the 905 ppm series (Fig. 6). The same tendency was found when a 100 ppm F solution was used, i.e. approximately 90 % and 35 % more fluoride was lost in solutions containing formalin treated specimens than in the control samples after one, respectively five days' exposure to the fluoride solution.

Only minor differences in fluoride loss were observed between solutions containing enamel specimens formerly treated with acid or neutral formalin solutions. In all cases, however, the fluoride loss was slightly higher when the unbuffered formalin, pH 3.40, had been used.

The pretreatment of enamel specimens with sodium lactate in all cases increased the subsequent fluoride loss in the solution. Contrary to what was found in the formalin series, the lowering of pH in the lactate buffers used for pretreatment greatly influenced the subsequent fluoride loss in solutions. The relative influence was greater at one day observation than at later time intervals (Fig. 6).

The immersion of dentin specimens caused a more pronounced reduction, of longer duration, in the [F^-] in the solutions than did the immersion of enamel specimens (Fig. 5). The pretreatment of dentin with sodium lactate buffers resulted in an increase in fluoride loss in solutions. The increase was more pronounced when the pH was changed from 7.00 to 5.00. Paradoxically, when dentin specimens were

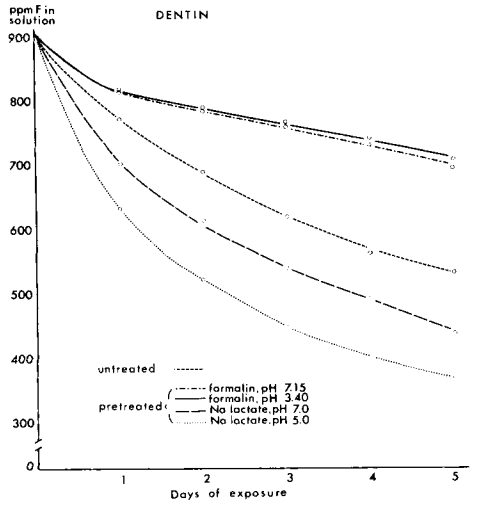

Fig. 5. Fluoride concentration in solutions containing dentin specimens (Exposed area 100 mm^2). Daily analyses. Original [F^-] 905 ppm. No stirring.

formalin fixed prior to being exposed to 905 ppm F, the fluoride loss in the solutions was greatly reduced, and the influence of pH changes was small (Figs. 5, 6). The same tendency of reduced F^- loss was observed in 100 ppm F solutions containing dentin specimens pretreated with formalin (Fig. 4).

No changes in fluoride concentration occurred in the fluoride solution containing dental specimens totally covered by varnish and wax.

The effect of TISAB on fluoride analyses

The fluoride analyses were, under the given conditions, only to a minor degree influenced by the presence or absence of total ionic strength adjustment buffers (Table 2).

DISCUSSION

Nail varnish, sticky wax and polystyrene bottles seemed to cause no changes in the fluoride concentration of sol-

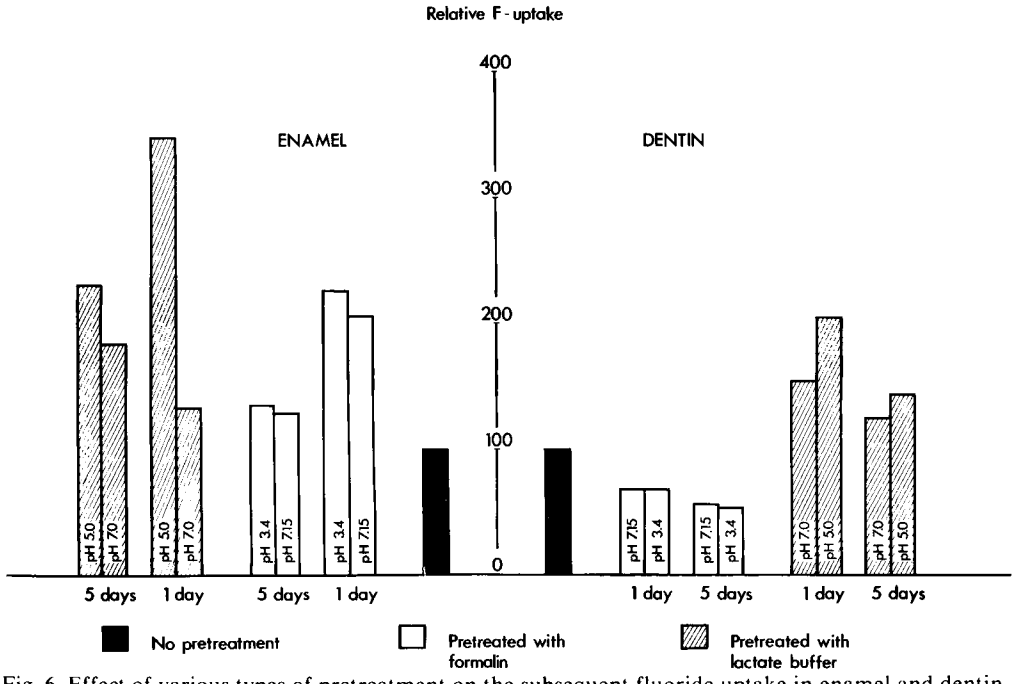

Fig. 6. Effect of various types of pretreatment on the subsequent fluoride uptake in enamel and dentin. Mean uptake in untreated (control) samples = 100.

Table 2. The effect of total ionic strength adjustment buffers (TISAB) on fluoride analysis. Mean [F⁻] in solutions previously exposed to untreated or pretreated enamel and dentin

	Treatment	No. of samples	ppm F No TISAB	ppm F TISAB III	Δ
Enamel	No formalin exposure	4	861	864	+ 3
	10 % buffered form., pH 7.15	2	871	878	+ 7
	10 % formalin, pH 3.40	2	872	870	− 2
	5 % Na-lactate, pH 7.0	2	785	782	− 3
	5 % Na-lactate, pH 5.0	2	766	782	+ 16
Dentin	No formalin exposure	4	419	435	+ 16
	10 % buffered form., pH 7.15	2	612	629	+ 17
	10 % formalin, pH 3.4	2	630	637	+ 7
	5 % Na-lactate, pH 7.0	2	239	245	+ 6
	5 % Na-lactate, pH 5.0	2	184	188	+ 4

utions. The reduction in [F⁻] observed in all fluoride solutions containing tooth specimens must, consequently, be caused by F uptake in, or adsorption to, enamel or dentin. Some fluoride may also be present in the solutions in the form of complexed ions. As only F⁻ is registered by the fluoride selective electrode, all complexation of fluoride ions will be recored as a reduction in

concentration. In the present study the addition of TISAB increased the observed [F^-] by approximately 1 %, which leads to the conclusion that the degree of complexation is slight under the described conditions.

As relatively high concentrations of fluoride in contact with hydroxyapatite favor the formation of calcium fluoride (7), it may be assumed that CaF_2 is the main reaction product resulting from the exposure of enamel and dentin specimens to 905 ppm F. A relatively greater amount of fluorapatite might be expected in the 100 ppm F solution. The formation of $Ca_5(PO_4)_3F$ is known to be speeded up by a lowering of the pH(6), but is nevertheless a much slower process than the formation of CaF_2 This may, in part, explain the slower decline in [F^-] (ppm F/day) observed in the 100 ppm F solution as compared to the 905 ppm F solution.

The fluoride uptake was higher in dentin than in enamel. This difference may be explained on the basis of the dentin's higher content of water, its greater porosity, smaller crystalites and possibly also its higher content of organic matter.

By exposing enamel surfaces to formalin the rate of fluoride uptake was enhanced. This may be due to alterations of the surface cuticle of the enamel, or it may be related to the observation that formalin, even at neutral pH, causes a slight demineralization of hydroxyapatite(2). Formalin may, consequently, leave the surface slightly demineralized and more reactive.

The observed reduction in fluoride uptake in formalin treated dentin is in accordance with the reduced rate of Ca release observed in similarly treated dentin specimens (2). It is likely that a slight demineralization takes place also in dentin during formalin fixation. The increase in fluoride uptake to be expected in a demineralized tissue, however, seems to be effectively counteracted by some specific formalin action, probably involving the organic stroma of the dentin. The reduced reactivity in Ca release as well as in F uptake may be connected to the slight swelling normally observed in formalin fixed protein material (1), which may change the permeability of the dentin.

The initial pH of the formalin solution seemed to have a minor effect on the fluoride uptake in enamel and dentin. This may partly be due to the fact that the pH of the acid, unbuffered formalin solution, during ten days exposure to enamel and dentin changed from 3.40 to 5.30 and 6.20, respectively, while the buffered formalin retained its original pH. Lowering of the pH from 7.00 to 5.00 in the lactate buffer, however, resulted in sharp increase in subsequent fluoride uptake in both tissues.

Lactate, like formalin, may form complex ions with calcium, CaL^+, thereby increasing the solubility of enamel and dentin (5). A slight surface etching, therefore, is the probable cause for the increased fluoride uptake observed after pretreatment, even with a neutral lactate solution.

The reported findings strongly indicate that the effect of formalin treatment should not be disregarded in laboratory experiments, and stress the importance of using experimental teeth with a known history. The buffering or lack of buffering of formalin solutions seems to be of minor importance as far as fluoride uptake is concerned.

REFERENCES

1. Baker, J.R. In: Cytological technique. The principles underlying routine methods. Methuen & Co LTD., London 1966, p. 28
2. Bjorvatn, K. The effect of formalin fixation on extracted human teeth. Ca^{2+} release from fixed and non-fixed enamel and dentin. *Unpublished results*
3. Crabb, H.S.M. The pattern of mineralization in human dental enamel. Thesis. University of Bristol, 1958, p. 25
4. Frisbie, H.E. & Nuckolls, J. Caries of the enamel. J. Dent. Res. 1947, 26, 181 – 202
5. Hauge, S. & Bjorvatn, K. Hydroxyapatite – acid dissolution and complex formation in sodium lactate/lactic acid buffers. In preparation
6. Larsen, M.J. *In vitro* studies of fluoride uptake in human enamel. Scand. J. Dent. Res. 1974, 82, 448 – 454
7. McCann, H.G. & Bullock, F.A. Reaction of fluoride ion with powdered enamel and dentin. J. Dent. Res. 1955, 34, 59 – 67