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Crystal growth on enamel in relation to acid etching

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SUMMARY

Crystal formation may occur during etching of enamel surfaces with phosphoric acid. In this in vitro study we observed, from a morphological standpoint, some crystals left after etching, on the surfaces, using the scanning electron microscope. More often, after water-spraying, a thin generalized precipitate remains on the surface. This precipitate may be harmful for the retention of composite resins. On few specimens we obtained needle-shaped or petal-like crystals. Crystal deposits were spread evenly, on the surface, or nucleated from different points. We tempted to identify these crystal formations by X-ray diffraction and microprobe analysis. Chemical identification seems very important because calcium phosphates solubility varies. Crystal dissolution in saliva can lead to marginal leakage and impairs the quality of esthetic restorations. On the contrary insoluble crystals may ensure microscopic retentions and crystal growth is now considered as an alternative for enamel pretreatment in bracket bonding. Crystal formation, in these first experiments, is too scarse to be used for crystal bonding. But it appears that two factors may enhance the crystal number: a preliminary topical application of fluoride and adsorption of an acidic protein, on the surface, before etching. However, further investigations are still necessary.

KEY WORDS:

Crystals, Enamel, Surfaces, Etching, S.E.M.

RÉSUMÉ

On peut observer la formation de cristaux, à la surface de l'émail, après un mordançage à l'acide phosphorique. Dans cette étude, effectuée in vitro, nous avons observé, en M.E.B., d'un point de vue strictement morphologique un certain nombre de cristallisations déposées, à la surface de l'émail, après mordançage. Le plus souvent, après rinçage, persiste en surface un fin précipité; celui-ci est généralement considéré comme néfaste pour la bonne tenue des résines composites. Sur quelques échantillons nous avons obtenu des cristaux en forme d'aiguilles ou de larges pétales. Ces cristaux peuvent être disposés au hasard ou regroupés autour d'un centre de nucléation. Nous avons essayé d'identifier certains de ces cristaux en pratiquant une analyse en microsonde et une diffraction aux rayons X. Il paraît très important de connaître la nature chimique de ces cristaux car les différents sels phospho-calciques ont des taux de solubilité variables. A long terme, la dissolution de ces cristaux, dans la salive peut conduire à la formation d'un hiatus qui risque de compromettre la qualité esthétique des restaurations. Au contraire, de telles cristallisations, si elles s'avéraient insolubles pourraient constituer des microrétentions. La croissance d'une phase cristalline, à la surface de l'émail, étant déjà considérée comme une alternative valable au mordançage, en orthopédie dento-faciale pour le collage des verrous. D'après les résultats de cette étude préliminaire il semble que la formation de cristaux soit encore trop aléatoire pour favoriser la rétention des résines. Deux facteurs semblent favoriser cette formation de cristaux: l'application topique préalable, sur l'émail, d'un fluorure et l'adsorption d'une protéine acide (ici, l'albumine) avant mordançage. Il semble toutefois que ces observations doivent être confirmées.

MOTS CLÉS:

Cristaux, Email, Surfaces, Mordançage, M.E.B.

INTRODUCTION

It has been shown that both secondary and primary calcium phosphates may form during treatment of enamel with phosphoric acid (Chow and Brown, 1973 b; 1975). More often these salts form a fine deposit. For many authors this precipitate has a markedly deleterious effect on the bond-strength of self-curing resins and composites (Soetopo et al., 1978; Beech and Jalaly, 1980; Silverstone et al., 1985). During a conventional etching it is generally considered that thorough washing with copious amounts of clean water is essential to remove this precipitate and ensure optimum bonding (Soetopo et al., 1978; Beech and Jalaly, 1980; Diedrich, 1981). Sometimes water-spraying is not efficient enough to remove a tenaceous surface coating and crystal precipitates may occlude the microporosities of etched enamel.

Crystal nucleation may also, occasionally, occur during an usual acid-etching. Some authors have mentioned the presence of large crystalline structures that can be seen with the S.E.M. (Beech and Jalaly, 1980; Diedrich, 1981; Gwinnett and Smith, 1982; Duprez et al., 1985).

On the other hand, for few years, it has been claimed that crystal growth conditioning might be a better alternative especially for enamel pretreatment in bracket bonding (Smith and Cartz, 1973; Smith et al., 1980; Artun and Bergland, 1984; Mac Phee et al., 1985; Farquhar, 1986; Maijer and Smith, 1986; Read et al., 1986). Crystal growth was first observed with polyacrylic acid that contains a sulfate component, actually it can be achieved with different ion solutions containing sulfate (Artun and Bergland, 1984).

In previous experiments we used the S.E.M. to examine the effects produced by the acid-etch technique on human enamel surfaces (Rodde, 1989). We observed also a lot of acid-etched enamel surfaces from teeth of different animal species especially fossil rodents (Rodde and Mein, 1988). These observations of several hundreds surfaces has revealed on a lot of specimens some deposits. More often they appear as a generalized thin covering, sometimes as lamellar crystals spread evenly over the enamel surface or as cactus-like formations nucleated from a point. This crystal growth seems to be linked with two parts of these experimental studies. Part 1 is an evaluation of various mechanical pretreatments performed on enamel samples, the second part is relative to the influence of albumin on acid-etching. In experimental models albumin was used as a substitute for salivary proteins (Hlady and Füredi-Milhofer, 1979; Juriaanse et al., 1981; Eggen and Rölla, 1983; Arends et al., 1986; van der Linden et al., 1987). We attempted to apply this model to the etch process.

The aim of this paper was to examine if crystal formation, in our experiments, was only fortuituous or if it may be related to some special treatment performed before or during acid application. We attempted also to identify some crystals by X-ray diffraction and microprobe analysis.

MATERIALS AND METHODS

Preparation of test specimens:
Freshly extracted human teeth which had been cleaned and stored, in a refrigerator in distilled water, until needed were used in this in vitro study. After the tooth root had been cut off with a diamond disk, 286 macroscopically sound enamel surfaces were chosen (54 specimens were temporary teeth, T.E., 232 permanent teeth, P.E.).

Part 1: Mechanical pretreatments of tooth enamel surfacres (Fig. 1).

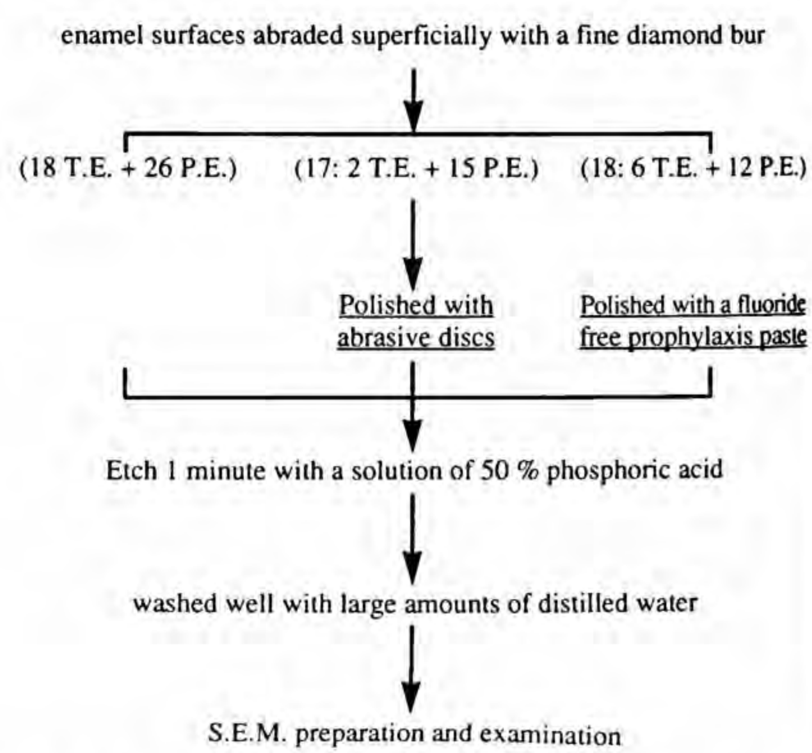

Fig. 1: Materials and procedures used in part 1 of the study.
Fig. 1: Description schématique des matériels et méthodes utilisés dans la première partie de cette étude.

The surfaces were mechanically treated, prior to etching, using one of the following:
Group 1: polishing with a suspension of flour of pumice in distilled water,

Group 2: abrasion of the superficial layer of the enamel with polishing discs (Soft-lex Pop-on contourning and polishing discs n° 1980, Dental products 3M) of medium up to ultrafine grit sizes,
Group 3: abrasion of the superficial layer of the enamel with an aluminium oxide wheel (Dedeco Ref. 5006 7/8×1/8) followed by a wet grinding preparation using the polyester abrasive polishing discs described just above,
Group 4: step 3 at which was added a final polishing with a fluoridated prophylaxis paste (Gencinol, Dental products Dentoria),
Group 5: step 3 followed for this group by a final polishing with a non fluoridated prophylaxis paste (Sitsaciline, dental products P. Rolland, France),
Group 6: the surfaces were prepared using a fine diamond bur (Horico, Ref. 221/014 F6),
Group 7: step 6 followed by a wet grinding preparation using the polyester abrasive discs Soft-lex as for groups 2, 3, 4, 5,
Group 8: step 6 followed, for this group, by a final polishing with the fluoride-free prophylaxis paste.

The surfaces, in group samples 1, 4, 5, were polished with a soft rubber prophylaxis cup (Mandred mounted rubber polishing cups, Dental products Vevey, Switzerland) rotating at low speed, for about 2 minutes. Rinsing and drying procedures, for all samples, were identical. The specimens were carefully rinsed with distilled water for 2 minutes and dried with a blast of oil-free compressed air. The enamel surfaces were then etched, for one minute, with aqueous solutions of phosphoric acid 10, 50, 70% vol., the acid was applied with a cotton pellet. The acid was then removed with a large amount of distilled water. The specimens were air-dried before mounting and coating.

Part 2 consists of the evaluation of the effects produced by an acidic protein (bovine serum albumin, fraction V n° A 8022, Sigma) on acid-conditioning. Experimental procedures are represented in text-figures 2 and 3.

Three concentrations for albumin solutions in distilled water were used: 0.5 g/l, 1 g/l and 2 g/l. When bovine serum albumin (BSA) was added to 50% phosphoric acid, even for a concentration as low as 1 g/l a slight precipitate was formed. Therefore, real concentrations were 0.5 g/l or contained between 0.5 and 1 g/l.

S.E.M. study:

Specimens were mounted on aluminium stubs and vacuum-coated with gold by ion sputter with a JEOL

Mechanical abrasion of the enamel surfaces of
16 permanent human teeth with a fine diamond bur
↓
the specimens were cleaned up
↓
immersed, for 5 minutes, in a solution of bovine serum albumin
in distilled water. The 3 protein concentrations used were:

0,5 g/l	1 g/l	2 g/l
(6)	(5)	(5)

↓
the specimens were washed carefully, air dried
↓
Etched for 1 min. with 50 % phosphoric acid
↓
washed and air dried
↓
S.E.M. preparation and examination

Fig. 2: Adsorption of serum albumin on the enamel surface prior etching. Group specimens 9.
Fig. 2: Groupe 9. Adsorption de la serum albumine à la surface de l'émail, avant la phase de mordançage.

Mechanical abrasion of the enamel surfaces of
16 permanent human teeth with a fine diamond bur
↓
the specimens were cleaned up
↓
specimens were immersed, for 1 min. in a 50 % phosphoric acid
solution that contained 0.5 g/l bovine serum albumin
↓
the specimens were washed carefully, air dried
↓
prepared for S.E.M. examination

Fig. 3: Addition of serum albumin to the etching agent. Group specimens 10.
Fig. 3: Groupe 10. Pour cette série d'échantillons la serum albumine a été ajoutée à l'agent de mordançage.

Plate 1.

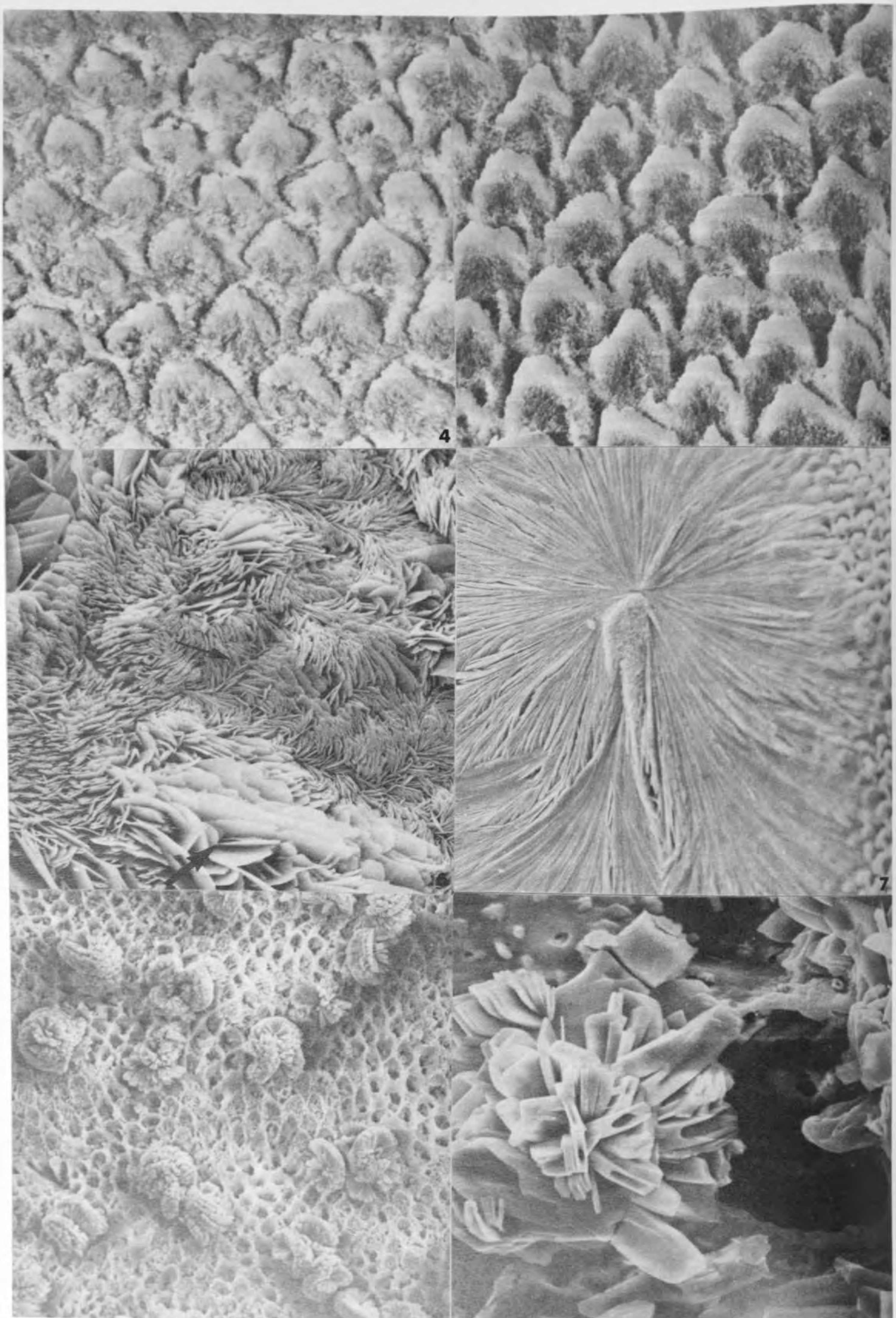

Plate 1.

Fig. 4: Labial surface of a young human premolar. Preliminary mechanical abrasion was performed with a diamond bur. The surface was etched for 1 min. with 50% vol. phosphoric acid. After a conventional water-spray rinsing of 1 min. we obtained a classical etched surface (etching pattern 2 in Silverstone classification). We can see, at the enamel surface, numerous microscopic crystals.
Magnification: × 2600.

Planche n° 1:

Fig. 4: Surface vestibulaire d'une prémolaire jeune. L'abrasion mécanique préalable a été effectuée avec une pointe diamantée. Le mordançage avec un acide phosphorique titré à 50% en volume a été pratiqué pendant 1 minute. Un rinçage classique avec un spray air-eau d'une durée de 1 minute a été effectué. Le mordançage est de type 2 (selon la classification établie par Silverstone). Nous pouvons noter, à la surface de l'émail, la présence de très nombreux cristaux microscopiques.
Agrandissement: × 2600.

Fig. 6: Labial surface of a lower incisor from an old person. Mechanical abrasion was performed successively with a diamond bur and sandpaper discs. 50% vol. phosphoric acid, 1 min., was used for etching. Etching was efficient with an etching pattern 2. At some places, on the surface, we can see numerous flake-like crystals. Near the nucleation center we observed small crystals (small arrow), at the periphery they are larger forming plate-like crystals (large arrow).
Magnification: ×2300.
Fig. 6: Surface vestibulaire d'une incisive inférieure appartenant à une personne âgée. L'abrasion mécanique, avant mordançage, a été pratiquée successivement avec une pointe diamantée et des disques abrasifs émerisés. Le mordançage à l'acide phosphorique, 50% en volume, a une durée de 1 minute. On obtient un mordançage de type 2. On peut voir, en plusieurs endroits, à la surface de l'émail de grands cristaux plats. Au centre, près de ce qui apparaît être un point de nucléation, les cristaux sont plus petits (voir flèche); en périphérie les cristaux sont plus grands (flèche plus épaisse).
Agrandissement: ×2300.

Fig. 8: Labial surface of a second lower human temporary molar. Mechanical abrasion was performed with a diamond bur. The surface was etched, for 1 min., with 50% vol. phosphoric acid. On a pattern 1 etched enamel we observed almost two hundred round calcifications. Crystal units were nucleated from a central point and formed pompon-like formations.
Magnification: ×700.
Fig. 8: Surface vestibulaire d'une deuxième molaire temporaire inférieure. L'abrasion mécanique a été pratiquée avec une pointe diamantée. La surface a été mordancée, pendant 1 minute avec une solution d'acide phosphorique (50% vol.). La surface présente un mordançage de type 1. On peut observer un grand nombre (de l'ordre de 200) formations cristallines de forme arrondie. Les cristaux sont regroupés autour d'un point central et forment une sorte de pompon.
Agrandissement: ×700.

Fig. 5: Labial surface of a young human premolar. Mechanical and chemical treatments are the same as for the previous sample. After etching the tooth specimen was soaked for 25 min. in increasing concentrations of acetone. The surface appears cleaner and cleared of calcium phosphate salts.
Magnification: ×2300.
Fig. 5: Surface vestibulaire d'une prémolaire jeune. Les traitements physique et chimique effectués sont identiques à ceux pratiqués pour l'échantillon précédent. Après mordançage, l'échantillon a été immergé, pendant 25 minutes dans des bains d'acétone de concentrations croissantes. La surface apparaît plus propre, comme débarrasséée de tout dépôt cristallin.
Agrandissement: ×2300.

Fig. 7: Labial surface of a permanent human upper canine. Mechanical pretreatment was performed successively with a diamond bur and a non-fluoridated prophylaxis paste on a rotating rubber cup. Large petal like crystals are nucleated from a central point.
Magnification: ×780.
Fig. 7: Surface vestibulaire d'une canine permanente supérieure. L'abrasion mécanique a été pratiquée successivement avec une pointe diamantée et une pâte abrasive non fluorée appliquée avec une cupule de caoutchouc. On observe de grands crystaux plats, en forme de pétales, rayonnant autour d'un centre de nucléation.
Agrandissement: ×780.

Fig. 9: Labial surface of a permanent lower incisor from a middle-aged person. Mechanical abrasion was performed successively with a diamond bur, paper discs and a fluoridated prophylaxis paste. The surface was etched, for 1 min. with 50% phosphoric acid. We can see, on the enamel and also on the neighbouring dentin some crystal formations. Here, crystal units showed a rectangular central hole.
Magnification: ×1700.
Fig. 9: Surface vestibulaire d'une incisive permanente inférieure ayant appartenu à une personne d'âge moyen. L'abrasion mécanique a été pratiquée successivement avec une pointe diamantée, des disques émerisés et une pâte fluorée utilisée en prophylaxie. Le mordançage d'une durée de 1 minute a été effectué avec de l'acide phosphorique (50% vol.). On observe, sur l'émail et aussi sur la dentine avoisinante quelques formations cristallines. Ces cristaux, de forme rectangulaire, présentent une cavité centrale.
Agrandissement: ×1700.

Plate 2.

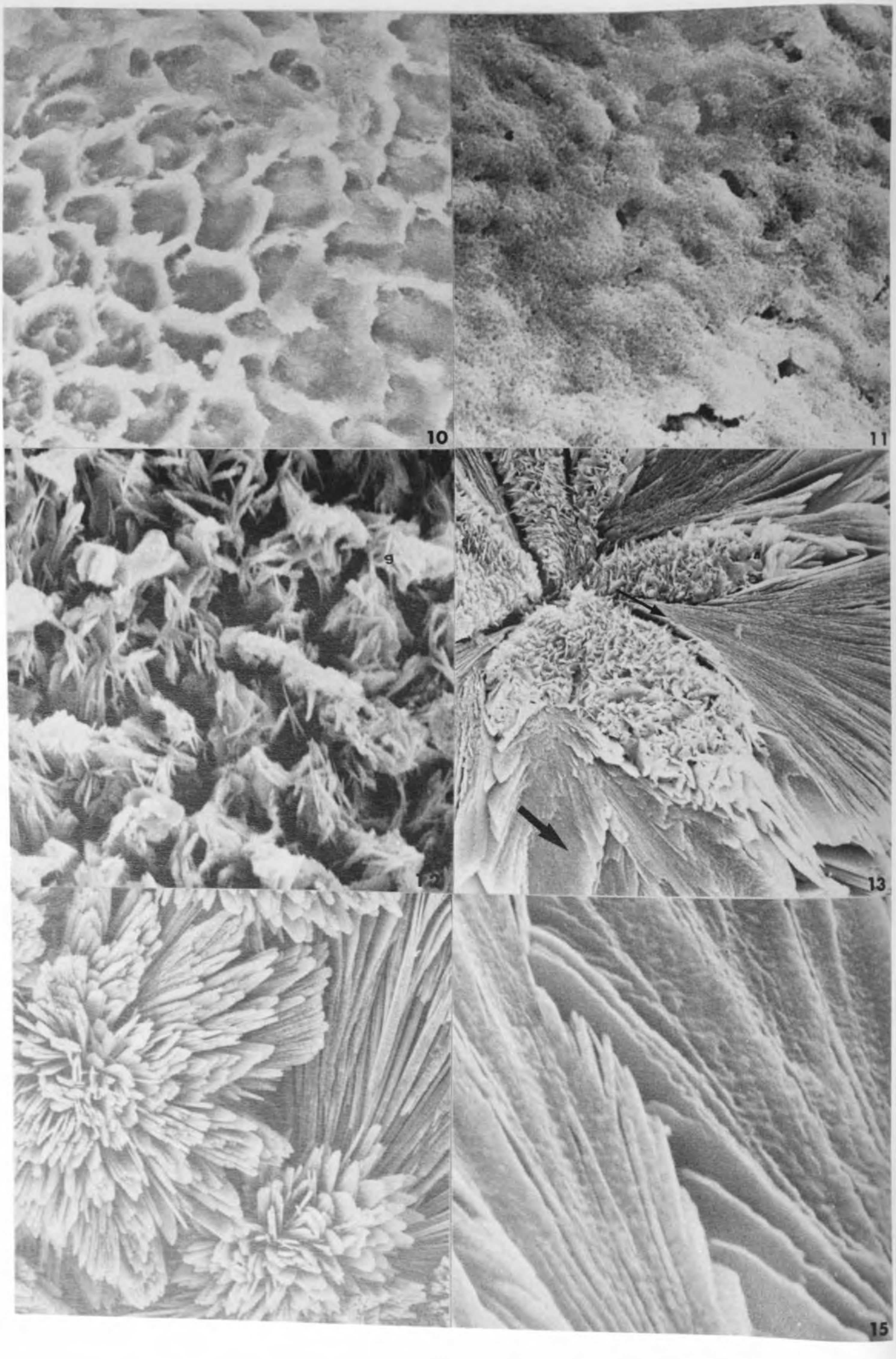

Plate 2:

Fig. 10: Labial surface of a permanent uper central human incisor. Mechanical abrasion was performed with a diamond bur. Bovine serum albumin, 1 g/l, was added to the 50% vol. phosphoric acid etchant. Etching was efficient but, in some places, a protein film occlude partly the microporosities.
Magnification: × 2600.

Planche n° 2:

Fig. 10: Surface vestibulaire d'une incisive supérieure permanente. L'abrasion mécanique a été pratiquée avec une pointe diamantée. A la solution acide de mordançage (ac. phosphorique 50% vol.) on a ajouté une protéine acide: la serum albumine bovine (la concentration étant de 1 g/l). Le mordançage reste efficace pourtant, en certains points, on observe la formation d'un film protéique qui vient obstruer en partie les microrétentions.
Agrandissement: ×2600.

Fig. 11: On the same tooth, in other places, on the surface, the microporosities are completely occluded by the protein precipitate.
Magnification: × 2600.
Fig. 11: Sur la même dent, sur d'autres portions de la surface de l'émail les microcavités créées par mordançage sont complètement obstruées par le film protéique.
Agrandissement: ×2600.

Fig. 12: Vestibular surface of a first upper permanent human molar. Mechanical pretreatment was performed with a diamond bur. Prior to etching the sample was immersed in a 1 g/l solution of bovine serum albumin in distilled water, for 5 min. Then etching was performed, for 1 min. with 50% vol. phosphoric acid. On a large part of the surface sample, appeared numerous needle-shaped or small flaky crystals which occlude partly the microporosities.
Magnification: × 3900.
Fig. 12: Surface vestibulaire d'une première molaire permanente supérieure humaine. L'abrasion mécanique a été effectuée avec une pointe diamantée. Avant mordançage l'échantillon a été immergé dans une solution (dans l'eau distillée) de serum albumine bovine (concentration 1 g/l), ceci pendant 5 minutes. Le mordançage a ensuite été pratiqué pendant 1 minute avec une solution d'acide phosphorique 50% vol. Sur une grande partie de l'échantillon on observe des formations cristallines en forme d'aiguilles et quelques petits cristaux plats, en forme de pétales. Tous ces cristaux obstruent partiellement les microrétentions obtenues par mordançage.
Agrandissement: ×3900.

Fig. 13: Vestibular surface of a permanent upper human incisor. Mechanical abrasion was performed with a diamond bur. Bovine serum albumin, BSA, 0.5 g/l was added to the 50% vol. phosphoric acid etchant. On this sample many petal-like formations were observed. In the center there were many small flaky crystals. Large petal-like crystals formed either from the center (small arrow) or at the periphery (large arrow).
Magnification: × 1950.
Fig. 13: Surface vestibulaire d'une incisive supérieure permanente humaine. Abrasion mécanique effectuée avec une pointe diamantée. Cette fois la serum albumine bovine a été ajoutée à la solution de mordançage, ceci pour une concentration de 0,5 g/l. Sur cet échantillon on peut observer de nombreux cristaux en forme de pétale. Au centre les cristaux sont plus petits. Certains grands cristaux plats partent eux-aussi de ce centre de nucléation (petite flèche) d'autres sont disposés plus en périphérie (flèche plus large).
Agrandissement: ×1950.

Fig. 14: On the same tooth, we observed other petal-like formations. Here, from a morphological standpoint all the crystals appear to be identical. Their size only vary.
Magnification: × 1300.
Fig. 14: Sur la même dent, on observe d'autres formations en forme de pétale. Tous les cristaux présentent des formes identiques, seules les dimensions varient.
Agrandissement: ×1300.

Fig. 15: Details of large plate-like crystal formations.
Magnification: ×7800.
Fig. 15: Figure présentant, plus en détail, ces cristaux plats en forme de pétales.
Agrandissement: ×7800.

JSC 1100 apparatus. These specimens were examined in a JEOL JSM 35 C scanning electron microscope, operating at 15 kV beam voltage.

X-ray and microprobe analysis:

X-ray diffraction was employed on one specimen only because, as it was discussed later, this method does not allow precise identification of the nature of crystals since stoechiometry can not be characterized. Three specimens with large nucleated crystals were used for microprobe tests. The apparatus was a Camebax VFS 65536, setting 2 operated at 15 kV accelerating voltage and 6 nA current. Number of impulses was recorded for 100 seconds.

RESULTS

Morphology of crystal deposits:

Part 1:

We can differenciate three kinds of crystal deposits: 1°) More often deposits appeared as a generalized thin covering which probably had not been completely removed by a thorough washing. These dissolved calcium salts covered only partially the retentive microporosities but they did not occlude all the micropores (Fig. 4, Plate 1). The presence of such a deposit appeared clearly when we compared surfaces which had been rinsed by a conventional washing regimen to surfaces which had been soaked for 25 minutes in increasing concentrations of acetone solutions. A longer time of immersion in acetone solutions seemed remove the calcium salts spread on the surface, the specimens appeared entirely cleaned and cleared of residual crystal precipitates (Fig. 5, Plate 1); 2°) Small well-formed lamellar, petal-like or needle-shaped crystals which were present as a generalized covering (Fig. 6, Plate 1); 3°) Lamellar crystals appeared, on some specimens, as close packed configurations. These formations nucleated from a point on the enamel surface, resulted in flower or cactus-like formations (Fig. 7, 8, 9, Plate 1).

Part 2:

Adsorption of albumin prior to etching or addition of this protein to the acid-etch solution generally leads to the formation of an organic deposit which altered dramatically the surface topography of the etched enamel surfaces. This organic coating can only cover the underlying well etched enamel (Fig. 10, Plate 2) but it can also mask all the microporosities (Fig. 11, Plate 2). Adsorption of albumin, prior to etching, can also lead to the formation of numerous, needle-shaped crystals (Fig. 12, Plate 2) or, sometimes to large nucleated crystals (Fig. 13, 14, 15, Plate 2).

Frequency of crystal formations:

Well shaped crystals formations were relatively scarse. They appeared on 9/192 enamel surfaces of fossil rodents treated by 50% vol. phosphoric acid. We have observed well-shaped crystals on 13/286 human enamel surfaces. The repartition of crystal deposits within the different sample groups may be of some interest:

- 4/6 treated surfaces in group 4
- 1/5 treated surface in group 5
- 2/13 treated surfaces in group 6
- 1/17 treated surface in group 7
- 2/18 treated surfaces in group 8
- 2/16 treated surfaces in group 9
- 1/16 treated surface in group 10.

In our experiments we observed a crystal growth on 15/478 samples. If we consider these results as a whole, crystal formation appears very occasionaly. But if we take into account the variation among the different groups we can observe that in groups 4 and 5 crystal growth was not so incommon. When some polishing processes were successively performed the frequency of crystalline deposits increases.

X-ray identification:

Fig. 16 shows the X-ray diffraction pattern of crystals presented in Fig. 9, Plate 1. Except small peaks corresponding to aluminium (Al) and gold (Au) probably due to aluminium stub and gold coating we observed two well delineated peaks corresponding to phosphorus (P) and calcium (Ca). Although the different types of crystals could not be separated or identified the P/Ca ratio observed on the X-ray diffraction pattern suggests that the precipitate was calcium apatitic.

Microprobe analysis was performed on large petal-like crystals similar to those presented in Fig. 14, 15, Plate 2. Under the conditions used in this experiment (15 kV accelerating voltage and 6 nA current) the energy peaks were for P: 2 keV, for Ca: 3.92 keV. The number of impulses for 100 seconds recorded for three crystals was presented in Table I.

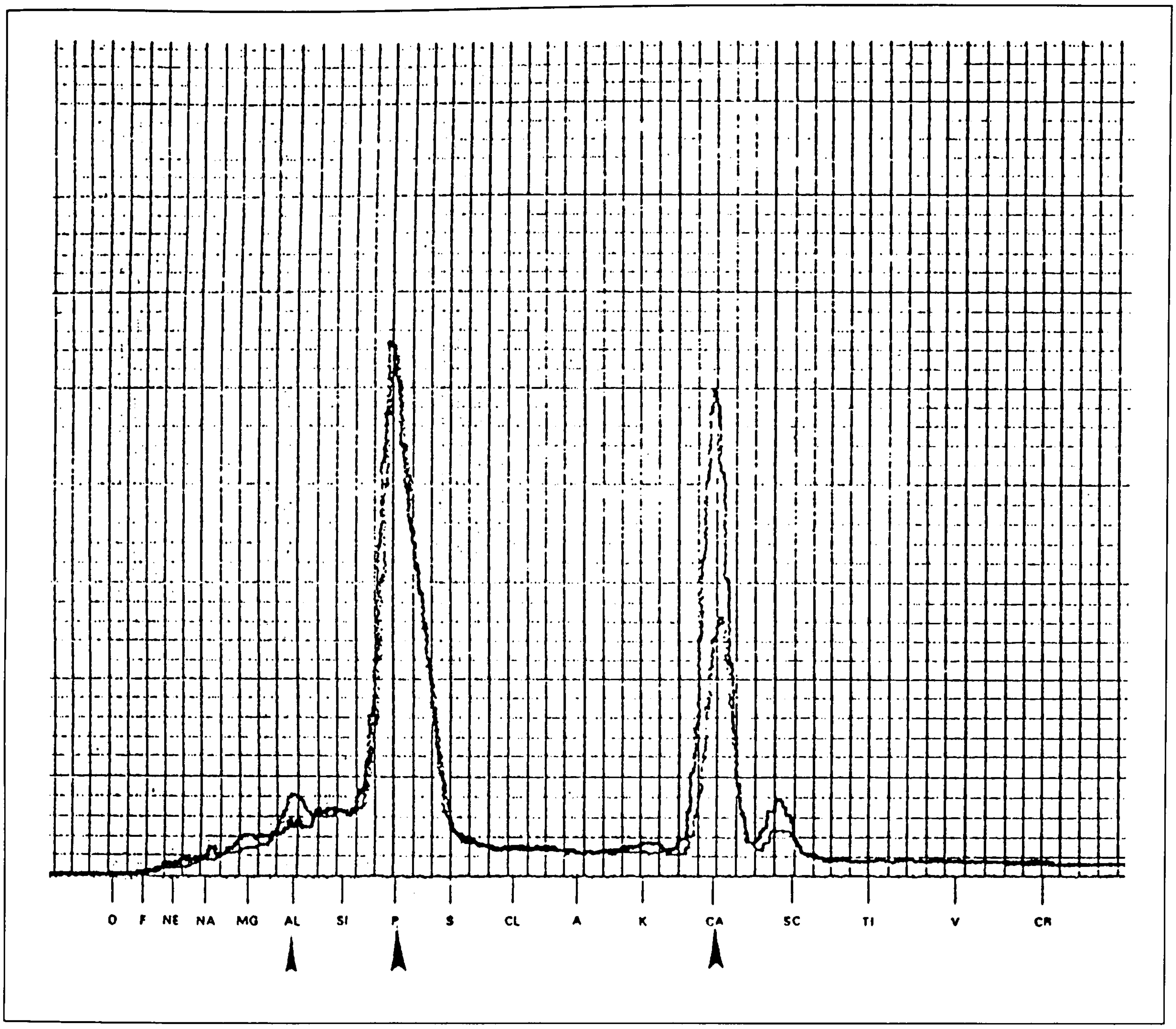

Fig. 16: X-rays diffraction diagram for the cristals presented Fig. 9.
Fig. 16: Diagramme de diffraction aux rayons X obtenu pour les cristaux présentés Fig. 9.

Table I
Microprobe analysis of 3 crystals. Number of imulses for 100 seconds.
Tableau I
Analyse en microsonde sur 3 cristaux. Nombre de coups émis pendant une durée de 100 secondes.

	crystal 1	crystal 2	crystal 3	mean
peaks P	48.080	47.910	50.494	48.828
peaks Ca	16.730	15.947	17.248	16.642

These measurements compared to the number of impulses transmitted by hydroxyapatite (OHAp) allow to think, after correlation between the formula of OHAp and the number of impulses, that the crystals studied were probably dehydrogen phosphate monohydrate $Ca(H_2PO_4)_2 . H_2O$.

DISCUSSION

It was surprising to note that only four papers have revealed crystal formation on the enamel surface after etching: Beech and Jalaly (1980); Diedrich (1981); Gwinnett and Smith (1982); Duprez et al. (1985) but without giving the proportion of crystal formations.

Generally crystal deposits are considered as a consequence of improper cleaning following the etch process (Soetopo et al., 1978; Hormati et al., 1980; Gwinnett and Smith, 1982; Silverstone et al., 1985). Beech and Jalaly (1980) observed such deposits after rinsing with a small amount of water (0.2 ml) but, in their experiments crystal precipitates disappear when the amount of water is 0.5 ml. Diedrich (1981) found that 2 seconds water-spraying was efficient: «three out of five teeth were not completely clean, whereas after 5 seconds of air-water no residues or crystal precipitates are no longer visible». But an improper cleaning seems not to be the only reason if we consider that Duprez et all. (1985), on the contrary, observed crystal formations after the specimens were rinsed, under running tap water, for one hour.

Since Maijer and Smith (1979) demonstrated that crystalline structures produced a bondable surface to which orthodontic brackets were attached with sufficient strength the phenomenom of crystal growth has been examined by several researchers (Smith et al., 1980; Artun and Bergland, 1984; Mac Phee et al., 1985; Farquhar, 1986; Maijer and Smith, 1986). This technique appears as a very interesting alternative because no mechanical abrasion has to be used in debonding. A sulfate component reacts with the calcium in the enamel surface to form a dense growth of small needle-shaped crystals. The crystals were found to be gypsum (Smith and Cartz, 1973; Read et al., 1986). Was this gypsum crystal builup a better retentive mechanism than crystals observed, after etching, with phosphoric acid? To answer this question it would be necessary to identify the crystals obtained in the previous experiments and also to test their solubility. Microprobe analysis indicate they may be $Ca(H_2PO_4)_2 . H_2O$. According to Brown (1975) monocalcium monohydrate appears when 50% phosphoric acid is applied on enamel surfaces. However Brown indicates that this salt is very soluble. In this case we think that it would have disappeared during a one hour rinsing under running tap water as performed by Duprez et al. (1985). Crystals shown in the literature were not identified. Diedrich (1981) indicates only that they are «calcium phosphate crystals»; from a morphological standpoint they resemble closely our Fig. 9. Gwinnett and Smith (1982) told of «a crystalline reaction product», this product resembles our Fig. 13 or 14 with large, lamellar petal-like formations. Newesely (1971) identified these crystals as octacalcium phosphate: $Ca_4H(PO_4)_3 .2\, H_2O$. This possible precursor of hydroxyapatite would be a more suitable crystal framework because its solubility is very low (Brown, 1975). Further investigations would be needed to determine precisely the composition and the stoechiometry of crystals. We attempted to analyze the procedures which appeared to facilitate formation of crystal deposits. Crystal formation occurs principally when abrasive pretreatments are numerous, as in groups 4 and 5 when successively an oxide wheel, paper abrasive discs and a prophylaxis paste were used. Is the increase of heat produced by these polishing process which facilitate crystal deposits? Is the inorganic component (silica 60% w/w) in Sitsaciline paste which lead to crystal nucleation? or fluoride contained in Gencinol prophylaxis paste? Some data seem to indicate that fluoride can effectively induce a crystal formation. A fluoride concentration as low as 0.1 ppm leads to an increase in the rate of apatite crystal growth in vitro (Moreno et al., 1978; Varughese and Moreno, 1981). Formation of synthetic flaky or petal-like crystals was also observed by Okazaki et al. (1984). They thought that these petal-like crystals similar to those described in Fig. 6 could be fluoridated apatites. Thus crystal formation could be related to the presence of fluoride. It would be tempting, in vivo, to facilitate the growth of fluoridated apatites because of their low solubility in the buccal environment.

A possible link between presence of protein and crystal formation has to be investigated.

Is it possible to consider that these crystal formations may be usefull to bind resin composites to enamel? From this preliminary study, it seems that crystal nucleation, even after a prophylaxis with a fluoridated paste, was too fortuituous to give a suitable way to increase the retention of the enamel surfaces.

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