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Effect of Surface Zone Deproteinisation on the Access of Mineral Ions into Subsurface Carious Lesions of Human Enamel

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Abstract. It has been proposed that the so-called intact surface zone of carious lesions of enamel could restrict the ingress of mineral ions and hinder remineralisation. The present study was intended to determine the role of organic (proteinaceous) material in restricting the movement of mineral ions into carious lesions in vitro. Natural carious lesion surfaces were divided into two halves. The experimental half was de-proteinised using hypochlorite, the control half remained untreated. The whole tooth was exposed to ^{45}Ca in solution, and ^{45}Ca uptake into experimental and control tissue was measured by image analysis of autoradiographs prepared from lesion sections. The results indicated that uptake was improved by removal of organic material.

Remineralisation has been defined as the repair of acid-damaged enamel by mineral ions of salivary origin [Silverstone, 1977]. Remineralisation can arrest recently active carious lesions and reduce white spot caries [Anderson, 1938; Muhler, 1961; Backer Dirks, 1966; Silverstone and Poole, 1968].

In vivo, subsurface lesions often fail to remineralise and go on to require restoration despite excess calcium and phosphate in saliva. The reasons for this are unclear. Continual acid challenge is undoubtedly important and mineralisation inhibitors from the saliva may be present [Hay et al., 1987]. However, where the surface zone has been fractured away [Artun and Thylstrup, 1986; Holmen et al., 1987], enhanced redeposition of mineral is noted, suggesting that lesion surfaces could restrict the ingress of remineralising ions [Silverstone, 1977, 1983].

Since surface zone porosity is extensive and the pores are of large size relative to mineral ions [Haikel et al., 1983], it is difficult to envisage how inward diffusion of calcium and phosphate ions might be impeded. The surface zone pores might, however, be partially occluded by protein components from the acquired pellicle or plaque matrix [Frank and Brendel, 1966]. Such deposits might not only reduce the access of ions to the interior of the lesion but also restrict remineralisation to the outermost lesion surface, further impeding ionic penetration. Removal of surface zone protein might, therefore, enhance the possibility of in vivo lesion repair.

In the present study, these possibilities have been investigated by treating natural lesions with hypochlorite ion, ClO^-, a non-specific proteolytic agent, in vitro. The effect of such treatment on mineral ion penetration into lesions was monitored by incubating intact lesions in ^{45}Ca-labelled saline and then comparing, by novel autoradiographic/image analysis techniques, ^{45}Ca distribution in ground sections prepared from control and test portions of the lesion.

Materials and Methods

Carious Lesions

Thirty human permanent, premolar and molar teeth with white-spot or brown-spot carious lesions in the approximal enamel were collected from patients aged 7–19 years, resident in the Leeds, W. Yorkshire area (fluoride content of the drinking water <0.1 ppm).

Twenty-eight of the teeth were stored dry in air at about 20 °C for several months prior to use. Two carious teeth were fully hydrated in thymol-saturated 0.9% w/v NaCl solution at about 20 °C and processed within 5 days of extraction. All teeth were cleaned prior to lesion window preparation using pumice powder and water on a slowly rotating bristle brush.

Lesion Windows

Dry-Stored Teeth. Each enamel lesion was divided into control and experimental halves of approximately equal area by a 200-μm wide slot, cut through the lesion's longitudinal axis with a peripheral diamond disc. The slot was sufficiently deep to isolate each half of the lesion, its base lying in either sound interior enamel, or, in the case of deeply penetrating lesions, in sound dentine. The slot was filled completely with red nail varnish. The remainder of the tooth surface was then varnished twice, such that the two halves of the lesion formed windows of equal area, each half-lesion being flanked, cervically and occlusally, by narrow strips of sound-surface enamel (fig. 1).

Saline-Stored Teeth. These were kept fully hydrated throughout the entire experimental period until after ^{45}Ca incubation and water-washing. The tooth surfaces around these lesion windows were protected by soft dental wax rather than by nail varnish since (a) the latter would not adhere firmly to damp surfaces, and (b) after application, nail varnish must be allowed to dry in air for several hours – a requirement incompatible with maintaining lesions in a fully hydrated state. Each saline-imbibed tooth was cut longitudinally through the lesion centre with a water-cooled peripheral diamond disc. The control and experimental tooth halves were blotted with paper tissue and coated (apart from the lesion windows) with molten soft red dental wax at 110–115 °C. Care was taken to seal the sectioned faces of the lesion, cut dentinal surfaces and the occlusal enamel. Excess wax on the lesion window was gently removed with a scalpel blade or chloroform-moistened paper points.

All lesion windows were thoroughly re-imbibed in 0.9% w/v NaCl (thymol-free) for 72 h prior to treatment with sodium hypochlorite solution or 30% phosphoric acid.

Deproteinisation with Hypochlorite

Twenty-seven teeth were used. The control lesion windows were first sealed against access of the deproteinisation reagent by small pieces of soft dental wax pressed onto the lesion surfaces and overlapping their varnished borders. These teeth and the experimental halves of the saline-stored teeth were then immersed in sodium hypochlorite solution (BDH Chemicals Ltd.; 10–14% w/v available Cl) at 20 °C for 4 h with periodic removal of gas bubbles from lesion surfaces.

The teeth were removed, rinsed briefly with tap water, and wax was removed from the lesion control windows. The teeth were then washed in running tap water for 30 min and re-equilibrated in 0.9% w/v saline (thymol-free) for 72 h prior to ^{45}Ca incubation.

Acid-Etching of Lesion Surfaces

Three teeth were used. The lesion control windows in 3 varnished, saline-imbibed teeth were sealed with wax as described above. The experimental windows were blotted dry with paper tissue and etched for 60 s with 30% w/w orthophosphoric acid, followed by rinsing in running tap water. Wax was then removed from the control windows. The lesion windows were re-imbibed in 0.9% saline for 72 h before ^{45}Ca incubation.

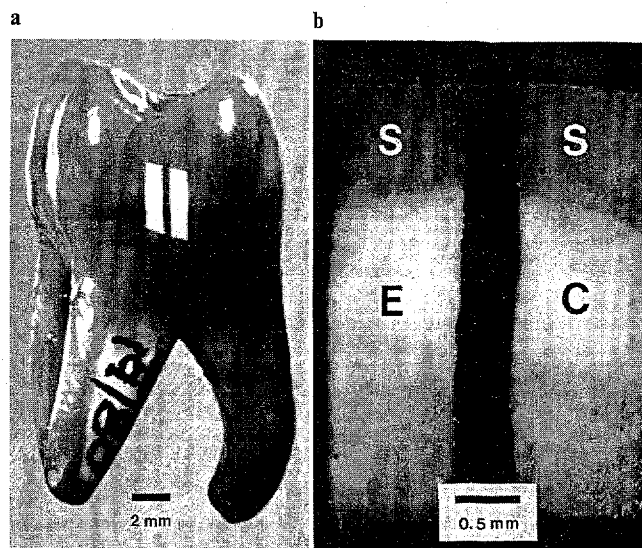


Fig. 1. a Whole tooth, covered with nail varnish, leaving exposed panels of enamel including experimental and control surfaces of an interproximal lesion. **b** Enlargement showing experimental (E) and control (C) lesion surfaces and adjacent sound enamel (S).

^{45}Ca Incubation of Lesions

The control and experimental lesion windows were incubated at 20 °C in 10- to 12-ml portions of 0.9% w/v NaCl solution containing 7.4 MBq of carrier-free $^{45}CaCl_2$ (about 10–12 μg/ml Ca^{2+}) for 16 h with gentle agitation. The teeth were then washed in running tap water (Ca^{2+} concentration about 17 μg/ml) for 72 h when ^{45}Ca activity was no longer detected in the washings. The washed teeth were allowed to dry in air for 24 h prior to sectioning.

Preparation of Lesion Window Ground Sections

One to three longitudinal slices, 200–300 μm thick, were cut through each lesion window with a *dry* peripheral diamond disc using a vacuum attachment to collect ^{45}Ca-contaminated tooth dust. Cuspal enamel containing the lesions was separated from the tooth slice, 1 mm of dentine being retained to provide mechanical support [Hallsworth et al., 1972]. These part sections were fixed to ground-glass microscope slides with clear nail varnish and polished to a thickness of 100 ± 10 μm using silicon carbide papers without lubricant. They were then removed from the slides and rinsed with amyl acetate to remove nail varnish and dried in air.

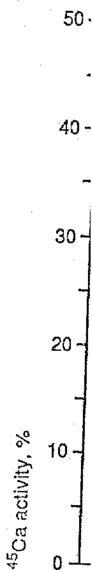
Autoradiography

The lesion ground sections were placed on a Kodak High-Resolution Plate, emulsion type 1A for 36–48 h, the experimental and control sections being exposed for identical periods. The autoradiographs were developed with Kodak D-178 (2 vol:1 vol water) for 6 min at 20 °C and fixed with standard Kodafix.

Microradiography

The lesion sections and an aluminium step-wedge (12 steps of 10-μm thick Al foil) were contact-microradiographed (80 min) on a Kodak High-Resolution Plate, type 1A by Ni-filtered Cu-K radiation excited at 20 kV, 4 mA. The microradiographs were processed as described above. All lesion sections showed microradiographic

Fig. 2. **a** Microradiograph of 100-μm section for interproximal carious lesions. **b** Digitised image of above microradiograph used to delineate boundary of carious enamel. **c** ^{45}Ca autoradiograph of carious lesions surface of enamel denoted S. **d** Digitised image of above autoradiograph used to calculate extent and degree of ^{45}Ca labelling.



surface zones (fig. 2a). Digitisation (fig. 2b) was employed to determine lesion area.

Quantitation of ^{45}Ca Uptake

The ^{45}Ca content of each lesion was determined by processing the autoradiographs (fig. 2c) using a Magiscan image analyser. The lesion boundaries were defined from an outline of the microradiograph traced on a transparent overlay affixed to the Magiscan VDU and matched with the topography of the ^{45}Ca isodensitrace image. A grey band contour map of ^{45}Ca distribution was produced for each lesion (fig. 2d). ^{45}Ca concentrations were assumed to be directly proportional to the optical density of the autoradiograph. The grey level of the ^{45}Ca-free enamel (clear background) was taken as 0% ^{45}Ca, and absolute black as 100% ^{45}Ca saturation. The total area, in square micrometres, of each ^{45}Ca 'isoconcentration' band *within the lesion* was then computed. ^{45}Ca content was measured as the product of band area and its 'numeric grey band' ^{45}Ca concentration. Summation of ^{45}Ca content over all bands gave the total ^{45}Ca content of the lesion. Total radioactivity was expressed as optical density units per area of lesion.

Results

Deproteinisation and ^{45}Ca Uptake

In general, the effects of hypochlorite treatment were: (1) to increase the depth of ^{45}Ca penetration into the interior of the lesion, (2) to increase ^{45}Ca uptake by the lesion body, which is illustrated in a typical line scan picture (fig. 3), (3) in most lesions, enhanced ^{45}Ca acquisition by the surface zone appeared to occur.

Table 1 shows the overall ^{45}Ca uptake for the control and experimental halves. The lesions are arranged in order of decreasing difference from the controls. Twenty-five lesions (90%) showed significant overall increases in total ^{45}Ca uptake after deproteinisation compared with adjacent control tissue. Two showed a decrease. The observed increases in ^{45}Ca up-

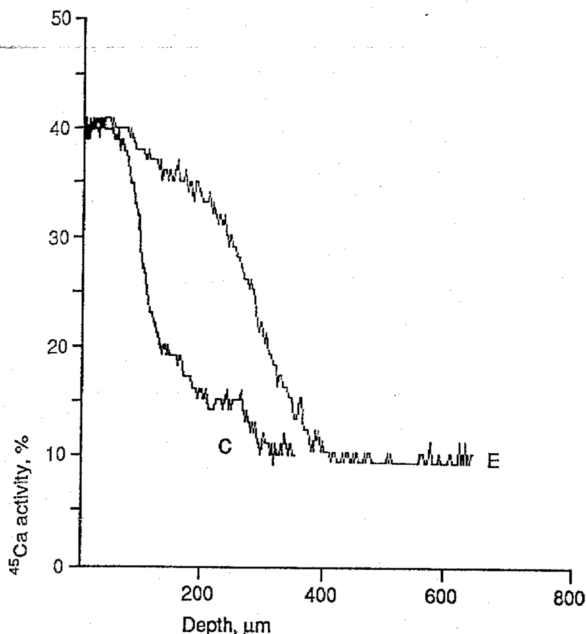


Fig. 3. ^{45}Ca activity line scans from surface to interior of typical experimental (E) and control (C) halves of an inter-proximal carious lesion.

take varied widely, however, ranging between 1.8 and 284.8%. The mean increase for 25 lesions was 51.2%.

The two lesions showing the highest increases in ^{45}Ca uptake, lesion 1 (284.8%) and lesion 2 (194.6%) had both been kept continuously hydrated and saline-imbibed prior to deproteinisation.

Microfractures present in the *undeproteinised* lesion surfaces did not seem to increase ^{45}Ca uptake by the interior of the lesion.

Surface-Zone Etching and ^{45}Ca Uptake Distribution

The uptake of ^{45}Ca by the acid-etched lesions (table 1, No. 28–30) was similar to that of the surface-deproteinised lesions and significantly higher than in the unetched controls.

Table 1. Effect of surface zone deproteinisation/acid etching on ^{45}Ca uptake by subsurface carious lesions of human enamel

Lesion No.	Autoradiograph OD units (digitised), $\times 10^{-2}$ OD units/unit area		Difference %
	control lesion	experimental lesion	
1	2,587	9,954	+284.7 (hydrated lesion)
2	22,945	67,600	+194.6 (hydrated lesion)
3	10,369	24,293	+134.3
4	11,497	24,189	+110.4
5	29,726	52,257	+ 75.8
6	29,002	48,224	+ 66.3
7	38,361	59,468	+ 55.0
8	15,188	23,267	+ 53.2
9	1,314	2,004	+ 52.5
10	17,246	24,532	+ 42.2
11	30,552	43,432	+ 42.2
12	27,042	37,400	+ 38.4
13	36,467	44,892	+ 23.1
14	23,742	27,304	+ 15.0
15	27,156	31,180	+ 14.8
16	49,450	56,462	+ 14.2
17	15,586	17,474	+ 12.1
18	29,628	32,735	+ 10.5
19	50,614	55,557	+ 9.8
20	38,998	42,581	+ 9.2
21	41,595	43,818	+ 5.3
22	15,161	15,945	+ 5.2
23	31,208	32,721	+ 4.8
24	38,515	40,016	+ 3.9
25	44,340	45,142	+ 1.8
26	60,599	45,609	− 24.7
27	48,097	37,228	− 22.6
28	19,119	53,263	+178.6 (acid-etched)
29	35,343	65,448	+ 85.2 (acid-etched)
30	63,138	69,307	+ 9.8 (acid-etched)

Discussion

Subsurface carious lesions of human enamel treated in vitro with an efficient deproteinising agent, hypochlorite ion, have been shown to acquire significantly increased amounts of $^{45}Ca^{2+}$ ion compared with controls. The data presented indicate that the access to the interiors of carious lesions is determined to a considerable extent by organic material. A brief, 1-min etch of lesion surfaces by 30% phosphoric acid seemed, in spite of very limited data available, to have a similar effect. The results support the view that material present in the surface zone of the enamel lesion can act as a diffusion barrier, impeding the access of remineralising ions to the lesion interior [Silverstone, 1977, 1983].

It is not clear from the data what kind of material this might be. Since hypochlorite effectively destroys protein, *it is likely* that it may be proteinaceous. In the surface zone this may be derived from pellicle or plaque. It could also represent enamel proteins, particularly in the interior of the lesion [Robinson et al., 1989].

Judging from the present information it may thus be possible to improve access to enamel lesions for calcium and phosphorus by deproteinising the tooth surface. This would have the advantage over similarly effective acid treatments in that no tooth mineral would be removed.

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