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THE EFFECT OF UNDISSOCIATED ACETIC-ACID CONCENTRATION OF BUFFER SOLUTIONS ON ARTIFICIAL CARIES-LIKE LESION FORMATION IN HUMAN TOOTH ENAMEL

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Summary—A chemical system for lesion production was used. The influence on lesion characteristics of the concentration of undissociated acetic acid in a calcium and phosphate-containing buffer solution was investigated. Artificial lesions obtained after demineralization in buffers with a pH of 4.0, 4.5, 5.0 or 6.0 at 5 or 6 different acid concentrations for different demineralization times were investigated microradiographically. The lesion characteristics studied were: (a) the mineral content of the surface layer; (b) the mineral content of the body of the lesion; (c) and (d) the depth at which these mineral levels were reached; (e) the depth of the lesion. The concentration of undissociated acetic acid had little effect on the lesion characteristics at low pH. When lower concentration buffers at pH 6.0 were used, the effect was more pronounced. At this level, the buffer capacity of the acetic acid/acetate buffer is small.

INTRODUCTION

Several investigators, such as Enright, Friesell and Trescher (1932), Besic (1953) and Coolidge, Besic and Jacobs (1955) used buffer solutions to which calcium and phosphate were added to produce caries-like lesions in human enamel. van Dijk, Borggreven and Driessens (1979) formulated the theoretical minimum set of conditions needed to form subsurface lesions. They confirmed this by an *in vitro* artificial caries experiment. The effect of factors such as the degree of saturation and the pH of buffer solutions was investigated by Theuns *et al.* (1983, 1984).

We have studied the effect of acid concentration on the demineralization process. As undissociated acid is the potential source of hydrogen ions inside the enamel, this seems to be more relevant in studying the factors influencing artificial demineralization than the total acid concentration. Our aim was to determine the influence of the undissociated acid concentration during lesion progression on some microradiographic characteristics of the lesions.

MATERIALS AND METHODS

The preparation of the teeth before exposure to the buffer solutions was similar to that described by Groeneveld, Theuns and Kalter (1978). The buffer solutions were made by adding appropriate amounts of monetite ($CaHPO_4$) to an acetic-acid solution of the desired concentration. Potassium hydroxide or hydrochloric acid was added to adjust the solution to the desired pH.

The negative logarithm of the ion-activity product (pI) of the solutions for hydroxyapatite can be used as an expression of the degree of saturation.

$$pI_{(OHA)} = -\log[(Ca)^{10}(PO_4)^6(OH)^2]$$
$$= 10.pCa + 6.pPO_4 + 2.pOH$$

The solution is undersaturated if the pI_{OHA} is higher than the negative logarithm of the solubility product for that mineral: 117.2 (Driessens, 1982). For fluorapatite (FA), a similar formula holds true and the solution will be undersaturated if the pI_{FA} is higher than the negative logarithm of the solubility product for FA: 121.2 (Driessens, 1982). The complexes and activity corrections taken into account are the same as those given by Moreno and Zahradnik (1974).

The composition of the solutions is given in Table 1. Although analytical grade chemicals were used, all solutions contained trace amounts of fluoride which predominantly originated from the monetite. Of all ingredients of the buffer solutions, the fluoride concentration was measured using gas chromatography. Subsequently, the fluoride concentrations given in Table 1, column 4 were calculated. The amounts of fluoride were low although high enough to cause a supersaturation of the solution with respect to FA in most cases.

The experiment was done at four pH levels. Each solution was applied to two or three windows (see demineralization time in Table 1) at the buccal surface of six sound human premolars. All teeth (126 in all) were exposed to 5 ml of solution for time periods indicated in Table 1. A total of 306 lesions were made. After demineralization, the teeth were sectioned and the lesions investigated by microradiography. From the density of the microradiograms measured using a Leitz densitometer with a slit width of 100 μm, the volume percentage of mineral was calculated using the density of the aluminium stepwedge as a reference and the mineral composition as described by Angmar, Carlström and Glas (1963). In the schematic drawing of Fig. 1, the most interesting points of the tracing are indicated. The lesions were scanned, the depths and mineral content of all curves summed to give average-curves.

The lesion characteristics investigated were: the maximal mineral content of the surface layer (MSL),

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Table 1. Composition of the solutions and used demineralization times

pI_{OHA}	pH	Ca and PO_4 conc. (mmol l^{-1})	F^- conc. (μmol l^{-1})	pI_{FA}	Undissociated H-Ac conc. (mmol l^{-1})	$Ac^- + H\text{-}Ac$ conc. (mmol l^{-1})	Demineralization time (days)
126	4.0	8.2	2.0	117.4	21	25	4, 8
126	4.0	8.3	2.2	117.4	41	50	4, 8
126	4.0	8.5	2.3	117.3	50	61	4, 8, 16
126	4.0	8.7	2.5	117.2	62	75	4, 8
126	4.0	9.1	2.7	117.1	82	100	4, 8
126	4.5	2.8	0.8	119.2	15	25	4, 8, 16
126	4.5	3.1	1.0	119.0	30	50	4, 8, 16
126	4.5	3.4	1.3	118.8	45	75	4, 8, 16
126	4.5	3.5	1.4	118.7	50	85	4, 8, 16
126	4.5	3.6	1.5	118.6	59	100	4, 8, 16
126	5.0	1.1	0.4	120.8	8	25	8, 16
126	5.0	1.2	0.6	120.4	16	50	8, 16
126	5.0	1.4	0.8	120.1	23	75	8, 16
126	5.0	1.5	1.0	120.0	30	100	8, 16
126	5.0	1.9	1.6	119.6	50	171	8, 16, 32
126	6.0	0.15	0.2	123.4	1	25	8, 16
126	6.0	0.18	0.4	122.8	2	50	8, 16
126	6.0	0.21	0.6	122.5	3	75	8, 16
126	6.0	0.23	0.8	122.2	4	100	8, 16
126	6.0	0.63	5.7	120.4	25	800	8, 16, 32
126	6.0	0.96	12.1	119.8	50	1710	8, 16, 32

The degree of saturation with respect to hydroxylapatite (OHA) and fluorapatite (FA) indicated by the negative logarithm of the ion-activity product (pI_{OHA} and pI_{FA} respectively) (columns 1 and 5) is obtained by the pH (column 2) and the calcium, phosphate and fluoride concentrations given in columns 3 and 4. The fluoride concentration (column 4) was present in the solution as an impurity from the analytical grade chemicals used (1 part per million $F^- = 52.6\,\mu$mol l^{-1}). The pI_{FA} can be calculated from the pI_{OHA}, pH and the pF (see Driessens *et al.*, 1980b). The activity coefficients of the ions were calculated using the equation of Guntelberg (Robinson and Stokes, 1970).

the depth at which this maximum was reached (DSL), the minimal mineral content of the body of the lesion (MB), the depth at which this minimum was reached (DB), and the depth of the lesion (DL). The depth of the lesion was defined as the depth at which no further increase in mineral content was observed.

RESULTS

For enamel demineralized in a pH 4.5 solution, Fig. 2 shows the effect of undissociated acid on the maximal mineral content of the surface layer and on the minimal mineral content of the body of the lesion. The lines are drawn using the mineral content of sound enamel (assumed 88 vol per cent) at the start of the experiment as a reference level (Theuns, Arends and Groeneveld, 1980). The maximal mineral content of the surface layer gradually decreased to about 70 vol per cent. The mineral loss was on the average smaller in the teeth used in the 30 mmol l^{-1} undissociated-acid experiments (Fig. 2B). The vertical lines denote the standard deviation of the individ-

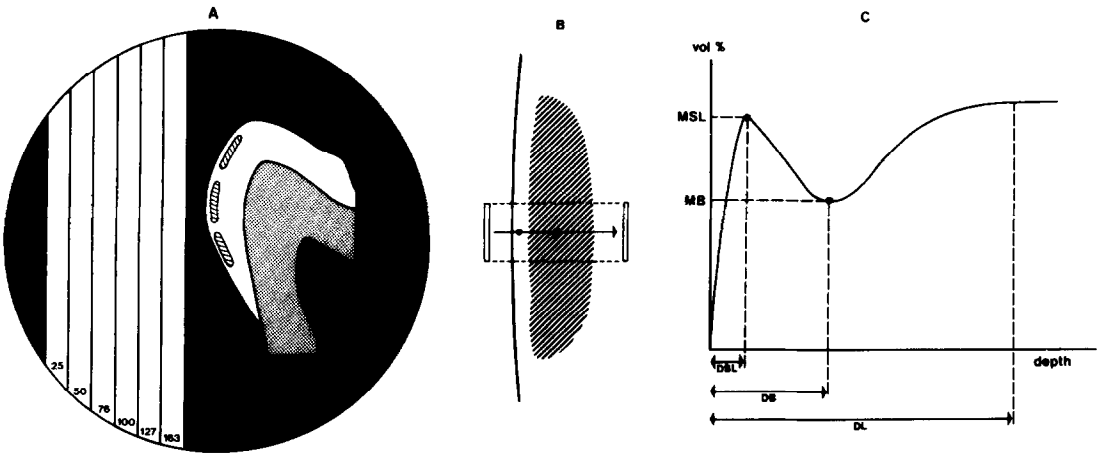

Fig. 1. Schematic drawing of the microradiographic procedure. (A) Schematic drawing of a microradiogram, with the aluminium stepwedge (left) and about 80 μm thick the tooth section (right). The numbers in the stepwedge denote the thickness of the aluminium. (B) Schematic drawing of a densitometric tracing through a lesion. The slit and its pathway are indicated. (C) After calculation, the mineral content is plotted against the depth in the enamel. Several typical points on this graph are indicated.

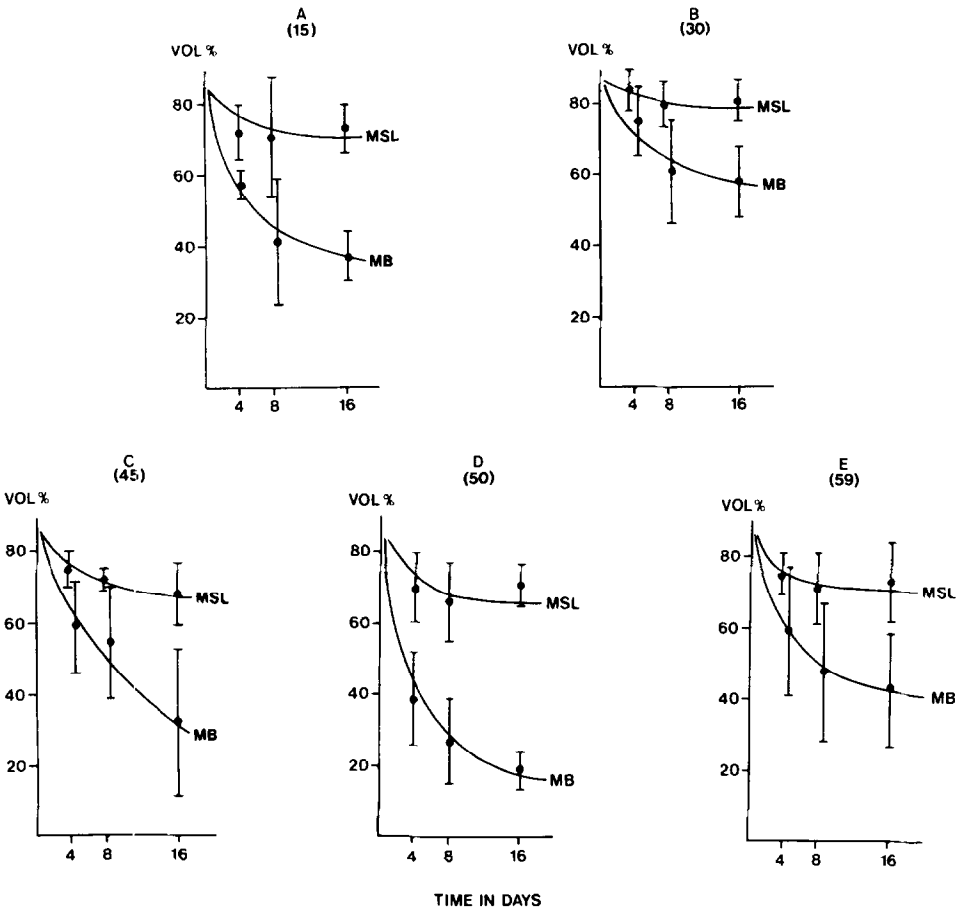

Fig. 2. (A–E) The effect of the concentration of undissociated acid on the mineral content of the surface layer (MSL) and of the body of the lesion (MB) during demineralization at pH 4.5. The mineral content in the lesion at these points is plotted against the demineralization time. A = 15, B = 30, C = 45, D = 50 and E = 59 mmol undissociated acetic acid l^{-1}. The bars represent the SD of the individual values (n = 6).

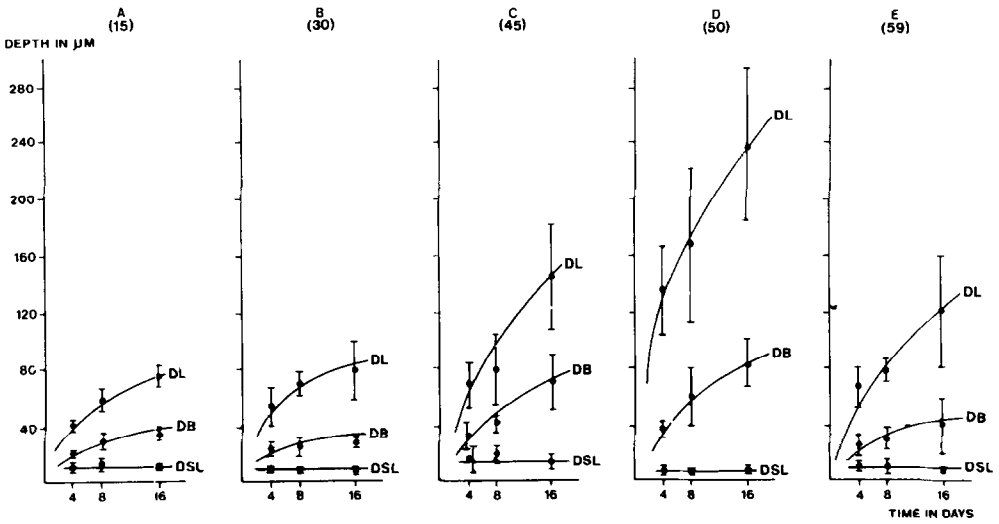

Fig. 3. The effect of the concentration of undissociated acid on the depth at which the maximal mineral content of the surface layer (DSL) was reached, the depth at which the minimal mineral content of the body of the lesion (DB) was reached, and the depth of lesions (DL) during demineralization at pH 4.5 The depth in the lesion is plotted against the demineralization time. A = 15, B = 30, C = 45, D = 50 and E = 59 mmol undissociated acetic acid l^{-1}. The bars represent the SD of the individual values (n = 6).

ual values ($n = 6$); they clearly showed that the range in the degree of demineralization was larger for the body of the lesion than for the surface layer.

The distance of several characteristic points in the lesion to the surface is shown in Fig. 3. In the lesions exposed to $pI_{OHA} = 126$ (pH 4.5) solutions, it appeared that after four days the depth at which the maximal mineral content of the surface layer was attained was maintained during the further 12-days demineralizing process. The average depth of this point appeared not to be different for the various concentrations of undissociated acid. The depth of the lowest mineral content of the body of the lesion increased with time, and there was some variation with increasing undissociated-acid concentration. The same was observed for the lesion depth.

In Figs 4–6 the mineral content in the lesion is plotted against the distance from the anatomical surface inwards. At pH 4.0 (Fig. 4) and pH 5.0 (Fig. 5), the results were slightly different from those described for pH 4.5 in Figs 2 and 3. The lesions, demineralized in the lowest undissociated-acid concentration (21 mmol l^{-1} for pH 4.0 and 8 mmol l^{-1} for pH 5.0), showed a slightly-smaller mineral loss than the lesions demineralized in buffers having a higher undissociated-acid concentration. From the pH 6.0 lesions (Fig. 6), a totally different picture was obtained. It appeared that, in the case of 25 and 50 mmol l^{-1} undissociated acetic acid (800 and 1710 mmol l^{-1} total acid respectively), normal subsurface lesions were obtained; but, in the low concentration range from 1 to 4 mmol l^{-1} undissociated acid (25–100 mmol l^{-1} total acid), only about half the lesions were of the subsurface type; the other half showed only superficial demineralization (studied in detail by Theuns *et al.*, 1982). The depth of the pH 6.0 lesions increased with increasing acetic-acid concentration.

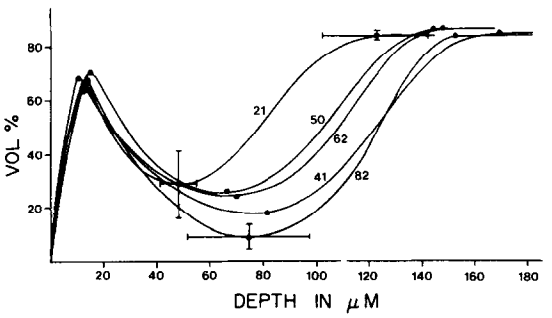

Fig. 4. The mineral content in the lesions plotted against the depth in the lesions after 8 days of demineralization in a pI_{OHA} 126, pH 4.0 buffer solution. The numbers on the graphs denote the concentration of undissociated acid. The bars represent the SD of the individual values ($n = 6$).

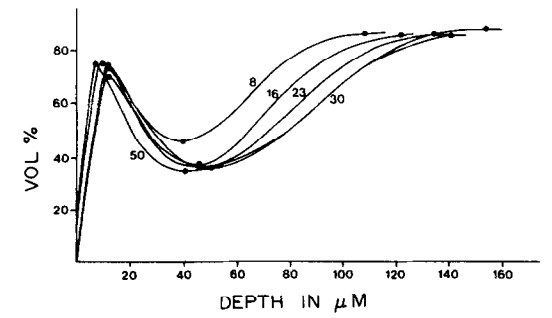

Fig. 5. The mineral content in the lesions plotted against the depth in the lesions after 8 days of demineralization in a pI_{OHA} 126, pH 5.0 buffer solution. The numbers on the graphs denote the concentration of undissociated acid. In this Figure no bars for the SD of the individual values ($n = 6$) are given because this would render the Figure unclear. The SD are, however, similar as those given in Figs 4 and 3.

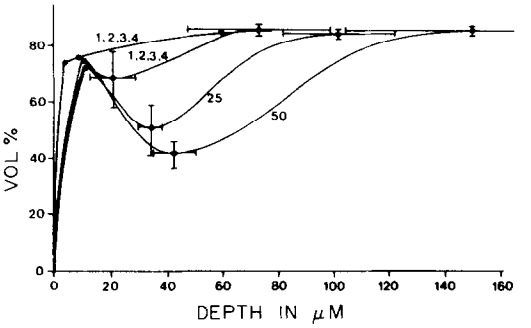

Fig. 6. The mineral content in the lesions plotted against the depth in the lesions after 8 days of demineralization in a pI_{OHA} 126, pH 6.0 buffer solution. The numbers on the graphs denote the concentration of undissociated acid. The bars represent the SD of the individual values ($n = 6$).

DISCUSSION

The acetic-acid concentrations used are in the range occurring in plaque as reported for the total acid concentrations (Geddes, 1975; Gilmour *et al.*, 1976), at least in the pH 4.0, 4.5 and 5.0 experiments. In the pH 6.0 experiments, only the lowest concentrations possibly apply to *in vivo* conditions.

Kapur, Fischer and Manly (1962) showed that the concentration of total lactate up to 1000 mmol l^{-1} had a pronounced effect on the lesion depth at low pH, and had far less influence at high pH. They used much higher concentrations of undissociated acid in the low pH range than we did; this may explain the difference between their results and ours. Furthermore, the buffer capacity of lactate at pH 5.0 and 6.0 is less than that of acetate, and this could be a further explanation of the difference.

Featherstone and Rodgers (1981) showed that acetic acid plays an important role in the carious attack. At a given pH and a given total acid concentration, acetic acid is less dissociated than lactic acid and produces deeper lesions in the same time period. This is in accord with mathematical simulation (van Dijk *et al.*, 1979). Gray and Francis (1963) reported that the undissociated form of a weak acid determines the rate of the demineralization process.

From our findings (Fig. 2), it can be concluded that the undissociated-acid concentration at pH 4.5 had no effect on the mineral content of the surface layer and a variable effect on the mineral content of the body of the lesion.

Figure 3 shows that a change in undissociated-acid concentration from 15 to 50 mM at pH 4.5 resulted in an increase in the depth of the lesion by a factor

3, whereas a further increase to 59 mM caused it to be halved. This unexpected drop in lesion depth with the highest H-Ac concentration cannot be explained. At pH 4.0 and 5.0 this result was not obtained, which perhaps indicates that this is an anomalous result. Perhaps the 12 teeth used in this part of the study differed to some extent from those of the rest of the experiment.

The demineralization process will be affected markedly only in the region in which the concentration of the buffer becomes too low to maintain the low pH in the enamel pores. This may be the case in the advancing front of the lesion, and to a smaller extent in the body of the lesion, but not in the surface layer (Driessens *et al.*, 1980a).

A higher concentration of the undissociated acid in the buffer solution would result in a greater influx. However, the outflux of reaction products was not affected. This might explain why the depth of the lesion and the depth at which the lowest mineral content of the body of the lesion is formed, is only slightly dependent on the buffer concentration (Figs 3–5). At pH 6.0 (Fig. 6) the undissociated-acid concentration becomes even more important. The mineral content in the body of the lesion, the depth at which this is reached, as well as the lesion depth, are affected. At low undissociated-acid concentrations (1, 2, 3 and 4 mmol l^{-}) half of the lesions did not show a subsurface type of demineralization; these demineralizations can be described as a priming (Koulourides *et al.*, 1980) of the enamel.

Probably in the case of our *in vitro* priming, the low undissociated-acid concentration was not able to maintain locally the pH at 6.0, by which the driving force to dissolve mineral decreased, and resulted in less demineralization.

The first action of the acid inside the enamel probably always results in this priming. The slow-demineralizing process at this high pH caused the appearance of this first outcome of the attack. In enamel which is less resistant to the acid (or with a steeper gradient in solubility), subsurface lesions were formed in the same time period. Thus the differences in mineral composition, solubility and porosity (van Dijk *et al.*, 1979; Wöltgens *et al.*, 1981) matched, in this case, the obvious histological differences in lesion formation.

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