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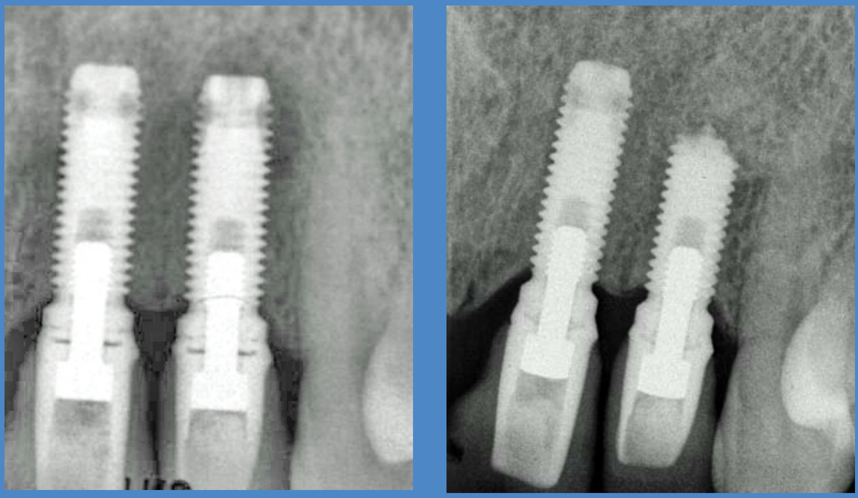

Implant periapical lesion

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Instructions to authors

Introduction

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Implant periapical lesion

A case series report

Kerstin Rosendahl[1], Gunnar Dahlberg[2], Jenö Kisch[3], Krister Nilner[1]

Abstract

◎ One complication in implant dentistry is the implant periapical lesion-IPL- which is a lesion around the apex of a stable implant diagnosed radiographically as a radioluscency in the bone at the apical part of an implant. The IPL can perform with or without clinical symptoms such as tenderness, swelling, suppuration and fistulation.

This report describes 4 cases of IPL which were treated surgically with sectioning and removal of the affected portion of a stable implant and thorough debridement of the granulomatous tissue around it. This treatment was, up to 4 years after treatment, successful in all 4 cases.

It can also from this report be concluded that IPL is a rather rare condition and that it can occur at any stage of implant treatment, in these cases from 4 months up to 11 years after implant installation.

Finally there is a discussion about the aetiology of IPL and a comparison to findings in other reports on IPL and it is concluded that it is difficult to claim that there is a single cause to IPL. Rather it is evident that the condition might be a sequel of the summation of many possible causes. This summation exceeds the local biological threshold for the individual patient.

Key words

Implant dentistry, fistulæ, implant periapical lesion, apical peri-implantitis

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Apikal peri-implantit
En rapport av fyra fall

Kerstin Rosendahl, Gunnar Dahlberg, Jenö Kisch, Krister Nilner

Sammanfattning

◎ En komplikation vid käkbensförankrad protetik är apikal periimplantit - IPL - från den engelska benämningen Implant Periapical Lesion. Det innebär att ett implantat som sitter stabilt och är väl osseointegrerat i sin coronala del, uppvisar en uppklarning på röntgen vid sin apikala del. Komplikationen kan uppträda med eller utan kliniska symtom såsom ömhet, svullnad, suppuration och fistelbildning.

Denna rapport redovisar fyra olika fall av IPL. Den redogör för när komplikationen diagnostiserats, hur den behandlats, utfallet av behandlingen och diskussion kring etiologin.

Samtliga fyra fall har haft kliniska symtom och har upptäckts vid mycket olika tidpunkter av behandlingen eller vid efterkontroll. Två av fallen diagnostiserades efter 11 år, ett fall 4 mån efter fixturinstallationen och ett fall 15 månader efter distansanslutningen (18 mån efter fixturinstallationen).

Alla fyra fallen har behandlats kirurgiskt med uppfällande av lambå, avlägsnande av granulationsvävnad och den affekterade delen av fixturen. I två av fallen spolades området med fysiologisk koksaltlösning innan återsutureringen av lambån och i två av fallen fylldes benkaviteten dessutom med benersättningsmedel som täcktes med ett resorberbart membran innan återsutureringen. Samtliga fyra fall läkte efter behandling och de kliniska och röntgenologiska symtomen försvann och återkom ej under fyra års uppföljningstid.

Vid jämförelse med andra rapporter, kan konstateras att någon enskild orsaksfaktor till IPL svårligen kan hävdas. Det tycks snarare vara följden av flera olika samverkande faktorer vilka sammantaget gör att det biologiska tröskelvärdet överskrids och lesionen uppstår.

Introduction

Unexpected complications and failures in implant dentistry can occur at any stage. A complication is distinct from a failure. The former is amenable to treatment and - if left untreated - leads to the latter (1). One complication cited in the literature is the implant periapical lesion – IPL, which has also been termed periapical implant lesion, apical peri-implant lesion, and retrograde or apical peri-implantitis (4,5,12,18,23,24,28).

Esposito et al stated that these lesions often are found around long implants placed in dense bone (12). Radiographically, the coronal portion of the implant is supported by "normal" bone in contact with a stable implant. The lesions may be completely asymptomatic or have been discovered in relation to tenderness or persistent pain or swelling and fistulation (25). The aetiology seems to be multifactorial.

In 1995, *Reiser & Nevins* on one hand reported the prevalence of IPL to be 0.26% (25). They included the inactive form of IPL, which they meant is a periapical radiolucency visible on radiographs similar to an apical scar without clinical symptoms. Any radiolucency should be followed up radiographically to decide if it is increasing in size (25). The active form must be treated aggressively otherwise it will lead to failure (25).

In 2005, *Quirynen et al* on the other hand reported the prevalence of IPL to be somewhat higher - 1.9% on single implants, where the lesions were revealed on radiographs before or at abutment connection combined with or without clinical symptoms (24).

Finally *Balshi et al* in 2007 reported a prevalence of 9.9%, where the lesions were identified either radiographically, by clinical observation or by a combination of these (3).

Alsaadi et al indicated in 2008 a prevalence of IPL again to be in the lower range (0.7%) when studying the impact of local or systemic factors on the incidence of failures. The lesions in their report were detected radiographically before or at abutment-connection (2).

Failures and complications in implant dentistry can biologically be classified as early or late, where early (before loading) means inability to establish osseointegration and late (after loading) means inability to maintain the achieved osseointegration (10).

Four cases of IPL and their treatment are described in this report and the outcome of the treatment seems to fulfil the proposed criteria of success for implants (1).

The aims of this report are to increase the awareness of IPL, to describe our treatments, and to speculate about the possible aetiologies of these four cases.

Case 1

A 30-year-old healthy man with almost all teeth intact was traumatized (in his upper jaw) by an iron chain. The pulp of the central incisor on his right side was exposed, the tooth was immediately treated endodontically and a ceramic crown was bonded to it. The lateral incisor on the same side was 3 months after the injury still tender to percussion and at test of its mobility. It was by then treated endodontically and the tenderness disappeared (Fig 1a). Seven months later the tooth fractured (Fig 1b) and was replaced with an implant (Self-tapping fixture; 15x3,75mm; Nobel-Biocare, Göteborg, Sweden) four weeks after extraction to preserve as much crestal bone as possible. V-penicillin was prescribed on the day of installation. The patient had severe pain for 1–2 weeks otherwise the healing process was uneventful.

Four months later a fistula appeared buccally of the implant. The fixture was stable and the fact that the cover screw was loosened seemed at first sight to

Figure 1a. Radiographical appearance of the central incisor with the ceramic crown and the lateral incisor still functioning five months after the injury.

be the explanation of the fistula. An abutment connection was made. The fistula, however, persisted without other symptoms. Different antibiotics were prescribed but they kept the fistula away only for a few weeks and finally a very discreet radiolucency of about 5mm around the apical part of the fixture was disclosed by fistulography (Figs 1c-d). Surgery was performed and access to the apical part established. Four mm of the apical part of the implant was removed, the granulomatous tissue was thoroughly debrided, the flap was sutured back without any bone allograft or membrane in the lesion and antibiotics were again prescribed. The fistula finally

Figure 1b. Radiographical appearance eleven months after the injury. The coronal part of the lateral incisor removed.

Figure 1c. Clinical appearance of the fistula at the lateral incisor about seven months after its first appearance.

disappeared. The pathological anatomic diagnosis (PAD) was "Connective tissue, granulation tissue, and round cells. No pus, no epithelium. Diagnosis: 'peri-implantitis'."

Six months later the conditions were clinically healthy and intra-oral radiographs showed complete healing (Fig 1e).

Four years later the conditions were still healthy (Figs 1f-g).

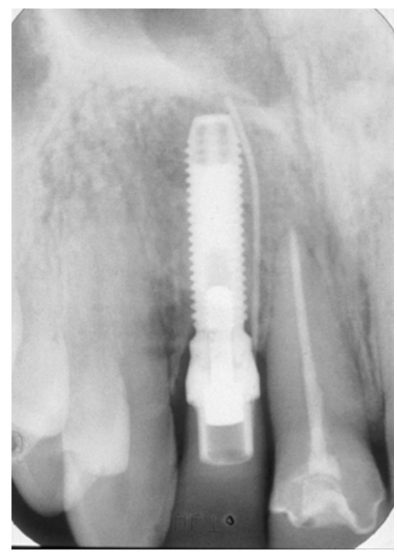

Figure 1d. Fistulography, 13 months after implant installation.

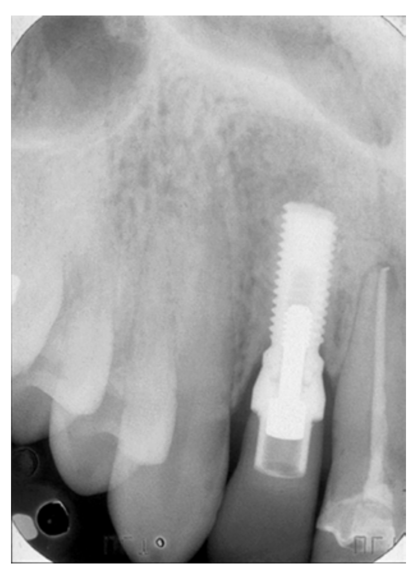

Figure 1e. Radiographical appearance six months after the resection of the apical part of the fixture. The radiograph reveals complete healing of the surrounding bone.

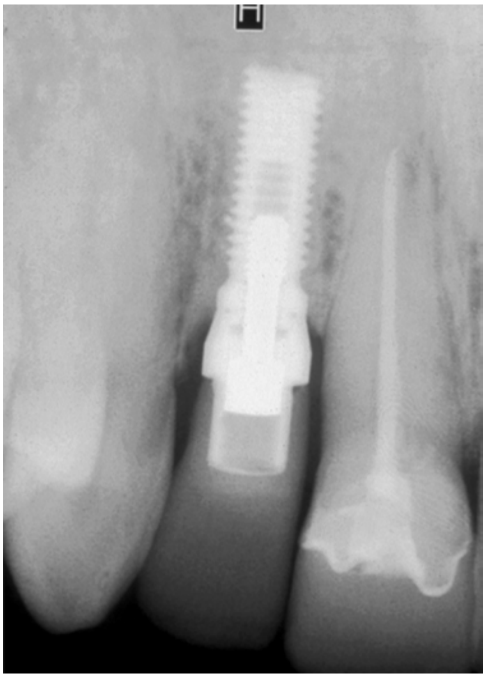

Figure 1f. Radiographical appearance four years after resection, the surrounding bone still remains healthy.

Figure 1g. Clinical appearance four years after resection, showing scars only.

Case 2

A young man, with aplasia of both upper lateral incisors, was habilitated with two implants (Self-tapping fixture; 13x3,75mm; Nobel-Biocare, Göteborg, Sweden) when he was 20 years of age. Eleven years later a purulent swelling was found in the hard palate and an IPL was identified radiographically at the right hand side implant (Figs 2a-b). Surgical treatment, with removal of the apical fourth of the implant and debridement of the granulomatous tissue, made the lesion heal and four years later the conditions were still healthy (Figs 2c-d).

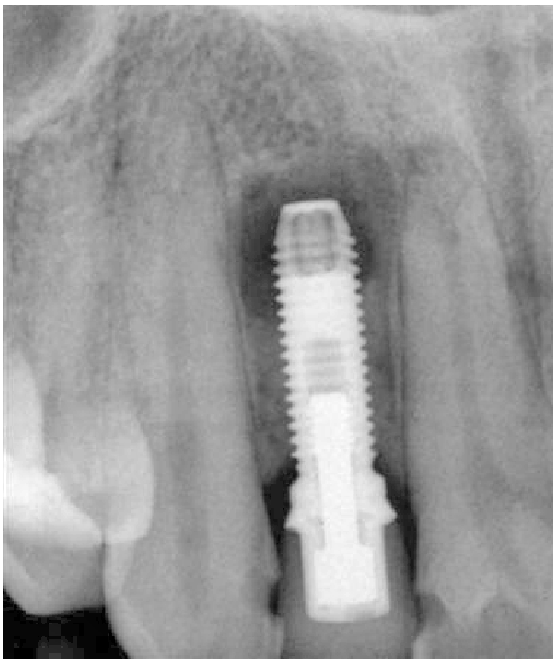

Figure 2b. Radiographical appearance of the IPL on the right lateral incisor.

Figure 2a. Clinical appearance with a palatal swelling 11 years after implant installation for the lateral incisors.

Figure 2c. Clinical appearance four years after treatment.

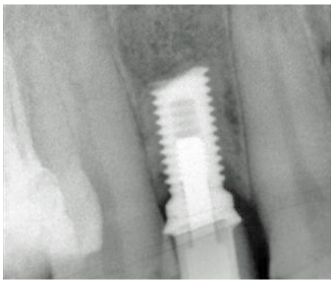

Figure 2d. Radiographical appearance four years after removal of the apical fourth of the implant indicating healing of the IPL.

Case 3

A young healthy woman who had her right central incisor in the upper jaw replaced with an implant (Self-tapping fixture; 10x3,75mm; Nobel-Biocare, Göteborg, Sweden) when she was 18 years old. The tooth had been injured at an accident followed by endodontic treatment that had failed. Eleven years after implant installation she had a swelling with tenderness on the buccal side of the ridge and an IPL could be identified radiographically (Fig 3a). During these 11 years regular check–ups had been made without discovering any signs of pathology (neither clinically nor radiographically). The surgical treatment consisted of flap-elevation, thorough debridement of the lesion, sectioning and removal of the affected apical portion of the implant, grafting with bone-substitute (Frios Algipor; Dentsply Friadent, Mannheim, Germany), covering with a resorbable membrane (Resolut Adapt; W.L. Gore&Associates Inc, Flagstaff, Az, USA) and resuturation. Normal clinical conditions were present one year later and complete healing was revealed via radiographs (Fig 3b). Four years later the tissues were still healthy and the implant-stability good (Fig 3c).

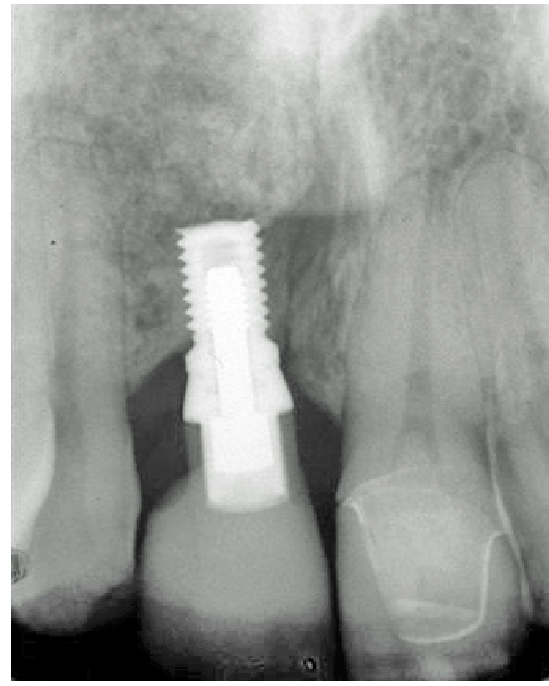

Figure 3b.
Radiographical appearance of the completely healed IPL one year after surgery of the right central incisor.

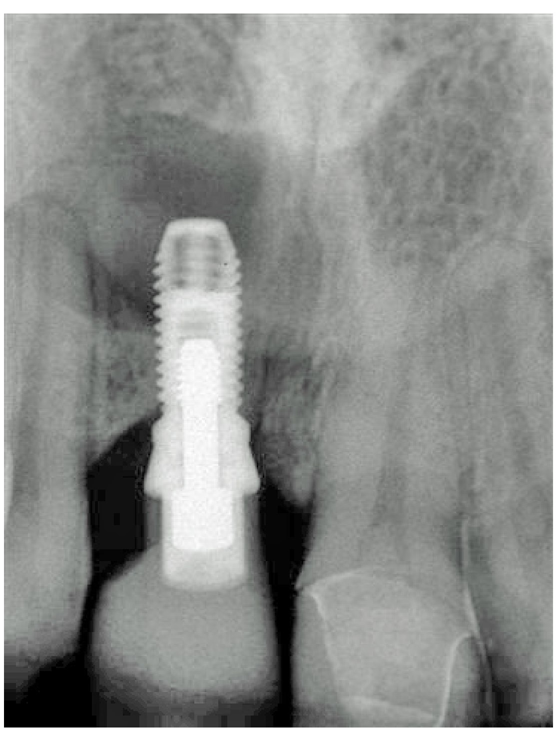

Figure 3a.
Radiographical appearance of the IPL on the right central incisor 11 years after implant installation.

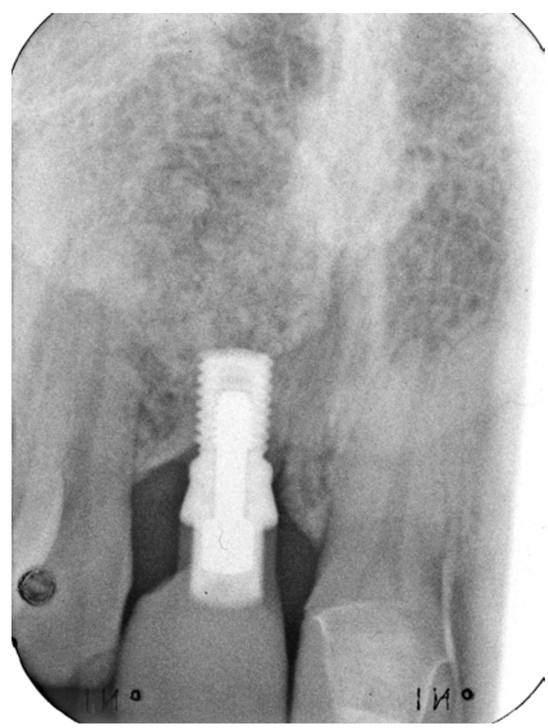

Figure 3c.
Radiographical appearance four years after surgery, showing that healthy conditions are remaining.

Case 4

A woman, 53 years of age, had her incisors in the upper jaw injured at an accident. The central and lateral incisors of her left side were later extracted because of extensive root resorbtions. The teeth were replaced with two implants (Standard fixture; 13x3,75mm; Nobel-Biocare, Göteborg, Sweden). The installation of the implants was preceded by bone transplantation with a healing period of six months. Fifteen months after abutment connection (18 months after implant installation) there was a swelling with tenderness at the buccal side of the ridge and an IPL was found at the lateral implant (Fig 4a). The condition was treated surgically with flap elevation, debridement of the lesion, sectioning and removal of the affected part of the implant and grafting with bone-substitute (Frios Algipor; Dentsply Friadent, Mannheim, Germany) and covered with a resorbable membrane (Resolut Adapt; W.L. Gore&Associates Inc, Flagstaff, Az, USA). Six months later radiographs revealed complete healing (Fig 4b). Six years later the conditions were still normal (Fig 4c).

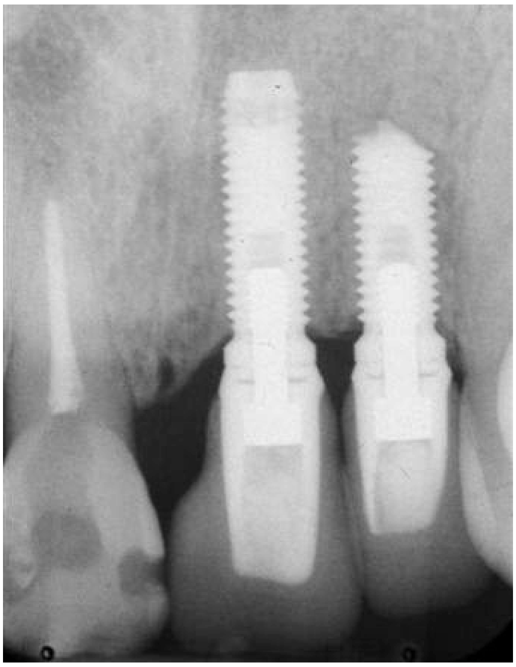

Figure 4b.
Radiographical appearance of the completely healed IPL six months after surgery.

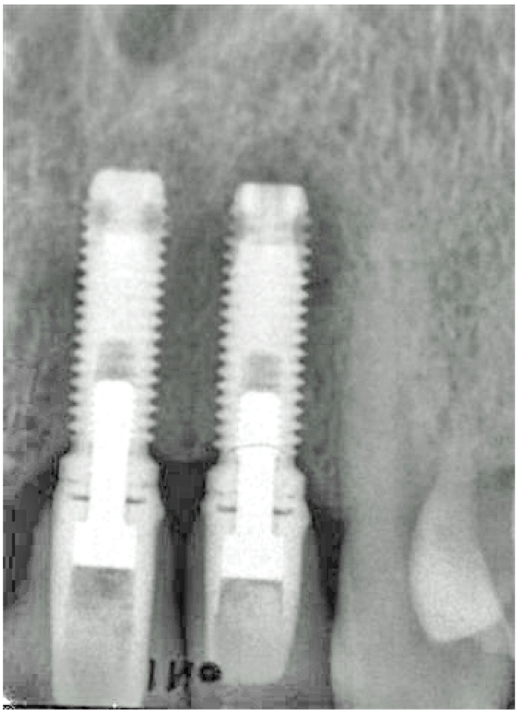

Figure 4a.
Radiographical appearance of the IPL on the left lateral incisor 15 months after abutment connection.

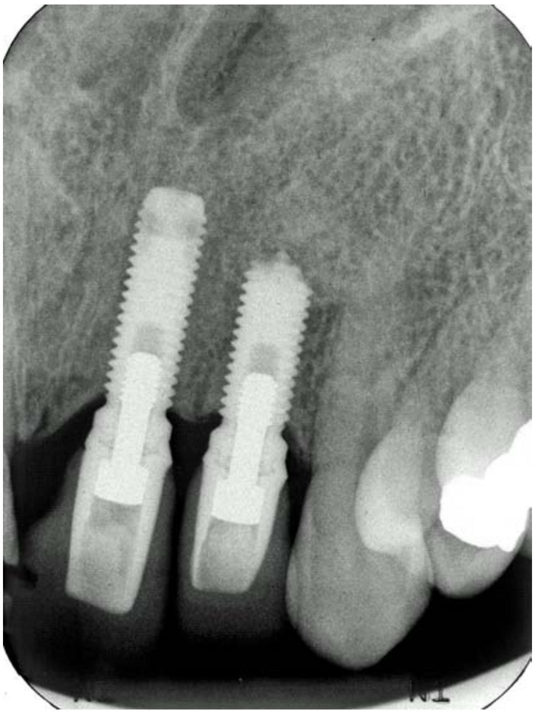

Figure 4c.
Radiographical appearance six years later, showing that healthy conditions are remaining.

Discussion

In the evaluation of the aetiology of the IPL in the first case it was found that many of the contributing factors mentioned in the literature could be excluded. The patient was a young, healthy non-smoker with all teeth intact except a few minor restorations in his molars, good oral hygiene, no parafunctions, no periodontitis and good quality of the jawbone (2-4,8,9,11,13,16-18,21-26). The circumstances for successful implant treatment were favourable. The neighbouring teeth exhibited no signs of pathology and there had never been any indication of periapical infection around the traumatically injured upper lateral incisor (6,8,16,18,20,22-25,27,28). Nothing indicated the presence of residual root fragments or foreign bodies (16,21,25,27).

The implant was installed one month after a careful extraction of the injured tooth, to save as much buccal and crestal bone as possible. The surgery was performed under strict antiseptic conditions, and the patient was under antibiotic coverage during implant insertion. The implant was installed with good stability.

Contamination of the implant by the manufacturer during production is possible but not probable (25). Nor could a contamination of the implant due to unintentional contact with the surrounding tissues during insertion be totally excluded as the cause of the complication, as has been discussed in similar situations (8,11,25). The healing process was uncomplicated except for a severe pain lasting for the first ten days after installation.

About four months after implant installation, a fistula appeared. Antibiotic therapy was administrated, but the fistula persisted. During abutment connection, it was found that the cover screw had loosened, which is considered a conceivable cause of fistulation (10,12,14,29). Different antibiotic treatments were prescribed without lasting effect. This finding is in agreement with observations in other reports (4,11,16,18,23) stating that "biomaterial-centred infections, as a rule, are extremely resistant to antibiotics" (11).

Once the fistulography revealed the apical peri-implant radiolucency, the treatment consisted of surgical elimination of the apical part of the implant and the surrounding granulomatous tissue. Antibiotics were again prescribed. This treatment was successful.

A delicate problem and a possible indirect aethiologic factor is the installation of a long fixture - 15mm. Effects like bone compression, bone fractures, or overheating at the apex of the implant might leave a small amount of necrotic bone in the apical hole of the fixture and in the adjacent tissues. Whether such necrotic tissue is significant in the development of an IPL is not fully understood (8,18,23,25).

It has also been claimed that epithelial tissue accidentally implanted might proliferate and cause the lesions (7). In our case, the PAD analysis of the removed tissue gave no support to such theories, thereby contradicting the commentary to the report of *Scarano et al* (7,27).

Finally it could be speculated that the close vicinity between the apex of the implant and the nasal cavity might have an influence on the development of the lesion although no findings in our report really support such a speculation (2,29).

In the second case, also a young man of good general health with favourable prerequisites for successful implant treatment, previous pathology in the area is excluded as explanation to the IPL as no teeth had ever been present in the region. The neighbouring teeth had no periapical pathology (8,14,16,20,24,25,28) and were adequately restored. There is unfortunately no radiographic documentation available after installation but a fistula appeared in the palate after 11 years. This makes it impossible to tell whether there has been an incomplete osseointegration around the apical part of the implant of the right hand side. The implant that replaced the lateral on the left hand side did not develop an IPL.

Overloading is sometimes mentioned to be a plausible explanation of IPL, but in this case the implants could be regarded as physiologically less loaded as they replaced lateral incisors (8,18,25).

In the third case, a young healthy woman had a central incisor in the upper jaw replaced with an implant because of unsuccessful endodontic treatment of the trauma-injured central. Her records testify that radiographs showed no signs of IPL before the clinical symptoms developed after 11 years. It seems strange that it does not appear until after 11 years. Rather it could be argued that for this case, as well as case 2, the IPL most probably was caused by agents to which the patient was exposed later on, or by organisms that may have remained dormant protected by their glycocalyxes until a coincidence of lower host defence (11).

The implant in case 3 shows an unfavourable crown-implant ratio which could make it tempting to claim that overloading might be a plausible explanation to the IPL(8). It is, however, remarkable that after shortening the implant more than one third of its length, it obviously works well despite the worse crown-implant ratio. This could lend support to the argument that loading conditions are of less importance for IPL.

In the fourth case the IPL appeared 18 months after implant installation at one of the two inserted implants. Notes from the patient's records state that radiographs, taken before the fistula appeared, showed no signs of radioluscency at any of the implant apices. Surgical treatment in this case also led to a successful outcome.

Nedir et al in 2007 reported on a case where the aetiology of the IPL was a foreign-body related reaction to starch particles (19). Whether such recent theories are of any significance can not be concluded from our report – especially not as the PAD (of case 1 only) does not report on any such findings.

From these four cases it appears that IPL might develop between first- and second-stage surgeries, slightly more than one year after supra-constructions are connected (1,5 year after implant installation) as well as up to eleven years after installation, which emphasizes that the aetiology is complex.

The treatment of all four cases included a reduction of the implant length which implies the removal of any infected implant surface. There are on one hand no proofs that a contaminated titanium surface prevents osseointegration but on the other hand there are no unambiguous treatment modalities suggested for infections in the vicinity of titanium surfaces, such as peri-implantitis which altogether justifies this approach. All cases healed but although this case series does not explicitly explain the cause of the infection, it suggests a strategy for successful treatment (15,17).

It is from our cases and results presented in the literature difficult to claim that there is a single cause to IPL. Rather it is evident that the condition might be a sequel of the summation of many possible causes. This summation exceeds the local biological threshold for the individual patient. Such local conditions make in no way the prediction and treatment of these rare lesions easier. They obviously must be tackled from different angels, whereas debridement and removal of the apical part of a stable implant appear appropriate.

This report will also stress the importance of good radiographic quality, that fistulografy can be of help and that radiographs have to cover the apical as well as the marginal part of the implant, which easily can appear as the most important part because of the far more frequent complication - marginal periimplantitis (17).

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Can orthodontic treatment improve mastication?

A controlled, prospective and longitudinal study

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Abstract

◉ *Objectives:* To prospectively and longitudinally evaluate the self-perceived masticatory ability and the tested masticatory efficiency in orthodontically treated and untreated groups.

Design: Prospective observational cohort.

Subjects and methods: Three groups of age matched adolescent girls were included. Sixty-five Class II subjects received orthodontic treatment fixed appliance treatment (Orthodontic group), 58 subjects were orthodontically untreated (Class II group) and 60 subjects had a normal occlusion (Normal group). The self-perceived masticatory ability was assessed on a visual analogue scale while the masticatory efficiency was evaluated with a masticatory efficiency test using round silicon tablets. Registrations were performed at the start and after two years when all subjects in the Orthodontic group had finished orthodontic treatment.

Results: Over the two-year period the self-perceived masticatory ability increased significantly in the Orthodontic group. After treatment, the Orthodontic group perceived their masticatory ability as high as the Normal group did.

The masticatory efficiency increased significantly, during the two years, in all three groups. However, the normal occlusion group presented a significantly better masticatory efficiency than both the Orthodontic group and the Class II group on both registrations.

Conclusions: Orthodontic treatment was beneficial for the self-perceived masticatory ability. The masticatory efficiency increases with age during adolescence. Normal occlusion subjects had a better masticatory efficiency than subjects with orthodontically treated as well as untreated Class II malocclusion.

Key words

Mastication, orthodontic treatment, occlusion

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Förbättrar ortodontisk behandling tuggförmågan? En kontrollerad, prospektiv och longitudinell studie

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Sammanfattning

◎ *Syftet* med studien var att prospektivt och longitudinellt utvärdera tuggfunktionen i ortodontiskt behandlade och obehandlade grupper.

Materialet bestod av tre grupper med åldersmatchade flickor, 11-15 år. Sextiofem flickor med distalbett fick ortodontisk behandling med fast apparatur (ortodontigruppen), 58 flickor med distalbett förblev ortodontiskt obehandlade (distalbettsgruppen) och 60 flickor hade ett normalt bett (normalgruppen). Individernas egen utvärdering av tuggförmåga bedömdes med en visual analog skala medan tuggeffekten utvärderades med ett tuggtest som använde runda silikontabletter. Registreringar utfördes vid studiens start samt efter två år när samtliga individer i ortodontigruppen var ortodontiskt färdigbehandlade.

Resultat: Över de två åren ökade den självbedömda tuggförmågan signifikant i ortodontigruppen som efter två år bedömde sin tuggförmåga i nivå med normalgruppen.

Den testade tuggeffekten ökade under studien i samtliga tre grupper men vid en jämförelse mellan grupperna, visade det sig att normalgruppen tuggade bättre än både ortodontigruppen och distalbettsgruppen vid bägge registreringarna.

Konklusioner: Den ortodontiska behandlingen var fördelaktig för den självbedömda tuggförmågan. Tuggeffekten ökar med åldern under tonåren. Individer med ett normal bett hade en högre tuggeffekt än både ortodontiskt behandlade eller obehandlade distalbett.

Introduction

Mastication is one of the main functions of the stomatognathic system and an important factor and a stimulus for normal craniofacial growth (5, 16). Masticatory efficiency, a subject's capacity to break down food or test materials, is often tested by fractional sieving (1, 2, 6, 7). *Anehus-Pancherz & Pancherz* (2) found that masticatory efficiency increased with age when comparing 10-year-olds with 16-year-olds and concluded that a harmonious interplay between the occluding teeth and the muscles influencing them were important factors. *Gaviao et al.* (9) found a higher masticatory efficiency in subjects with normal occlusion than in subjects with posterior cross-bite and in open bite subjects in the primary dentition. While *English et al.* (7) concluded that malocclusion negatively affects subjects' ability to process and break down foods when comparing normal occlusion subjects with subjects with Class I, II and III malocclusions. *Tzakis et al.* (14) found a reduced masticatory efficiency in adult patients with signs and symptoms of temporomandibular disorders (TMD).

An individual's own self-perceived assessment of the masticatory function was defined as masticatory ability by *Carlsson* (4). The knowledge about the self-perceived masticatory ability in children and adolescents is limited and in addition only a few studies have investigated the masticatory efficiency in children and adolescents.

Since some orthodontic patients can have expectations of an improved stomatognathic function there is a need for prospective and longitudinal studies to increase the knowledge about masticatory efficiency and ability in children and adolescents to be able to compare changes to untreated subjects.

This study is a prospective two-year follow up of the masticatory efficiency and ability in two groups of girls with either Normal occlusion or Class II malocclusion. The present study is also part of a series of studies by *Henrikson et al.* (10-13) evaluating the relationship between orthodontic fixed appliance treatment and TMD. At baseline in this material *Henrikson et al.* (10) found that girls with normal occlusion had a higher masticatory efficiency and ability than subjects with Class II malocclusion. It was also found that few occlusal contacts and a large overjet predicted a reduced masticatory efficiency and that subjects who reported symptoms of TMD had reduced masticatory efficiency.

The aim of the present study was to longitudinally study the masticatory efficiency and the self-perceived masticatory ability in girls with Class II malocclusion, receiving orthodontic fixed appliance treatment, in comparison with subjects with untreated Class II malocclusion and normal occlusion subjects of the same age.

The hypothesis was that an orthodontic treatment of Class II malocclusions aiming at normal occlusion leads to an improved masticatory ability and efficiency.

Material and methods

Subjects

One hundred and eighty three girls, aged 11-15 years at the start of the study, were included. Sixty-five subjects with Class II malocclusion received orthodontic fixed appliance treatment *(Orthodontic group)*; 58 subjects with Class II malocclusion were orthodontically untreated *(Class II group)* and 60 subjects had a normal occlusion *(Normal group)*. The subjects in the Normal and the Class II groups were included by screening school classes attending clinics of the Public Dental Service in the Malmö region (Sweden), while the subjects in the Orthodontic group were consecutively included among those on the waiting-list for orthodontic specialist treatment in the Public Dental Service of Malmö.

Orthodontic group: Sixty-five girls (mean age at start: 12.8 years; SD 1.1) were included in this group. Subjects with an earlier history of orthodontic treatment were excluded. The inclusion criterion was a bilateral or unilateral Class II relationship of at least half a cusp (3).

Class II group: Fifty-eight girls with Class II malocclusion (mean age at start: 12.9 years; SD 1.0) were included in this group. The inclusion criterion was the same as for the orthodontic group, but without any planned orthodontic treatment.

Normal group: Sixty subjects with normal occlusion (mean age at start: 12.7 years; SD 0.7) were included in this group. The inclusion criteria for the Normal group were a bilateral neutral sagittal relationship for molars, premolars and canines and a normal transverse relationship. The overjet and overbite was to be between 1 and 4 mm. Less than 2 mm of crowding or spacing in each jaw and midline discrepancies less than 2 mm were accepted. Subjects with aplasia of teeth were excluded, as were subjects with an earlier history of orthodontic treatment.

Methods

All subjects were examined and all registrations were performed at the beginning of the study and

2 years later. All subjects in the Orthodontic group were out of active treatment at the examination after two years.

Questionnaire
The subjects rated their self-perceived masticatory ability on a visual analogue scale (VAS) 0-100 mm with the end points bad (0 mm) and good (100 mm). Data about preferred chewing side was obtained through a questionnaire.

Test of masticatory efficiency
To quantify the masticatory efficiency, a test developed by *Edlund and Lamm* (6) was used. The test uses round tablets of silicon impression material (Optosil ® Bayer). Briefly, the method involves chewing for twenty strokes of five separate tablets with a standardized weight. Each of the samples of chewed material was fractionated in a system of sieves with coarse, medium and fine meshes. Essentially, the more efficient the mastication, the greater quantity of material will pass through into the finest sieve. The quantity of material is estimated by weight. The masticatory efficiency for a test person can be characterized by the distribution of the test material among the three fractions. A masticatory efficiency value, by proportion of weight, was calculated (6) for each test portion and the mean of the best four values out of five was used as the masticatory efficiency index (MEI). The index ranges from 0-100, the highest number corresponding to the highest efficiency value.

Statistical methods
Differences within the groups between the first and second measurement were calculated as follows; for ordinal data the Wilcoxon´s matched pairs signed rank test, and for numerical variables the pared T-test. Differences between the groups were calculated as follows; for ordinal data the Mann-Whitney rank sum test, and for numerical variables the analysis of variance (ANOVA).

The tested masticatory efficiency index was considered a numerical variable while the self-perceived masticatory ability on a VAS was considered ordinal data. 95% *confidence intervals* (CI) were also calculated when comparing numerical variables. *P*-values below 0.05 were required for the differences to be accepted as statistically significant.

Ethical approval
The study was approved by the Ethical Committee of University of Lund, Sweden (Ref no LU 489-03).

Results

Only 2 subjects out of 183 dropped out during this two year longitudinal investigation. One subject from the Class II group moved away from the region

Table 1. Percentage distribution of occlusal characteristics and the mean overjet and overbite (mm) in the three groups, at the start of the study and after two years.

Morphological occlusion	Class II Orthodontic group		Class II group		Normal group	
	Start (n=65)	2 years (n=64)	Start (n=58)	2 years (n=57)	Start (n=60)	2 years (n=60)
Dental relations						
Sagittal relationship						
Normal	0	94	0	0	100	100
Bilateral Class II	69	3	41	40	0	0
Unilateral Class II	31	3	59	60	0	0
Overjet mean in mm (SD)	6.4 (± 2.3)	2.3 (±1.1)	4.4 (±2.0)	4.2 (±2.0)	2.5 (±1.0)	65 2.4 (±0.9)
Overjet ≥ 6 mm	65	2	22	20	0	0
Vertical relationship						
Overbite mean (mm)	3.1 (±2.1)	1.7 (±0.8)	3.8 (±1.7)	3.7 (±1.3)	3.0(±0.8)	2.8 (±1.1)
Overbite < 0 mm	14	0	7	7	0	0
Overbite 0-4 mm	56	100	52	54	100	98
Overbite > 4 mm	30	0	41	39	0	2
Transverse relationship						
Normal	86	100	79	81	100	100
Cross bite	12	0	16	14	0	0
Scissors bite	2	0	5	5	0	0

and was not able to take part in the two year registration and another subject discontinued the orthodontic treatment and did not want to take part in the second registration. At the first examination the MEI from 2 subjects were missing due to lost data.

Occlusal characteristics at the start and after two years in all three groups are presented in Table 1.

Preferred chewing side

There were no significant differences between the groups concerning preferred chewing side. In the total sample, both at the start and after two years, around 50 % answered that they chewed on both sides, 20% answered right side and 10% left side, while 20% could not answer the question.

Self-perceived masticatory ability

A large individual variation of the masticatory ability was found both within and between the three groups (Figure 1). Over the two-year period the masticatory ability increased significantly in the Orthodontic group (P=0.001) while no statistical changes were found in the other two groups. At the start of the study the Normal group presented a significantly higher masticatory ability than the Class II group (P<0.001) and the Orthodontic group (P<0.001), while no statistical difference was found between the Class II group and the Orthodontic group. After two years there were no statistical differences between any of the groups (Table 2).

Test of the masticatory efficiency

There was also a large individual variation of the MEI, both within and between the groups (Figure 2). The masticatory efficiency increased significantly in all three groups over the two-year period; Normal group (95% CI = 2.1 -6.4; P<0.001), Class II group

Table 2. Mean and median masticatory ability, self evaluated on a visual analogue scale (VAS),at the start of the study and after two years.

	Masticatory ability. Self evaluated on a VAS					
	Start			Two years		
	Mean	Median	S.D	Mean	Median	S.D
Class II Orthodontic group	74.4	72.0	19.0	86.4	93.0	18.7
Class II group (untreated)	77.7	82.0	19.3	83.6	90.0	16.2
Normal group	88.0	97.0	15.7	88.4	94.0	12.1

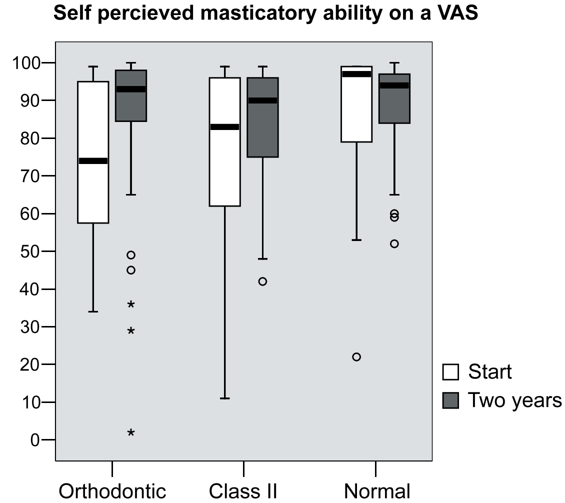

Figure 1. Self-perceived Masticatory Ability in all three groups at start and after two years. Fifty percent of the subjects have values within the box. The bar across the box represents the median. The whiskers show the largest and smallest observed value that is not outlier.
o = outliers, values more than 1.5 box-lengths from the box
* = extremes, values more than 3 box-lengths from the box.

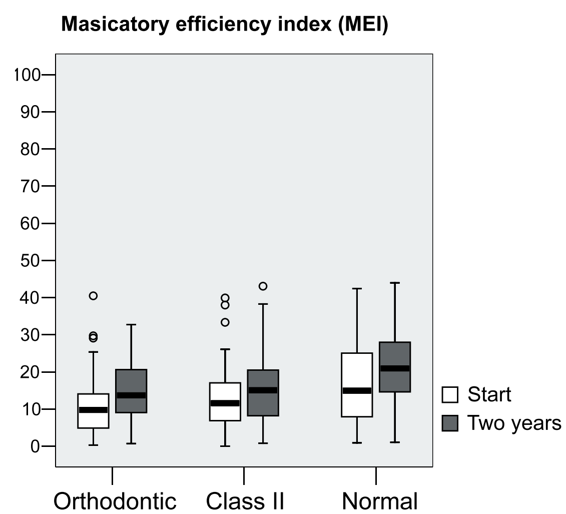

Figure 2. Masticatory efficiency index in all three groups at the start and after two years. Fifty percent of the subjects have values within the box. The bar across the box represents the median. The whiskers show the largest and smallest observed value that is not outlier.
o = outliers, values more than 1.5 box-lengths from the box

(95% CI = 1.7 -6.5; P=0.001) and the Orthodontic group (95% CI 1.9-7.1; P=0.001). At the start of the study the Normal group presented a significantly higher MEI than the Class II group (95% CI = 1.1-8.2; P=0.011) and the Orthodontic group (95% CI = 3.5-10.2; P<0.001), while no statistical difference was found between the Class II group and the Orthodontic group. Also after two years the Normal group presented a significantly higher MEI than the Class II group (95% CI = 1.2-9.1; P=0.011) and the Orthodontic group (95% CI = 2.0-9.7; P<0.001), while no statistical difference was found between the Class II group and the Orthodontic group (Table 3).

Table 3. Mean and median masticatory efficiency index (MEI) at the start of the study and after two years.

	Masticatory efficiency index (MEI)					
	Start			Two years		
	Mean	Median	S.D	Mean	Median	S.D
Class II Orthodontic group	10.3	9.1	8.1	15.2	14.0	8.3
Class II group (untreated)	12.4	11.5	9.0	16.4	14.8	10.7
Normal group	17.1	14.6	10.7	21.5	20.9	10.7

Discussion

The major findings in this longitudinal controlled study were that orthodontic treatment was beneficial for the self-perceived masticatory ability and that subjects with normal occlusion had a better tested masticatory efficiency than both orthodontically treated and untreated Class II malocclusion subjects, both at start and after two years. The hypothesis of the study was confirmed regarding the perceived masticatory ability but had to be rejected regarding tested masticatory efficiency.

An orthodontic treatment can be performed due to a variety of reasons. Except psychosocial and aesthetic reasons some subjects can have expectations of an improved stomatognathic function as a result of the orthodontic treatment. Since mastication is one of the main functions, it seems important to know to what extent these expectations are fulfilled by increasing the knowledge about how and if orthodontic treatment may influence mastication. Both by an objective evaluation by measuring the MEI and subjectively by the self perceived masticatory ability. This was the reason to carry out this controlled, longitudinal and prospective study.

For ethical and practical reasons, it was not possible to randomise subjects with Class II malocclusion into orthodontic treatment and non-treatment groups. The registrations in all three groups were merely a passive observation and the decision whether the subjects were offered orthodontic treatment or not was beyond our control.

Masticatory ability was in general rated high among the subjects in this study. The differences between the groups concerning the tested masticatory efficiency also existed in the self-perceived masticatory ability at the start of the study. However, after two years there were no differences between any of the groups concerning the masticatory ability. In comparison, English et al. (7) found a significantly higher self-perceived masticatory ability in subjects with normal occlusion than in subjects with Class II and Class III malocclusions. Another interesting finding was that the subjects in the Orthodontic group perceived a significant improvement of the masticatory ability after orthodontic treatment.

Our study showed divergent results when comparing the subjective self-perceived masticatory ability versus the objectively tested masticatory efficiency. The reason for this could be that a normal food intake does not require as much muscle force during mastication as when chewing Optosil ® tablets. It possible that morphological craniofacial and occlusal differences are more influential factors when testing with a relatively hard test material compared to the softer normal food intake.

The main requirement of an ideal test material for studying masticatory efficiency with fractional sieving is that the material is pulverized by chewing in such a manner that the degree of pulverization can be clearly established and that the material is unaffected by water and saliva. If this requirement is met, both the fractionating and the laboratory procedure can be simplified. In the present study a silicon material for dental impressions (Optosil ®, Bayer) was chosen, which fulfilled the above mentioned criteria (6).

Masticatory efficiency increased with age in all three groups in this longitudinal study. An increased masticatory efficiency with age in growing subjects has also been presented earlier in cross sectional studies (2, 9). Since both the treated and untreated groups showed a similar increase in masticatory efficiency, it is likely that the increase in the orthodontic group was more due to the general development and growth of the masticatory system than the orthodontic treatment per se.

Recent studies examining masticatory efficiency, using similar methods that were used in the present study, have shown that different kind of malocclusions negatively affects an individuals´ masticatory efficiency (7, 9). Their findings are in accordance with our findings.

Both at the start and after two years the Normal group had a higher MEI than the Class II group and the Orthodontic group. In Class II:1 subjects, *Anehus-Pancherz and Pancherz* (2) found an increased masticatory efficiency after activator treatment. They could, however, not conclude if it was the activator treatment per se that resulted in an increased masticatory efficiency or if it was an effect of the general growth and development in the masticatory system over time. At start in the present study, *Henrikson et al.* (10) found that subjects with an overjet ≥ 6 mm had a reduced masticatory efficiency. This finding was also confirmed in a multivariate analysis where the overjet was one out of two occlusal factors that significantly explained the variation in masticatory efficiency (10). An interesting finding in the present longitudinal study was that even if the overjet was normalized in the Orthodontic group the masticatory efficiency was not equal to that in the Normal group after two years. It is therefore possible that the different skeletal morphology and muscle strength in Normal occlusion subjects compared to Class II subjects play a larger role than occlusal variables when explaining differences in masticatory efficiency. This explanation seems to be in agreement with *van den Braber et al.* (15) who did not find an improvement in masticatory efficiency when evaluating masticatory efficiency in retrognathic patients before and after mandibular advancement surgery. *Garcia-Morales et al.* (8) found that children with greater hyper divergence (high angle) show a theoretically lower maximum bite force. Their findings pointed out the importance of vertical facial components regarding the function of the masticatory muscles and indirectly thereby on the masticatory efficiency.

From the results in this study it was concluded that:

- Orthodontic treatment was beneficial for the self-perceived masticatory ability.
- The masticatory efficiency increases with age during adolescence.
- The increased masticatory efficiency during the orthodontic treatment was probably more due to the general development and growth of the masticatory system than to the orthodontic treat ment per se.
- Normal occlusion subjects had a better masti catory efficiency than subjects with orthodonti cally treated as well as untreated Class II maloc clusion.

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Comparisons of similar patients treated by general dental clinicians and orthodontic specialists

Outcome and economical considerations

Björn Laag [1], Christer Ström [2]

Abstract

◎ The objective of this study was to evaluate and compare orthodontic treatment in two groups of patients in regard to treatment results and costs. One group was treated at a General Dental Clinic (GDC) with removable appliances and the other at a Special Orthodontic Clinic (SOC) using fixed appliances. Both groups had similar malocclusions. All treatment plans were determined by the same orthodontic specialist. Study models were taken before and after the treatment of the patients. Index of Orthodontic Treatment Need (IOTN- index) was used to determine the extent of treatment needed.

Weigthed Peer Assessment Rating (WPAR) was calculated for every model. The percentage of improvement in each group was calculated and results were compared. Chair time and treatment costs extracted from patient records were registered.

The group treated at the GDC had initially WPAR 22.2 and the percentage reduction in WPAR 69 was percent. The group from the SOC had initially WPAR 24.0 and was reduced by 81 percent. Treatment costs, with the exception of x-ray analyses, were 56 percent higher for the SOC. The results of the study indicated that it was economically advantageous to treat patients with removable appliances at a GDC, if the patients are sufficiently cooperative.

Key words

Orthodontic treatment, removable appliances, occlusal indices, dental economics, cost benefit analysis.

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Behandlingsutfall och ekonomiska aspekter på ortodontipatienter behandlade av allmäntandläkare eller specialist

Björn Laag, Christer Ström

Sammanfattning

◎ Syftet med denna undersökning var att jämföra två patientgrupper som behandlats ortodontiskt för liknande bettfel dels på en allmäntandläkarklinik (ATK) med avtagbara tandställningar, och dels på en specialistklinik (SPK) där det användes fastsittande apparatur. Behandlingsgruppernas resultat, behandlingstid och kostnad jämfördes. Behandlingsplanerna bestämdes av samma ortodontist, som även kontrollerade patienterna med jämna mellanrum. Studiemodeller togs före och efter behandlingen. Index of Orthodontic Treatment Need (IOTN – index) användes för att beskriva bettavvikelsens svårighetsgrad.

Weigthed Peer Assessment Rating (WPAR – index) beräknades för varje modell. Den procentuella förbättringen beräknades och gruppernas resultat jämfördes. Behandlingstiden och kostnaderna beräknades med hjälp av patientjournalerna.

Patientgruppen som fick sin behandling på ATK hade ett WPAR- värde på 22.2 från början och detta reducerades med 69 procent. Gruppen på SPK hade ursprungligen ett värde på 24.0 och detta reducerades med 81 procent. Behandlingskostnaderna, förutom kostnaderna för röntgen, var 56 procent högre på SPK. Det synes som om det är ekonomiskt fördelaktigt att behandla ortodontipatienter på hemmakliniken under övervakning om patienterna koopererar tillfredsställande.

Introduction

Orthodontic treatment for children and youths is very common in the Swedish Public Dental Health Service. It has been estimated that approximately 25 percent of all children need some orthodontic treatment to achieve acceptable alignment of their teeth both functionally and aesthetically. The extent of treatment required varies considerably, but it has been estimated that approximately 50 percent of these patients should be treated by a dentist specialised within orthodontics (2).

There are two major types of orthodontic devices for treating malocclusions. These are either fixed or removable appliances. The choice of device depends on the magnitude of the malocclusion and how the teeth should be moved orthodontically. Removable appliances are the first choice for minor problems. They are quite simple to install, but require the patient´s interest and cooperation. From a study in Gothenburg on Andresen activators it was stated that activators provided completely satisfactory results in only 33 percent of the cases. The fixed appliance Quad-Helix, used to correct cross-bites, produced satisfactory results in 97 percent of cases (1). Other studies regarding cross-bites treated with Quad-Helix or expanding plates produced nearly the same results, but the Quad-Helix gave 40 percent faster results (3) and was economically favourable (10). Similar results were also stated in a study in Malmoe regarding treatment time, and treatment results were more successful compared with removable plates (8). Another study when treating enlarged overjet showed that the overall costs were 17 percent more expensive using fixed appliances. (6)

It is important to diagnose and plan treatment of malocclusion carefully in order to choose the appropriate type of appliance. Any treatment with removable appliances should produce acceptable results if the cooperation is sufficient. To start treatment with a removable appliance and then continue with a fixed one is usually not economically. A study of patients who have had removable appliances showed it necessary to finish the cases with fixed appliances in 33 percent due poor patient cooperation (13).

Orthodontic treatment often requires considerable time with many return visits. As the district included within the responsibility of the Special Orthodontic Clinic (SOC) in Borås is extensive, there was an interest in receiving treatment at the local General Dental Clinic (GDC) to minimise long journeys, although treatment might be more time-consuming and results less satisfactory (12, 15). Besides, the waiting-time for fixed appliances was very long.

The results of orthodontic treatment have, during the last years, often been expressed in terms of a percental PAR- index reduction. This is not a perfect but generally accepted method (4, 5, 15, 16). PAR (Peer Assessment Rating) is a value that expresses the degree of deviation from a normal occlusion. The study models of the patients are measured with regard to the occlusion and the gap between the teeth on each jaw. The magnitudes of these measurements vary from 1 to 4 and are subsequently summed. Weighted PAR (WPAR) means that some deviations measured received a greater value. Earlier studies have shown improvements of 50 – 61 percent when you are using removable and minor fixed appliances (12, 13). The results of the orthodontic treatment are considered *greatly improved* when the WPAR scores are reduced by 70 percent. 30 percent reduction of treatment is considered to be *improved* (9, 12).

In order to determine the treatment need the IOTN-index (Index of Orthodontic Treatment Need) was used on the study models. This entailed patients being placed in one of five groups, where index 5 is *great need for treatment* and index 1 is *little or no need for treatment* (11, 14).

Discussions concerning at which level the orthodontic treatments are to be performed in order to obtain maximal benefit to the lowest costs are frequent (13, 16). Some orthodontists are convinced that the experienced general dentist may – under specialist supervision – obtain sufficiently qualitative results to a reasonable cost. Other orthodontists argue that it is more cost-effective to perform all orthodontic treatment at the specialist clinic (2, 16). The hypothesis in the present study is that trained general dentists are obtaining sufficiently qualitative results.

The purpose of this retrospective study was to see how successful the results of the treatments (expressed in PAR-Index reduction and IOTN changes) were at a GDC under the supervision of a specialised orthodontist only using removable appliances, and to compare the results and the economical aspects to patients treated at a SOC.

Materials and Methods

The test group in this investigation included all patients (n = 31, Table 1) who had orthodontic treatment completed at one of two smaller GDCs south of Borås. All treatments were planned and supervised by the same orthodontist. The patients were recruited during a period of two school-terms, and four general dentists responsible for treatment considered that they were able to cooperate adequately. In 52% of the cases crowding was the main diagnosis, 42% had increased overjet, and 6% had lateral cross-bite. Seventy- four percent of the patients were treated with removable plates in the upper jaw, and the rest of the patients were treated with Andresen activators. Prerequisites were study models of sufficient quality before and after treatment and obtainable high quality records. Some patients were excluded due to too many deciduous teeth at treatment start. Only these removable appliances were used, and final study models were taken several months after treatment completion to expose possible relapses. The start and final study models were used for calculations according to the PAR index and improvements were calculated by percent. In addition the IOTN groupings were performed on the models. All calculations were made by two orthodontists, and the mean values are shown in the Tables. From the records the number of appointments could be obtained as well as the length of the active treatment. Consequently chair time could be approximated. There was no need for additional treatment including fixed appliances for any patient.

A control group (n = 19) of patients who had finished their orthodontic treatment at the SOC in Borås at the same time as the test group were also calibrated similarly to the test group patients. The required age for patients in the group was below 15 years at the start of treatment, and the diagnoses similar to that of the test group. In 63% of the cases crowding was the main diagnosis, 21% had increased overjet, and 16% had dental displacement. The control patients were treated with fixed appliances in the upper jaw. Only three patients had minor fixed appliances in the lower jaw. Furthermore, orthodontic tooth extraction was noted in both groups (Table 1).

Economic calculations based on an hourly fee determined by the Dental Office of the Region in 2006 were used. In the GDC the fee was 1492 SEK/hour and in the SOC 1922 SEK/hour. Costs of necessary laboratory equipments were included. X-ray costs were not included.

The non-parametric Kruskal-Wallis test was used to compare the PAR-reduction between the test group and the control group.

Results

The number of patients including age and gender is shown in Table 1 where it is also stated whether extractions were included in the treatments. A significant difference in mean age was found between the groups (p=0.0001). The WPAR- results before and after treatment, number of visits and total treatment chair time is shown in Table 2. This table also includes the number of orthodontic specialist consultations for the test group.

The test group had an average WPAR value of 22.2 ± 7.1 (range 10-37) before treatment. After treatment the WPAR value was 7.2 ± 3.7 (range 2-16). The improvement was thus 69.0 ± 10.6 percent (range 41-89%). The control group had an originally WPAR-value of 24.0 ± 6.5 (range 9-37), and after treatment 4.6 ± 2.2 (range 1-10). WPAR was reduced by 81.4 ± 7.1 percent (range 66-90%). Before treatment no significant difference was found in WPAR-values between groups (p=0.37). After treatment a significant difference in WPAR was found between the groups (p=0.002). The difference in percentage im-

Table 1. Number of patients and gender in the groups. The test group was treated in a General Dental Clinic and the control group was treated in Orthodontic Specialist Clinic.

	Girls	Boys	Age Years	Range Years	Extraction n=20
Test group	20	11	11.4+1.6	8.2 – 14.2	8
Control group	16	3	13.1+0.9	11.1 -14.2	12

Table 2. Peer Assessment Rating (PAR) before and after treatment.

	Patients	PAR		Visits	Time	Consultations/pat
		before treatment	after treatment	n	(min)	n
Test group	31	22.2	7.2	11.3	97.3	2.8
Control group	19	24.0	4.6	18.5	172	

Table 3. Classification according to IOTN, before and after treatment.

	IOTN 1	IOTN 2	IOTN 3	IOTN 4	IOTN 5
Test group					
Before treatment			2	19	10
After treatment	8	19	4		
Control group					
Before treatment		2	17		
After treatment	9	10			

provement between the test and the control group was significant ($p<0.001$).

The calculated IOTN values of the groups before and after treatment are shown in Table 3. The patients were divided into IOTN classes. Before treatment 32 percent of the test group was classified as IOTN 5 *(great need for treatment)*, 61 percent IOTN 4, and 6 percent belonged to IOTN 3 *(moderate need for treatment)*. After treatment, 61 percent of the cases were classified as IOTN 2 *(minor need)* and 26 percent IOTN 1 *(no need for treatment)*. 13 percent of the patients still had a moderate need of treatment (IOTN 3).

Patients in the control group were classified as IOTN 4 in 89.5 percent of the cases and the remainder in IOTN 3 before treatment. After treatment 47 percent were classified as IOTN 2 and the remaining patients to IOTN 1.

The patients in the control group had an average chair time of 173 minutes and the test group 97 minutes, which is a significant difference ($p<0.0001$). Time for planning therapy, X-ray examinations and other administrative time were not included. The time for orthodontic consultation of the treating orthodontist was also calculated for the test group. The total cost of an average treatment in the test group was 3525 SEK and for the control group 5510 SEK. Treatment in the SOC can thus be expressed as 56 percent more expensive than at the GDO.

Another result of this study was that patients with extractions as a part of treatment (n=20) had a WPAR improvement of 72 percent. The non-extraction patients reached an improvement of 64 percent. The difference was significant ($p<0.001$).

Discussion

In this study patients having had orthodontic treatment at a GDC were compared to similar patients treated at a SOC. The study was not optimally designed, since it was not prospective. Furthermore, the number of not-sufficient cooperative patients was not registered. More patients in both groups had been preferable but all treatment details were planned and decided by the same orthodontist (BL). Thus, the design reflected the specific circumstances locally where one orthodontist had full responsibility for many children in a large geographic area and delegation of treatments to non-specialists was mandatory.

The two groups differed in age and gender. The age differences could be explained by the fact that all control patients were taken from a waiting list to the SOC. It was unfortunate that no patient of the control group was referred to IOTN 5, but limitations in age and diagnosis was the cause of this. Both groups consisted mainly of girls, common for orthodontic patients in the Borås district and in Sweden (4).

The calculated WPAR values of the patients before treatment were 22.2 in the test group and 24.0 in the control group. Overjet produced high values in WPAR, which is one explanation for the minimal difference between the groups. The IOTN – "value" was higher in the test group but the WPAR was lower so there did not seem to be a strong connection between a high IOTN –"value" and high WPAR.

Using different removable appliances can be often successful to treat an enlarged overjet. According to the literature the average WPAR reduction after orthodontic treatment varies between 30 – 60 percent when using removable appliances (9). The test group in this study had a WPAR-reduction calculated to 69 percent, the non-cooperative patients excluded. We have no figures of the ratio non-cooperative/cooperative patients.

The exact time required for treatment was difficult to estimate. The time estimated in this study does not include the length of examination, the necessary X-rays and analysis. The treatment costs for the control group may be higher, especially since an X-ray analysis tends to be more extensive at the SOC. The patients often had to travel longer distances to the SOC than to the GDC.

It seems that treatment becomes more expensive at the SOC. The PAR reduction was significantly better, but the difference was minor. Improvement for the control patients was 81 percent, compared to 69 percent for the test group patients. The improvements of patients treated at the SOC were on the same level as in other studies in Sweden and Norway (4, 7). The patients from these Clinics usually had a longer retention period, while the patients treated at a GDC might have had less supervision as they changed dentists when transferred to other schools. Perhaps this could lead to the difference becoming more pronounced for the patients over time. As the limit for "greatly improved" was set at 70 percent this implies that treatment has been favourable for both groups.

The results of this study are somewhat uncertain due to the difficulties of estimating exact chair time. Different dentists were involved and approximation of the duration for each visit according to the records of the patient could be difficult. All dentists participating in the study worked differently even with many years of experience. The level of cooperation also differed between patients.

However the results of this study indicate an economical advantage in treating patients with removable appliances in GDC, but there are some doubts. When patients are non-cooperative a non-favorable increase of treatment time may occur. Furthermore, certain orthodontic movements can only be performed using fixed appliances. Economically it is unfavorable to start with removable appliances and then change to fixed due to unsatisfactory cooperation. If early treatment is needed, the number of permanent teeth can, however, make this approach necessary.

An additional study of how many of a group of patients treated with removable appliances that shows sufficient cooperation would be interesting. The dentists of the GDC:s involved estimated the poorly cooperative patients to 15-20 percent.

Conclusion

The hypothesis that trained general dentists may obtain sufficiently qualitative results to a reasonable cost was confirmed. Patients with high levels of WPAR and IOTN can be successfully treated with removable appliances at a General Dental Clinic if the patient is sufficiently cooperative. The results tend to be better at the Special Orthodontic Clinic, but more expensive than at the General Dental Clinic.

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Elemental composition of normal primary tooth enamel analyzed with XRMA and SIMS

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Abstract

© There is an interest to analyze the chemical composition of enamel in teeth from patients with different developmental disorders or syndromes and evaluate possible differences compared to normal composition. For this purpose, it is essential to have reference material. The aim of this study was to, by means of X-ray micro analyses (XRMA) and secondary ion mass spectrometry (SIMS), present concentration gradients for C, O, P and Ca and F, Na, Mg, CI, K and Sr in normal enamel of primary teeth from healthy individuals.

36 exfoliated primary teeth from 36 healthy children were collected, sectioned, and analyzed in the enamel and dentin with X-ray micro analyses for the content of C, O, P and Ca and F, Na Mg CI, K and Sr.

This study has supplied reference data for C, O, P and Ca in enamel in primary teeth from healthy subjects. No statistically significant differences in the elemental composition were found between incisors and molars. The ratio Ca/P is in concordance with other studies. Some elements have shown statistically significant differences between different levels of measurement. These results may be used as reference values for research on the chemical composition of enamel and dentin in primary teeth from patients with different conditions and/or syndromes.

Key words

Elemental analysis, primary teeth, mineral composition, XRMA.

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Ämnessammansättning av normal primärtandsemalj analyserade med XRMA och SIMS

Nina Sabel, WolframDietz, Ted Lundgren, Sandor Nietzsche, Hans Odelius, Marianne Rythén, Sara Rizell, Agneta Robertson, Jörgen G. Norén, Gunilla Klingberg

Sammanfattning

◎ Det finns ett intresse av att analysera den kemiska sammansättningen i tänder från patienter med olika utvecklingsstörningar och syndrom för att utvärdera möjliga skillnader mot den normala sammansättningen. Därför är det viktigt att ha ett referensmaterial att jämför mot.

Syftet med denna studie är att med hjälp av röntgenmikroanalys (XRMA) och analyserande jonsond (SIMS) presentera koncentrationsgradienter för C, O, P och Ca respektive F, Na, Mg, Cl, K och Sr i normal emalj i primära tänder från friska individer.

36 exfolierade primära tänder från 36 friska barn insamlades och snittades, emaljen analyserades med XRMA och SIMS på 5 olika nivåer från emaljytan till emalj-dentin gränsen.

Denna studie uppvisar referensdata för C, O, P, Ca och F, Na, Mg, Cl, K och Sr i primära tänders emalj från friska barn. Inga statistiskt signifikanta skillnader förelåg i ämnessammansättningen när värden för incisiver och molarer jämfördes. Förhållandet Ca/P överensstämde med vad man funnit i andra studier. Vissa ämnen uppvisade statistiskt signifikanta skillnader mellan olika mätnivåer i emaljen. De presenterade värdena kan användas som referensvärden i forskning som rör den kemiska sammansättningen av emalj i primära tänder från patienter med olika tillstånd och/eller syndrom.

Introduction

Primary teeth have long been regarded as markers for events occurring during their mineralization, especially for enamel (18, 19, 10). Relationships have been established between developmental disturbances in enamel, as well as in the dentin, and a number of different conditions occurring during the neonatal period and the first year of life (12, 20). Some studies have also shown differences in the chemical composition between primary control teeth and primary teeth from children with different disorders (9). However, the number of teeth analyzed in these studies has often been limited.

The special properties of the dental hard tissues being hard and brittle, combined with their non-electric transmitting properties, have made it difficult to study the chemical composition related to the morphology. This is of relevance when studying possible differences between dental hard tissue from normal healthy individuals and from individuals suffering from, for example, perinatal disorders. Different analysis methods have been used such as microradiography, secondary ion mass spectrometry (SIMS), X-ray micro analysis and X-ray microtomography (16, 14, 5, 24, 7). These methods have, despite being time-consuming and expensive, proven to be suitable for elemental analyses of dental hard tissues. However, there is a need for basic data related to known morphological structures and levels from normal primary teeth of healthy individuals. It is known that the chemical composition and the density of enamel vary from the enamel-dentin-junction towards the surface and also from the incisal-cuspal area to the cervical area (21, 22, 12).

Of special interest is the content of some of the major elements (i.e., C, O, P and Ca) as well as other elements of importance for the mineralization of dental hard tissue. Further, there is an interest to analyze the chemical composition of enamel in teeth from patients with different developmental disorders or syndromes and evaluate possible differences from normal composition (20, 7). For this purpose, it is essential to have reference material. However, there are few studies dealing with the content of some elements in enamel and dentin in human primary teeth (24, 7, 8). Therefore, there is a need for presenting the chemical composition from a material of primary teeth from healthy individuals.

The aim of this study was to, by means of X-ray micro analyses (XRMA), present the values in weight % for C, O, P, Ca in normal enamel of primary teeth from healthy individuals, and by secondary ion mass spectrometry present values in weight % for F, Na, Mg, Cl, K and Sr in normal enamel from healthy individuals.

Material and methods

Tooth material

49 exfoliated primary teeth (40 incisors, 9 molars) from 49 healthy children were collected. 36 teeth (27 incisors, 9 molars) were used for the XRMA analyses and 13 incisors for the SIMS analyses. The study was approved by the Regional Ethical Review Board of the University of Gothenburg. After 24 hours in 70% ethanol, the teeth were embedded in an epoxy-resin (Epofix®, Electron Microscopy Sciences, Fort Washington, PA USA) and sagittal longitudinal sections, with a thickness of approximately 100 µm were prepared in an Leitz Low Speed Saw Microtome (Leitz®, Wetzlar, Germany). Central sections were used for the histological examination in a polarized light microscope (POLMI). All sections were examined in polarized light dry in an Olympus polarizing microscope employing strain free objectives.

XRMA

After POLMI-analysis the 36 samples were mounted on sample holders for XRMA with carbon tape. The sections were etched for 30 seconds with 30% phosphoric acid, carefully rinsed with de-ionized water, and coated with a 15-20 nm thick carbon coating by vacuum-evaporation.

The sections were analyzed in the enamel for the composition of C, Ca, P, and O in a Philips SEM 515 (Philips, Eindhoven, The Netherlands), equipped with an EDAX DX-4 ECON detector (EDAX Inc.,

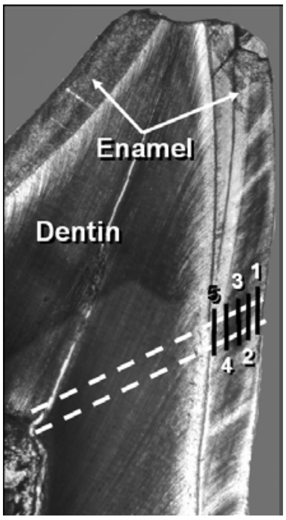

Figure 1. Photo of an un-decalcified section of a primary incisor showing the two principle lines for measurement in enamel for the five levels of the XRMA and SIMS analyses. (Black lines crossing the two white lines represent the 5 levels for measurements in the enamel, numbered 1-5).

Mahwah, USA). The measurements were performed along a line at five levels in the enamel followed by a repeat of the elemental analyses adjacent and parallel to the first line (Fig. 1). In the enamel the first of the two repeats of measurements were made 10 µm under the enamel surface and followed by measurements located ¼, ½, ¾ of the enamel thickness and 10 µm above the enamel-dentine junction (EDJ). All measurements were carried out on the buccal side of the tooth, always aiming to analyzing the morphologically same area, starting approximately at half of the crown height on a line towards the tip of the pulp. For all measurements, the emitted X-rays were detected during continuously fast scanning small window of 6.1 µm x 4.3 µm at a magnification of 650 x. The relative amounts of C, Ca, P, and O were obtained by semi-quantitative analysis of the EDAX DX-4 software. All values were considered semi-quantitative.

The proportion of the elements in enamel was calculated as the mean of each element throughout enamel, related to the means of the other elements, by finding the lowest common denominator.

SIMS analyses

In order to avoid build-up of surface charge during the analysis, the 13 specimens were coated with a thin layer of gold by vapour deposition, after being mounted in a sample holder for the ion probe.

The analysis was performed using an ion probe (Cameca IMS 3 F). The principles for the ion probe have been described in detail elsewhere, therefore only a brief outline will be given here (11). The surfaces of the specimens were bombarded with negative oxygen ions. Secondary positive ions were collected from the center of the bombarded area. The secondary ions then passed a magnetic analyser and separated according to mass/charge ratio. The counts of the different ion species were then analysed by means of an on-line computer. The diameter of the primary ion beam was 50 µm and the diameter of the analyzing area 10 µm. A continuous line scan was made from the enamel surface till the dentin was reached.

The following ion species were analyzed: F, Na, Mg, Cl, K and Sr. All raw data were normalized to the reference isotope of calcium (Ca44). The SIMS analysis was performed as a line scan starting at the enamel surface with 5 µm steps over the enamel-dentin junction. In order to have comparable data with the XRMA analyses, values from the same levels were extracted.

The proportion of the elements in enamel was calculated as the mean of each element throughout enamel, related to the means of the other elements, by finding the lowest common denominator.

Statistical analysis

The statistical analysis was performed using SPSS version 15.0 for calculating the statistical summaries and applying the Mann Whitney U-test, with results considered significant at $p<0.05$. The charts shown are based on the median values.

Results

POLMI examination

A short description will be given here of the POLMI examination. When examined in polarized light, the presence of a neonatal line made it possible to distinguish between prenatal and postnatal enamel (Fig. 2). The neonatal line appeared as a distinct, positively birefringent band extending from the enamel-dentine junction in the most cervical part, towards the enamel surface at the incisal part of the teeth. All sections showed a negatively birefringent, (less porous) zone at the surface suggesting a more mineralized surface. Prenatal primary enamel appeared with a positively birefringent inner zone indicating a higher degree of porosity in the tissue. In 30 of the 49 sections, the micro-porous zone of the prenatal enamel extended over the neonatal line, thus giving part of the postnatal enamel a positive birefringence. In the remaining teeth, the postnatal enamel appeared negatively birefringent.

XRMA analyses

A total of 180 measurements were made in the ena-

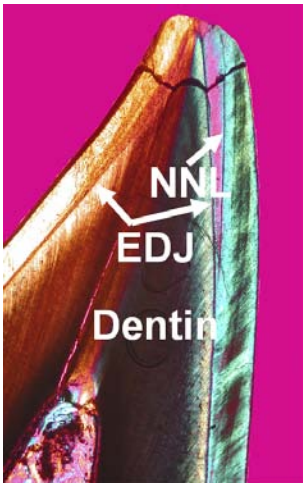

Figure 2. Un-decalcified section of a primary incisor seen dry in air in polarized light (EDJ=enamel dentin junction; NNL=neonatal line; original magnification 40x).

mel. Two measurements were made on each level (Fig 1.) and the mean value was calculated and used for the statistical analyses. When "values" are referred to in the following text, it is the mean of the two repeated measurements on line 1 and 2. The ratios for Ca/P and Ca/C were calculated and mean values, standard deviations and median values were calculated for all elements and ratios for each level in the enamel.

A statistical analysis using the Mann-Whitney U-test for medians was made in order to investigate possible differences between the values for molars and incisors. However, no statistical differences were found wherefore all 36 teeth were used for the analyses.

C, O, P, Ca

Carbon had its highest value at the surface with a slight decrease towards the bulk of the enamel and then with an increase towards the EDJ. Carbon was also found to have the largest standard deviation (Fig. 3; Table 1). Oxygen showed an increasing concentration gradient from the enamel surface to the EDJ. Both P and Ca had flat curves in the bulk of the enamel (Fig. 3; Table 1).
When comparing the median values for oxygen between the different levels, the value at the surface was significantly lower compared with level 4 and 5. There was also a difference between levels 2 and 5 (Table 2.). For phosphorous, the values in the first 4 levels were significantly higher than in level 5 (Table 2).

The proportion of the elements C:O:P:Ca in the enamel, calculated from the mean of the values throughout the entire enamel was 1.00:4.44:2.04:4.15.

Table 1. Mean values in weight %, standard deviations (SD) and range for carbon (C), oxygen (O), phosphorous (P), calcium (Ca) and the ratios Ca/P and Ca/C for the 5 levels in enamel and dentin, respectively. (Level 1 in enamel closest to the enamel surface, level 5 closest to the enamel-dentin junction; level 1 in dentin closest to the enamel-dentin junction, level 5 closest to the pulp.)

Level	C			O			P			Ca			Ca/P			Ca/C		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
1	10.88	5.12	5.02 -30.89	37.21	2.38	28.5 - 40.88	17.31	1.14	14.07 -18.79	37.40	3.68	24.75 -41.90	2.00	0.13	1.76 -2.28	3.92	1.83	0.85 -8.17
2	9.31	3.41	4.46 -20.02	37.63	2.21	33.26 -43.13	17.60	1.01	13.50 -19.24	35.45	3.52	23.36 -43.2	2.01	0.13	1.73 -2.35	4.45	1.87	1.17 -9.88
3	9.50	3.78	4.72 -21.05	37.63	2.02	32.67 -41.38	17.53	1.00	14.19 -19.16	35.03	3.19	25.41 -40.45	2.00	0.12	1.75 -2.27	4.35	1.81	1.35 -8.58
4	9.40	3.26	4.82 -19.19	38.55	1.94	34.46 -41.82	17.36	1.94	34.46 -41.82	34.69	2.95	24.99 -39.84	2.00	0.11	1.78 -2.24	4.24	1.74	1.30 -8.27
5	10.35	4.40	4.80 -23.78	38.98	2.52	33.74 -45.14	17.00	0.89	14.53 -18.78	33.82	3.06	26.13 -39.50	1.99	0.12	1.76 -2.20	3.94	1.80	1.13 -8.23

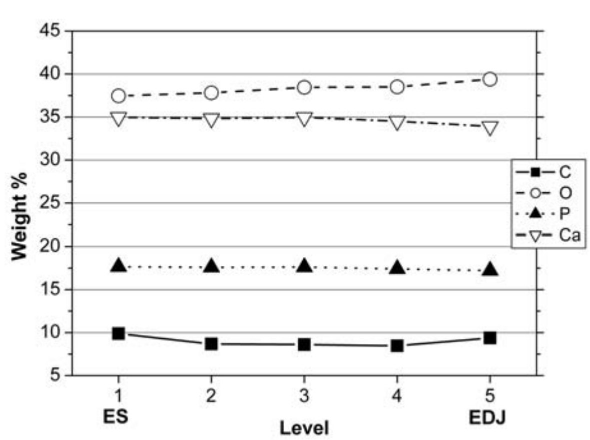

Figure 3.
Chart for the median values in weight % for C, O, P and Ca in the enamel for the five levels of the XRMA measurements (ES=enamel surface; EDJ=enamel dentin junction).

This could be a simple way to describe the relations between these four elements and might be useful when comparing enamel from healthy individuals with enamel from individuals suffering from different diseases or syndromes.

Ca/P, Ca/C
The ratio Ca/P had a flat concentration gradient through the enamel with small values for SD (Fig. 4; Table 1). The ratio Ca/C had its lowest values at the enamel surface and close to the EDJ (Fig. 4; Table 1). After a peak at level 2 the values decreased. The standard deviations where high in all levels (Table 1).

SIMS analyses
All mean values, standard deviations and ranges for the SIMS analyses are given in Table 3.

Fluorine, chlorine
Fluorine had its highest value at the level closest to the surface followed by a rapid decrease which remained flat through the bulk of the enamel (Fig. 8). The fluorine level was significantly higher at the surface compared with all other levels (Table 4). Chlorine had its highest value at the enamel surface and then decreased towards the EDJ (Fig. 8). The chlorine level was significantly higher at the surface level compared with all other levels, and at level 2 and 3 compared with level 5, closest to the EDJ (Table 4).

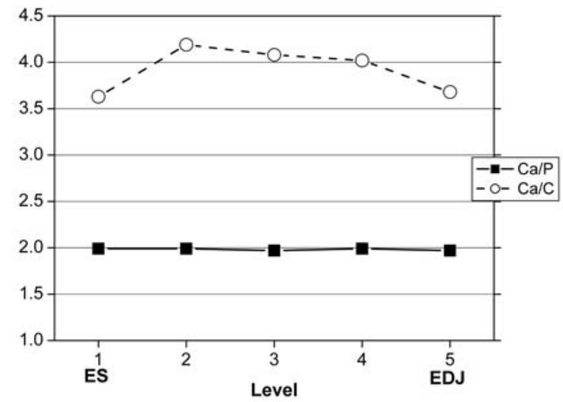

Figure 4.
Chart for the median values for Ca/P and Ca/C ratios in the enamel for the five levels of the XRMA measurements (ES=enamel surface; EDJ=enamel dentin junction).

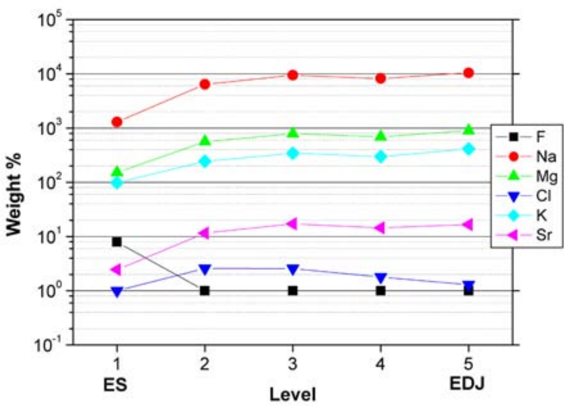

Figure 5.
Chart for the median values in weight % for F, Na, Mg, Cl, K and Sr in the enamel for the five levels of the SIMS measurements (ES=enamel surface; EDJ=enamel dentin junction).

Table 2. Analyses of the median values between different levels in the enamel and dentin, respectively. Statistically significant differences, calculated with Mann-Whitney are given in the table; *=p<0.05; **=p<0.01; ***=p<0.001. (DL= two levels compared; e.g. DL 1-3 in enamel: outer enamel compared with middle part of enamel). (Level 1 in enamel closest to the enamel surface, level 5 closest to the enamel-dentin junction; level 1 en dentin closest to the enamel-dentin junction, level 5 closest to the pulp.)

DL	C	O	P	Ca	Ca/P	Ca/C
1-2	9.88-8.67	37.46-37.81	17.65-17.59	34.97-34.83	1.99-1.99	3.63-4.19
1-3	9.88-8.60	37.46-38.45	17.65-17.60	34.97-34.96	1.99-1.97	3.63-4.08
1-4	9.88-8.46	37.45-38.45*	17.65-17.40	34.97-34.52	1.99-1.99	3.63-4.02
1-5	9.88-9.38	37.45-39.40**	17.65-17.21*	34.97-33.91	1.99-1.97	3.63-3.68
2-3	8.67-8.60	37.81-38.45	17.59-17.60	34.83-34.96	1.99-1.97	4.19-4.08
2-4	8.67-8.46	37.81-38.45	17.59-17.40	34.83-34.52	1.99-1.99	4.19-4.02
2-5	8.67-9.38	37.81-39.40*	17.59-17.21**	34.83-33.91	1.99-1.97	4.19-3.68
3-4	8.60-8.46	38.45-38.45	17.6-17.40	34.96-34.52	1.97-1.99	4.08-4.02
3-5	8.60-9.38	38.45-39.40	17.62-17.21**	34.96-33.91	1.97-1.97	4.08-3.68
4-5	8.46-9.38	38.50-39.40	17.40-17.21*	34.52-33.91	1.99-1.97	4.02-3.68

Table 3 Mean values in weight %, standard deviations (SD) and range for fluoride (F), sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K) and strontium (Sr) for the 5 levels in normal enamel of primary teeth. The F values for level 1 is within brackets due to large individual variations depending on posteruptive exposure of fluorine. (Level 1 in enamel closest to the enamel surface, level 5 closest to the enamel-dentin junction.)

Level	F			Na			Mg			Cl			K			Sr		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
1	(0.0115)	0.0241	(0,000205 -0,089867)	1.8727	0.6906	1.0602- 3.2285	0.2201	0.2700	0.0731 -1.0526	0.0014	0.0011	0.0003 -0.0033	0.1428	0.1577	0.0347 -0.5704	0.0036	0.0008	0.0024 -0.0051
2	0.0003	0.0005	0,000082 -0,001996	2.1694	0.5893	1.2225 -3.0871	0.1893	0.0943	0.0921 -0.4304	0.0009	0.0006	0.0002 -0.0019	0.0820	0.0251	0.0358 -0.1212	0.0039	0.0012	0.0027 -0.0068
3	0.0002	0.0003	0,000082 -0,001326	2.3246	0.4220	1.5389 -3.1615	0.1934	0.0788	0.0976 -0.3755	0.0006	0.0003	0.0002 -0.0012	0.0848	0.0213	0.0444 -0.1075	0.0042	0.0016	0.0025 -0.0086
4	0.0003	0.0003	0,000073 -0,000848	2.4527	0.2609	2.0454 -2.9381	0.2054	0.0785	0.1044 -0.3497	0.0005	0.0003	0.0001 -0.0010	0.0892	0.0216	0.0587 -0.1312	0.0043	0.0014	0.0027 -0.0081
5	0.0002	0.0002	0,000068 -0,000683	2.5077	0.4538	1.6026 -3.2047	0.2154	0.0816	0.1175 -0.3477	0.0003	0.0001	0.0001 -0.0005	0.0991	0.0266	0.0604 -0.1369	0.0040	0.0008	0.0028 -0.0055

Table 4. Analyses of the median values between different levels in the enamel. Statistically significant differences, calculated with Mann-Whitney are given in the table; *=p<0.05; **=p<0.01; ***=p<0.001. (DL= two levels compared; e.g. DL 1-3 in enamel: outer enamel compared with middle part of enamel). (Level 1 in enamel closest to the enamel surface, level 5 closest to the enamel-dentin junction.)

DL	F	Na	Mg	Cla	K	Sr
1-2	0.00452-0.000172***	1.58153-2.08575	0.106522-0.175454	0.001005-0.000750	0.06257-0.0818	0.003410-0.003648
1-3	0.00451-0.000126***	1.58153-2.32238	0.106522-0.191725	0.001005-0.000638 *	0.06257-0.08992	0.003410-0.004050
1-4	0.00452-0.000165***	1.58153-2.41856*	0.106522-0.190922	0.001005-0.000537**	0.06257-0.09435	0.003410-0.004136
1-5	0.00452-0.000144***	1.58153-2.55695*	0.106522-0.205241	0.001005-0.000284***	0.06257-0.09965	0.003410-0.004036
2-3	0.000172-0.000126	2.08575-2.32238	0.175454-0.191725	0.000750-0.000638	0.0818-0.08992	0.003648-0.004050
2-4	0.000172-0.000165	2.08575-2.41856	0.175454-0.190922	0.000750-0.000537	0.0818-0.09435	0.003648-0.004136
2-5	0.000172-0.000144	2.08575-2.55695	0.175454-0.205241	0.000750-0.000284**	0.0818-0.09965	0.003648-0.004036
3-4	0.000126-0.000165	2.32238-2.41856	0.191725-0.190922	0.000638-0.000537	0.08992-0.09435	0.004050-0.004136
3-5	0.000126-0.000144	2.32238-2.55695	0.191725-0.205241	0.000638-0.000284 *	0.08992-0.09965	0.004050-0.004036
4-5	0.000165-0.000144	2.41856-2.55695	0.190922-0.205241	0.000537-0.000284	0.09435-0.09965	0.004136-0.004036

Sodium, magnesium, potassium

Na, Mg and K were parallel to each other and all had their lowest values at the surface region followed by an increase to level 2 (1/4 of the enamel thickness) and then remained flat through the enamel to the EDJ (Fig. 8). There was a statistically significant difference for Na between the value at the surface and the two levels closest to the EDJ (Table 4).

Strontium

Strontium had in principle the same values in all locations in the enamel.

Discussion

This study has revealed values in weight % for C, O, P, Ca and ratios Ca/P and Ca/ and weight % for F, Na, Mg, Cl, K and Sr in weight % in the enamel of primary teeth from healthy individuals. Some statistically significant differences were exposed when a statistical analysis was performed for each measured element between the different levels in enamel and dentin, respectively. The most marked differences were found for F at the enamel surface compared with the levels in the bulk of the enamel. Marked differences were also found for P, Ca and the ratio Ca/P when the values in dentin close to the enamel-dentin junction were compared with values close to the pulp.

The POLMI examination is in concordance with what has earlier been shown for primary enamel (12), and thus verifies that the included tooth samples are representative of normal enamel in primary teeth.

The measured elements for XRMA were chosen since they all constitute the main elements of enamel and are easily measured by XRMA. Of special interest is the ratio Ca/P as it represents the mineralization of the hard tissues and the quality of the hydroxy apatite. The ratio Ca/C on the other hand then represents the relative content of organic matter.

XRMA has proven to be a useful tool for measuring some important elements in dental hard tissues and bone, owing to a high sensitivity for the main elements in dental hard tissues (17). One of the advantages with the method used in this study is the possibility of controlling where measurements are carried out, that is the exact location and being able to relate the chemical findings to the morphology of the enamel and dentin.

The elements measured with SIMS were chosen since they have proven to be particularly suitable for this technique due to its sensitivity for analysis of these elements in dental hard tissues (18, 13, 4). As for XRMA analyses, the location for measurements are well controlled in relation to the morphology.

It is known that the mineral concentration distribution may vary considerably between different levels in the same tooth, especially in the enamel, rather than tooth type (16, 6, 24). In the present study no statistically significant differences in elemental abundance could be found between incisors and molars.

The relative values in weight % for C, O, P and Ca are in good agreement with analysis of primary teeth from normal children (21, 11).

The increase of mineral concentration from the enamel-dentin junction (EDJ), towards the enamel surface, coincides with data from studies employing other methods (23, 24). The ratio Ca/P was comparatively constant at 2.00±0.1 in the enamel in all levels which is in concordance with previous studies, even though measurements have been performed in different ways (17, 1-3).

Despite variations in calcium and phosphorous, the ratio Ca/P appeared to be fairly constant throughout the enamel. However, the ratio Ca/C appeared more or less constant in the bulk of the enamel with a lower value at the enamel surface, at the same level Ca and C reaching their highest values. This might reflect on an influence of organic matter on the enamel surface.

The SIMS measurements are in agreement with the concentration gradients that have been shown in previous studies of primary teeth (13, 14, 9). However, the fluorine value heavily depends on the post eruptive exposure, which also explains the rapid increase close to the surface (15). Therefore, the fluorine values should not be regarded as reference values, at least not for the surface.

Conclusions

This study has supplied reference data for C, O, P, Ca and for F, Na, Mg, Cl, K and Sr in enamel in primary teeth from healthy subjects, utilizing XRMA and SIMS, respectively. No statistically significant differences in the elemental composition were found between incisors and molars. The ratio Ca/P is in concordance with other studies. Some elements have shown statistically significant differences between different levels of measurement.

These results may be used as reference values for research on the chemical composition of enamel in primary teeth from patients with different conditions and/or syndromes.

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Analysis of some elements in primary enamel during postnatal mineralization

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Abstract

The primary teeth start to mineralize in utero and continue development and maturation during the first year of life. The aim of this study was to investigate the concentrations of some elements, C, F, Na, Mg, Cl, K and Sr, by secondary ion mass spectrometry (SIMS) in human primary incisors at different stages of mineralization. The teeth derived from an autopsy material from children who had died in sudden infant death. The buccal enamel of specimens from the ages 1, 2, 3, 4, 6 and 19 months, respectively, was analyzed. It was evident that posteruptive effects play an important role in composition of the outermost parts of the enamel. Before the tooth erupts, the concentrations of the elements vary with the maturation grade of the mineralization in the enamel. Sodium was the element with the highest concentration of the measured elements and chlorine was the element of lowest concentration. The 19 month old specimen, considered as the only mature and erupted tooth, showed to differ from the other specimens. The concentration of fluorine, in the 19 month old specimen's outermost surface, is readily seen higher compared with the other specimens at this depth zone. In the 19 month old specimen the concentration of carbon is lower. Potassium, sodium and chlorine have higher concentrations, in general, in the 19 month old specimen compared with the immature specimens.

The thickness of the enamel during mineralization was calculated from data from SIMS. The thickness of the buccal enamel of primary incisors seemed to be fully developed between 3-4 months after birth, reaching a thickness of 350-400 μm.

Key words

Elements, enamel, mineralization, SIDS, SIMS, XRMA

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Analys av vissa grundämnen i emaljernas primära tänder vid postnatal mineralisering

Nina Sabel, Gunilla Klinberg, Sandor Nietzsche, Agneta Robertson, Hans Odelius, Jörgen G. Norén

Sammanfattning

De primära tänderna påbörjar sin mineralisering under omkring 4:e fostermånaden och är färdiga under slutet av det första levnadsåret. Syftet med studien var att undersöka koncentrationsgradienterna för vissa element, C, F, Na, Mg, Cl, K och Sr, med hjälp av analyserande jonsond (SIMS) i primära incisiver, som befann sig i olika stadier av mineraliseringen. Tänderna kom från ett obduktionsmaterial av barn som dött i plötslig spädbarnsdöd. Analyserna ägde rum i den buckala emaljen, från tänder representerande åldrarna 1, 2, 3, 4, 6 och 19 månader. Det var uppenbart att posteruptiva effekter spelar en viktig roll i emaljens sammansättning i den yttersta emaljen. Innan tanden erupterar varierar koncentrationsgradienterna med emaljens maturationsgrad. Natrium var det ämne som hade högst koncentration medan klor hade lägst över lag. Preparatet från den 19-månader gamla individen var det enda som var fullt mineraliserat och hade erupterat, dess emalj avvek från ur kemisk synpunkt från de andra preparatens. Fluorkoncentrationen i det 19-månader gamla preparatets yttersta ytskikt var betydligt högre, i samma preparat var kolkoncentrationen låg. Kalium, natrium och klor hade högre koncentrationen i 19-månaders preparatet jämfört med de mindre mineraliserade preparaten.

Emaljens tjocklek under mineraliseringen beräknades med hjälp av data från SIMS-analysen. Den buckala emaljens tjocklek hos de primära incisiverna var nådd omkring 3-4 månader efter födelsen och var då 350-400 μm tjock.

Introduction

At birth, all primary teeth are in the process of mineralization. This period of time is of great interest for human development and growth. Because of the growth pattern and stability of the structure, primary enamel supplies a unique opportunity of recording metabolic changes during development; from the latter half of the gestation period to the end of the first year of life (9). The primary central lower incisors start to mineralize between the 13th and 16th week of gestation and continue development and maturation during the first year of life (13). The inorganic ions are primarily transported through the enamel organ and are also controlled by the ameloblasts. Evidence that calcium and phosphate ions are nucleated in the newly deposited enamel matrix, shortly after secretion by the ameloblasts, is well established (1, 2, 5, 20, 8).

The concentrations of some elements in enamel and dentin have been presented in several studies utilizing secondary ion mass spectrometry (SIMS). Elements such as C, F, Na, Mg, K, Cl and Sr show characteristic concentrations through the tissue. However, it is not known when and how these concentrations are established. It is evident that posteruptive effects play an important role in the outermost parts of the enamel (6), but in the bulk of the enamel, and probably also in the enamel's adjacent parts of the dentin, the concentrations are established at a particular stage of the mineralization process.

Most studies of the mineralization of dental hard tissues have, for different reasons, been performed on specimens from other species than humans. The rat incisor, for example, provides an excellent model for studies of the dynamic phases of dentin and enamel formation (6). Nevertheless, studies on human material are of special interest but, for ethical reasons, it is not possible today to collect material. In a multicenter study of possible etiological factors behind Sudden Infant Death Syndrome (SIDS), autopsy material was collected and examined for mineralization disturbances of the dental hard tissues (25). As this tooth material represented a time cycle of 1 to 19 months, it was of interest to study the incorporation of some elements in enamel in different time periods.

The aim of this study was, by means of SIMS, to describe the concentrations of some elements during phases of enamel mineralization.

Subjects and Methods

Subjects

Tooth buds from 19 subjects were previously collected for the purpose of another study (25). A sample of 6 subjects was selected for this study (Table 1).

Immediately after extraction, all of the teeth were put in buffered 4% formaldehyde, before they were being embedded in epoxy resin (Epofix®, Electron Microscopy Sciences, Fort Washington, PA, USA), then the teeth were stored for 24 hours in ethanol. The teeth were then cut in two halves sagittaly, in a bucco-lingual direction with a Leitz low speed saw microtome (Leitz®, Wetzlar, Germany) and ground sections were prepared from each tooth (15). All teeth were previously examined histologically regarding the occurrence of developmental defects (25). Six central incisors were chosen from individuals with intervals of age; 1, 2, 3, 4, 6 and 19 months old,

Table 1. Age (in months) and sex of subjects in this study (F=female; M=male).

No	Age moths	Sex
12	1	F
10	2	F
7	3	M
9	4	F
2	5	M
4	6	F
3	19	F

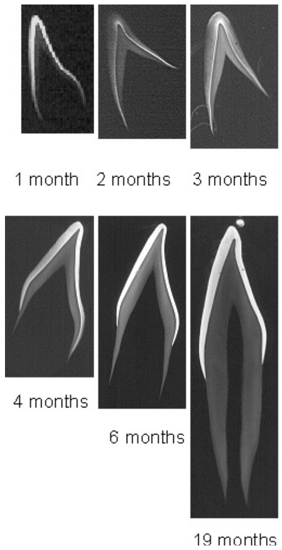


Figure 1. Microradiographs of specimen at different ages.

to represent the mineralizing period in this study (Fig. 1).

The eruption time of the first lower incisor is 7.5 ±1.7 months (18). Therefore, the only tooth considered erupted, belonged to the 19 month old individual.

SIMS analysis

After mounting in a sample holder for the ion probe, the specimens were polished and coated with a thin layer of gold by vapor deposition, in order to avoid buildup of surface charge during the analysis.

The secondary ion mass spectrometric analysis was performed in an ion probe (Cameca IMS 3 F). The principles for the ion probe have been described in detail elsewhere, wherefore only a brief outline will be given here (11, 10). The surface of the specimen was bombarded with negative oxygen ions. Secondary positive ions were then collected from the center of the bombarded area. The secondary ions were then passed through a magnetic analyzer and separated according to mass/charge ratio, the counts of the different ion species were then analyzed by means of an on-line computer. The diameter of the primary ion beam was 50 μm and the diameter of the analyzing area 16 μm. A step scan was performed from the enamel surface to the enamel-dentin-junction (EDJ) with 5 μm steps in the coronal part of the tooth (Fig. 2).

The mass numbers and their corresponding ion species are listed in Table 2. All raw data was normalized to the reference isotope of calcium (Ca44). All values are to be regarded as semi-quantitative. Graphs of the logarithmic values of the semi-quantitative concentrations of each element were made, in order to see the fluctuations across the enamel, comparing ages and elements, respectively.

Table 2. Recorded mass numbers and corresponding ion species analyzed. All values were normalized to those of 44Ca. The mass numbers 56 and 57 were used as auxiliaries for calculating a value for fluorine from the 59 peak.

Mass numbers of studied ions	
12/C	44/Ca44
19/F	56/57CaOH
23/Na	59/CaF
24/Mg	77/CaCl
31/P	88/Sr
39/K	

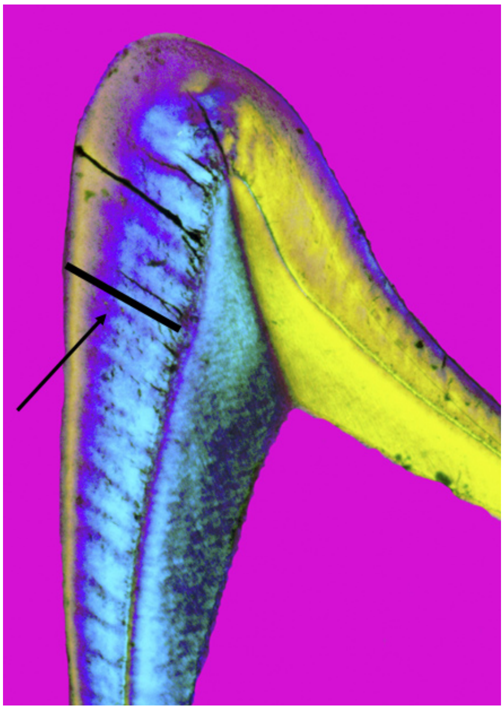


Figure 2. Image of tooth section in polarized light, black line across the enamel demonstrating position of SIMS step scan. Tooth from a 2 months old individual.

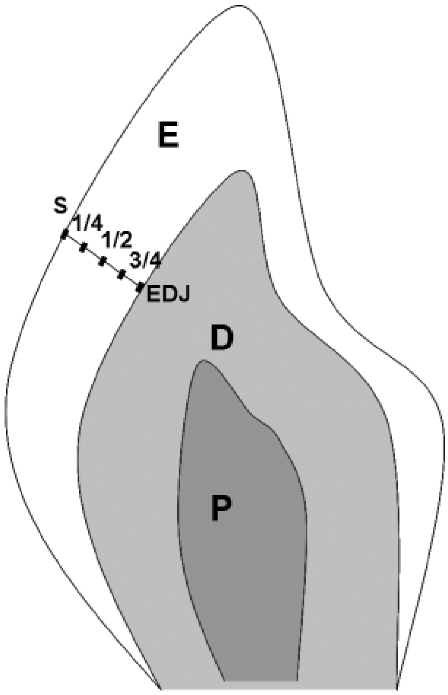


Figure 3. Image over the depth zones. S: surface, EDJ: enamel-dentin-junction, E: enamel, D: dentin, P: pulp.

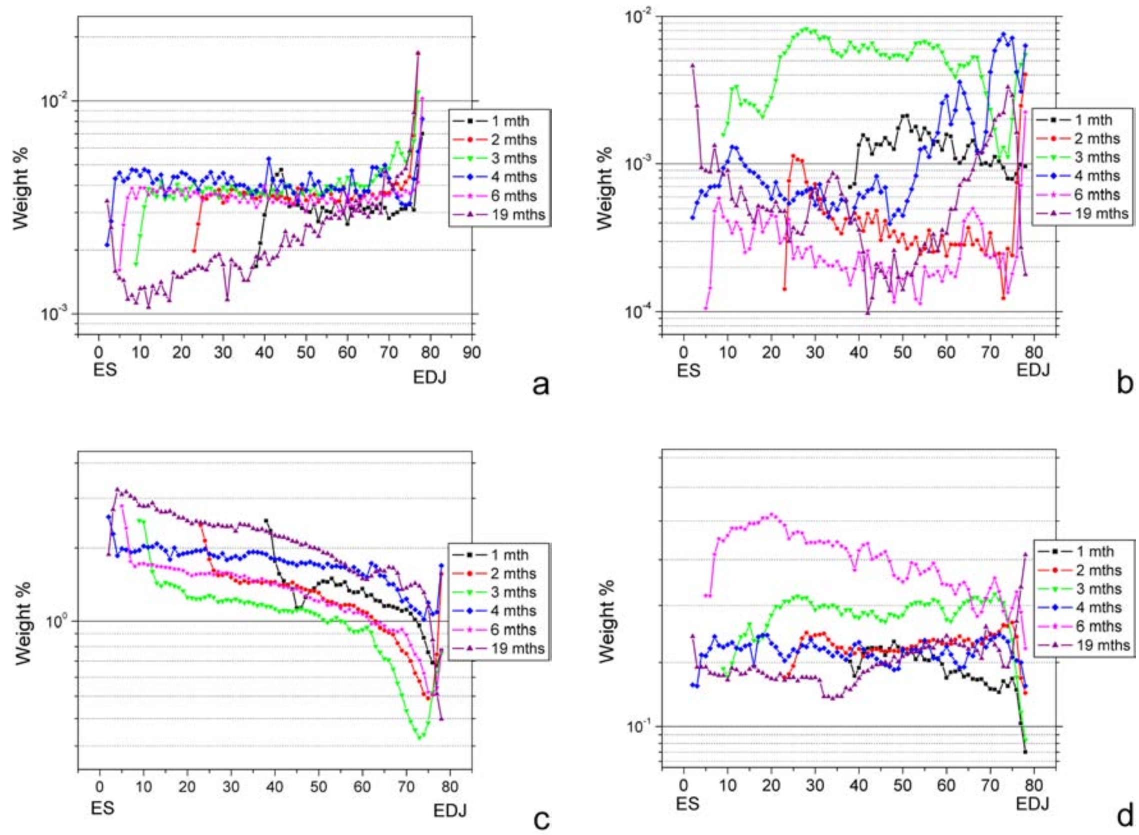


Figure 4. Graphs showing the concentration gradients across enamel of a. carbon, b. fluorine, c. sodium, d. magnesium. mth: month, mths: months. Measuring points at a distance correlating to 5µm.

From these values, tables of fluctuations were constructed to compare changes in the elemental composition at different depth zones of the enamel (Fig. 3). The depth zones of the enamel were made by dividing the total thickness of the enamel into equal quartiles in every specimen. The values at every depth zone/quarter of each element and specimen were used for comparison and analyzed for co-variations during the mineralization.

In each specimen, the concentration of each element at each depth zone was compared with the concentration in the surface in order to visualize the variation of the elements through the bulk of the enamel. Further, the relations in the concentration of each element of the immature specimens were compared with the values in the corresponding depth zones of the 19 month specimen, considered as fully mineralized enamel.

The variations in the different concentrations were made visible as an increase (+) or a decrease (-). The differences were classified into six intervals, Table 3c.

The length of the line across the enamel of every specimen was calculated by using the number of measuring points (5 µm apart) from the SIMS analysis.

Ethical Considerations

This study was approved by the ethical committee at the Sahlgrenska Academy at Gothenburg University, Göteborg, Sweden, registered 343-07.

Results

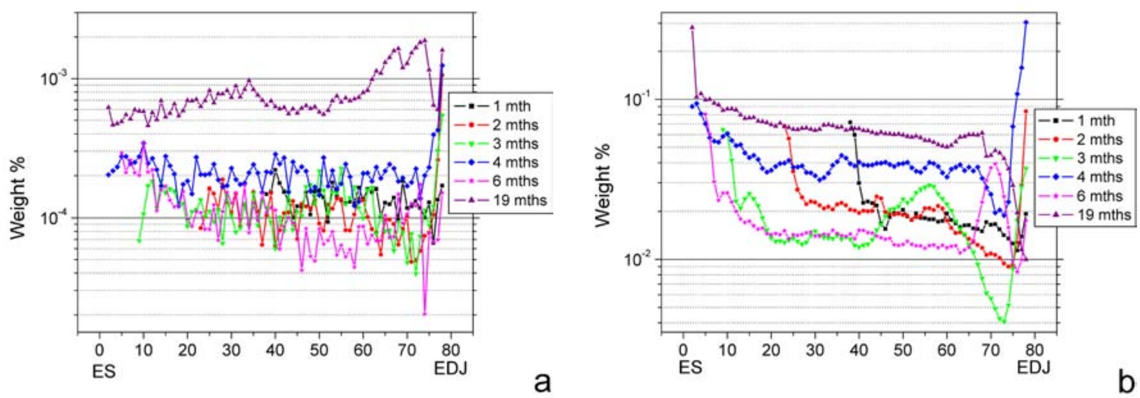
Graphs of the logarithmic values of the semi-quantitative concentrations of each element shows the fluctuations through the enamel.

Distribution of Carbon

Carbon had its lowest concentration values at the surface in all specimens, with the exception of the 19 month old specimen. The mature specimen had a lower carbon content in the bulk of the enamel, but EDJ, compared with its own surface (Fig. 4a).

Distribution of Fluorine

The concentration of fluorine fluctuated through



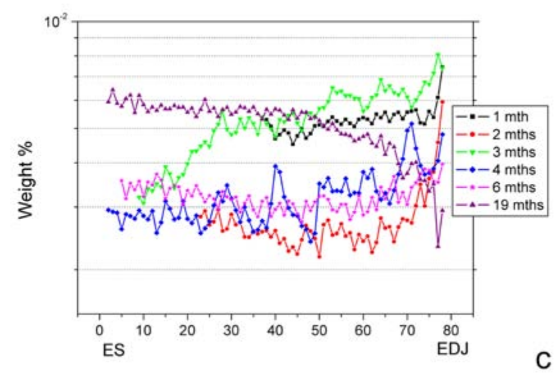


Figure 5. Graphs showing the concentration gradients across enamel of a. chloride, b. potassium, c. strontium. mth: month, mths: months. Measuring points at a distance correlating to 5µm.

the enamel in all specimens. Fluorine had a slightly lower concentration at the outer surface of the immature specimens, and a slightly higher concentration in the bulk of the enamel compared with the mature specimen.

At the surface, a decrease was seen in the concentration of the younger specimens, in contrast to the 19 month old specimen, where a steep increase of the gradient was noted (Fig. 4b).

Distribution of Sodium

Sodium was found to be the element with the highest content in the enamel of all the elements measured in this study. The highest concentration of sodium was found at the outer surface in all specimens. The sodium content in the outer part of the enamel in immature specimens was lower compared to the 19 month old specimen. At the EDJ, sodium was found to be higher in the immature specimens compared with the mature specimen (Fig. 4c).

Distribution of Magnesium

The concentration of magnesium was lower at the outer surface in all specimens compared to the enamel in the outer half. However, in the 19 month old specimen, the opposite pattern was observed. The concentration of magnesium was lower in the immature specimens at the EDJ in relation to the mature specimen (Fig. 4d).

Distribution of Chloride

The concentration of chloride at the different depth zones in the enamel showed minor variations. In all specimens, the concentration fluctuated or decreased in the bulk of the enamel in relation to the surface, except for the 19 month old specimen where the concentration increased in the inner parts, compared with the surface (Fig. 5a).

Close to the EDJ, the concentration rose in all specimens. Chloride was the only element of the immature specimens having the same relation to the mature specimen at all depth zones through the enamel.

Distribution of Potassium

Potassium had, in general, its highest concentration at the outer surface in all specimens. The concentration of potassium was fairly steady through the enamel. Potassium was found to a lower content in the immature specimens compared with the 19 month old specimen, at each depth zone, but at the EDJ (Fig. 5b).

Distribution of Strontium

Strontium fluctuated slightly between the specimens at the different depth zones compared to their re-

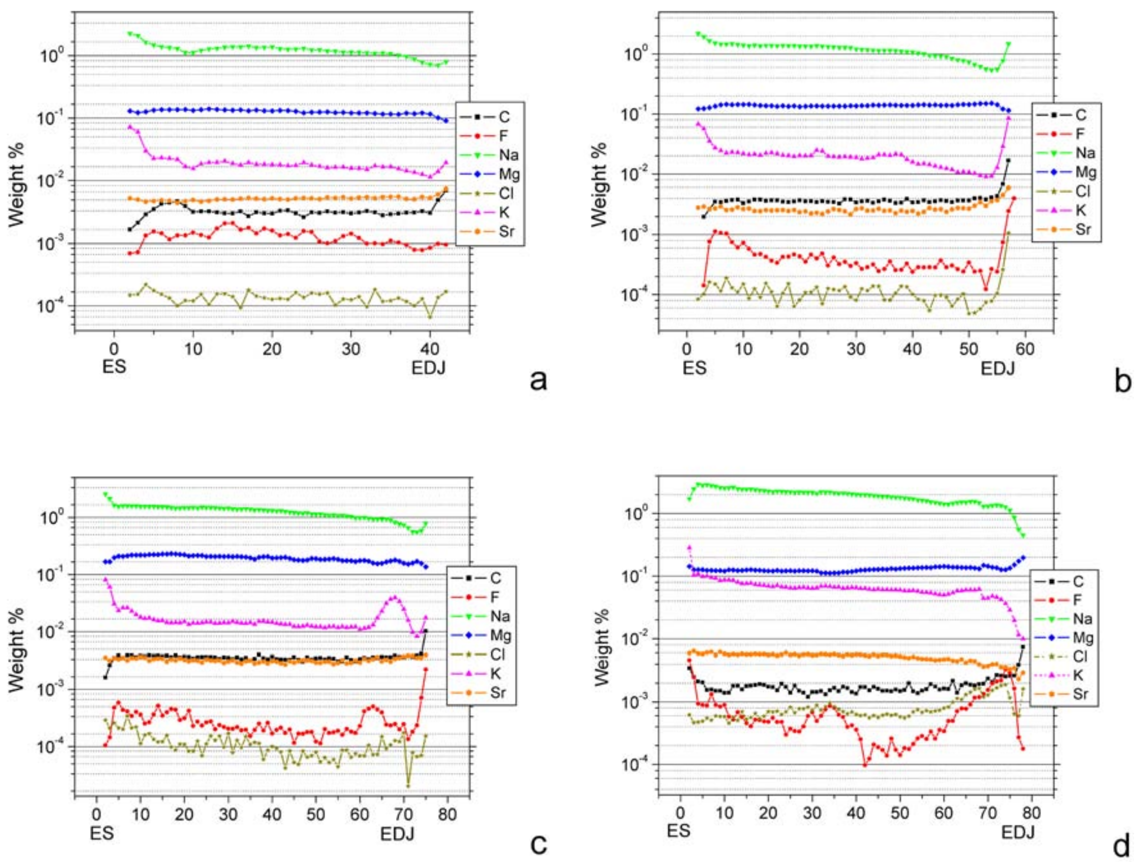


Figure 6. Graphs showing the concentration gradients across enamel of a. 1 month specimen, b. 2 months specimen, c. 6 months specimen, d. 19 months specimen. Measuring points at a distance correlating to 5μm.

spective surfaces, with the exception of the 19 month old specimen, where the concentration was highest at the outer surface (Fig. 5c).

Change of Elemental Concentrations at Various Ages

In order to describe the changes in the concentrations for the different ages, the elements were grouped according to their concentration depth zones.
Sodium, magnesium and potassium had the highest concentrations of all measured elements independent of age and stage of maturation, and appeared to remain relatively constant in all specimens in the logarithmic charts (Fig. 6). However, our analysis using non-logarithmic scales showed that these elements varied both in relation to the values on their surfaces and in relation to the respective concentration depth zone in the enamel of the 19 month old specimen. A co-variation was found between the concentration of sodium and potassium in the immature specimen. Sodium and potassium showed higher concentrations in the 19 month old specimen compared to the younger specimens (Figs. 5b and 6).

Strontium and carbon had a constant and almost equivalent concentration in the specimens from infants 2-6 months of age. In the 19 month specimen, carbon had a constant but lower concentration at all depth zones in the enamel compared with specimens from younger individuals, but at the surface, where the concentration was similar (Figs. 4a and 6). The concentration of strontium was equivalent in all specimens related to the 19 month old specimen and to their surface values.
In the oldest specimen, fluorine varied the most and had the lowest concentration in the bulk of the enamel. The highest concentration of fluorine was found in the surface of the oldest specimen, where chloride and fluorine had similar concentrations. Chloride had higher concentration in the oldest specimen compared to the younger ages, where chloride had the lowest concentration of all elements (Figs. 5a and 6).

Table 3 a. Table over carbon; the concentration of carbon changes through the enamel compared to the surface, of respective specimen

C	1 month	2 months	3 months	4 months	6 months	19 months
surface	/////	/////	/////	/////	/////	/////
1/4	++	++	++	++	++	-
1/2	+	++	++	+	++	--
3/4	+	++	++	+	+	-
EDJ	++	+++	+++	++	++	++

Table 3 b. Table over carbon, the concentration of carbon changes in all levels of all specimen compared to the 19 month specimen

C	surface	1/4	1/2	3/4	EDJ
1 month	-	++	++	++	-
2 months	-	++	++	++	++
3 months	-	++	++	++	++
4 months	+	++	++	++	++
6 months	-	++	++	++	++
19 months	/////	/////	/////	/////	/////

Table 3 c. The changes are classified in intervals

"+"	0- 0,001
"++"	0,001- 0,005
"+++"	≥0,005
"-"	0- (-0,001)
"--"	(-0,001)- (-0,005)
"---"	≤-0,005

Concentrations of Elements at Different Depth zones in the Enamel Compared with the Corresponding Depth zones in the 19 Month Old Specimen

Surface

At the surface, all immature specimens showed a lower content of all elements measured in this study compared to the surface of the 19 month old specimen, with the exception for the 6 month old specimen where magnesium had higher values and the 4 month old specimen, which had higher values for carbon at their respective surfaces (Table 3).

Depth zone 1/4

At the depth zone next to the surface, carbon and magnesium had higher concentrations in the immature specimens compared to the 19 month old specimen. Sodium, chloride, potassium and strontium had lower concentrations in the immature specimens. The values for fluorine fluctuated, at this depth zone, in the different specimens.
At this depth zone, magnesium and carbon had higher concentrations compared to their outer surfaces. Carbon was found to have a higher content in the immature specimens compared with the oldest specimen and also higher compared with the concentration at the surface for each specimen (Table 3).

Depth zone 1/2

In the middle of the enamel, higher depth zones of carbon and magnesium were seen in the immature specimens. Lower concentrations of sodium, chloride, potassium and strontium were found compared to the mature specimen. At this depth zone, the fluorine value varied between the specimens (Table 3).

Depth zone 3/4

At ¾ of the enamel thickness, higher concentrations of carbon and lower concentrations of chloride and potassium were seen in the immature specimens. Fluorine, sodium, magnesium and strontium were individually fluctuating at this depth zone. A shift in change of the concentration for these elements started to appear compared to the middle part of the enamel (Table 3).

EDJ

At the EDJ, higher concentrations of carbon, fluorine, sodium, potassium and strontium, and lower concentrations of magnesium and chloride were generally seen in the immature specimens. At the EDJ, a shift was seen in the concentrations for sodium, potassium and strontium. Higher concentrations for sodium, potassium and strontium were seen in the immature specimens close to the EDJ compared with the 19 month old specimen and in relation to the other depth zones.

A shift was also seen in the relation of immature specimens to the 19 month old specimen concerning magnesium. Magnesium had lower concentration values in the younger specimens at the EDJ and at the surface compared with the 19 month old specimen. However, at all other depth zones, the immature specimens showed higher concentrations of magnesium (Table 3).

Enamel Thickness

The enamel thickness increased from 205 µm at 1 month of age to 385 µm at 19 months of age (Fig. 7). Full thickness of the enamel was achieved between 3 and 4 months of age.

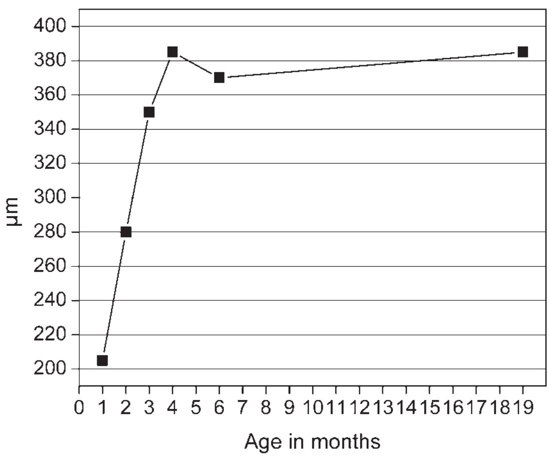


Figure 7. Graph showing thickness of enamel in relation to age of specimen.

Discussion

This study has shown the concentration of some elements in the enamel of the primary central incisors during the mineralization process after birth.

The number of teeth that became available for the SIMS analysis was limited, however, differences are seen according to the ages, though individual variations exist (29).

The SIMS technique has proven to be useful for measuring different elements, even in low concentrations, in dental hard tissues with excellent morphological resolution (1). Since the analysis may be performed with full control of the location in the specimens, the data may be correlated to the morphology. In order to avoid any surface contamination, measurements are not started before the sputtering process has penetrated through the gold layer and into the enamel. The achieved data is regarded as being semi-quantitative and the normalization to Ca44 makes it possible to compare data from different teeth, independent if the teeth are immature or mature. The logarithmic scale is more suitable for observing changes and reducing variations of the semi-quantitative concentrations.

The changes of concentration in Table 3 are not measured as percentage, therefore, the highest grades of changes were found in the elements having the largest concentrations, i.e. sodium, magnesium and potassium.

The lower concentration of carbon in the bulk of the fully developed enamel, compared with the enamel from younger teeth, indicates a full maturation without the presence of carbon-containing amelogenins. During the mineralization, amelogenins are participating in the process of creating crystallites. When the crystallites are formed, proteins are resorbed and the enamel thus will contain a lower concentration of carbon (21, 3, 26). The concentration of carbon in the mature enamel found in this study are in agreement of what has been found in mature, normal enamel in permanent first molars (7). Less content of carbon was found in fully mineralized enamel than enamel under mineralization, in accordance to the process of mineralization in enamel of teeth from rats (12). The high values of carbon close to the EDJ might reflect the presence of enamel tufts, containing more proteins than the rest of the enamel, while high concentrations of carbon at the surface of fully developed enamel possibly reflect a higher protein content of the lamellae structure at the surface of enamel (27).

Carbon is a constituent of carbonate (CO_3^{2-}) which is found in higher concentrations closer to the EDJ than in the outer surface, which has been suggested to be a result of bicarbonate diffusion down a concentration into the enamel fluid from the dentinal matrix (23). Bicarbonate is also thought to be the buffering system during the maturation phase, which establishes an environment with pH depth zones above demineralization (23).

The individual depth zone of fluorine was relatively steady, even though it varied between the different individuals, however, no age-dependent pattern was seen. This might reflect the individual exposure to fluorine in uterine life, since fluoridated water influences the fluorine concentration in prenatal and postnatal enamel (28).

The fluorine concentration in dental hard tissues varies with several aspects such as the level of fluorine intake, duration of exposure, stage of mineralization development, rate of growth, tissue porosity, vascularity, surface area of the tissue, crystallites, degree of mineralization, and in which region the specimen is sampled from (6). The prominent rise of the concentration of fluorine at the outer surface, which is seen in the mature specimen, agrees with other studies (16, 7). This indicates the uptake of fluorine in the oral cavity. *Norén* et al. 1984 found a higher concentration of fluorine, particularly at the outer surface, compared to this study (16).

Chloride is transported through the ameloblast. This was seen by *Prostak et al.* 1996 when inhibition of the anion transport was demonstrated, and chloride and bromide were found in higher concentrations in the enamel (19). Chloride was, in the present study, considerably lower in concentration under mineralization, compared to what was found in a previous study on exfoliated primary teeth (16). *Lundgren* et al. 1998 found that chloride increased in enamel during mineralization, which is also interpreted from the results in this study (12). Therefore, it might be suggested that chloride accumulates during the maturation phase and after exposure to the oral cavity, which would explain why the 19 month old specimen had a higher concentration of chloride at all depth zones in the enamel.

Magnesium had about the same concentration in the individual specimens throughout the thickness of the enamel, which is in accordance with previous findings (16). However, this is in contrast to what *Dolphin et al.* found, that there was a significant decrease of magnesium from the prenatal to the postnatal enamel (4). The concentration of magnesium in this study being approximately the same as observed earlier in permanent first molars (7).

Robinson et al. 1981 found that the concentration of magnesium increased from the EDJ and outwards in the enamel of permanent teeth (22). They found a correlation between enamel of low density in regions (often associated with high protein concentrations) with higher concentrations of magnesium and vice versa. This was explained to be due to either magnesium in the central core of the enamel crystallites during mineralization, or that magnesium is correlated to enamel proteins (22).

Potassium had approximately the same concentration as previously demonstrated in primary teeth (16). The pattern of the slope of potassium in the bulk of the enamel was not similar to what was found for magnesium, which is contradictory to findings stating that these two elements co-variate in fully mineralized primary enamel (14). The pattern of the slope in the bulk of potassium is similar for sodium, and this is confirmed to co-variate (14).

The values for strontium found in this study with individual variations are congruent to what have been found in other studies (30). The individual differences are proposed to depend on the diet of the mother, during pregnancy and breastfeeding (4).

In this study, the analysis was carried out in the same area of the tooth in all specimens. According to *Robinson et al.* 1981, the mineralization is divided into four different stages; stage 1 is reflecting the increase in enamel thickness, in stage 2 there is a selective replacement of matrix proteins, in stage 3 fluid in the matrix has replaced proteins and minerals starting to replace the fluid, in stage 4 the enamel becomes fully mineralized (21). The stages "grow" gradually from the incisal edge of EDJ toward the surface and, along the EDJ, toward the cervical region (21). This means that the stage of mineralization is different in the various samples analyzed in this study, depending on the age of the individual. Based on a study by *Deutsch et al.* 1982, the mineralization of the enamel in the different specimens analyzed is likely to be in either stage three, second mineralization, or in stage four, fully developed non-porous enamel (3). When full thickness of enamel is reached the enamel is considered to be in the maturation stage of mineralization (24).

Full thickness of the enamel appeared to be reached after approximately 4 months after birth, as measured in this study. This is principally in concordance with what has been found in other studies even if the figures vary to some extent. In a previous study, it was shown that the crown formation of the lower primary central incisors is completed around 3 months of age (17). The formation of enamel of primary central incisors is completed at 2.5 months, according to a study made by *Levine et al.* 1979 (9). Of the specimen which had reached the full thickness of enamel, only the 19 month old specimen shows a low concentration of carbon, though should the enamel not be seen as completed at 4 months of age, considering the finding of the diminished concentration of carbon. This is in agreement with *Dolphin et al.* 2005, stating that the enamel in primary teeth form until 9 months postnatally, thereafter becoming principally inert (4).

The curves of all elements from the 19 month specimen differ from the other specimen's. Whether this is just a coincidence or is a real difference, due to the stage of mineralization, is not possible to explain, because of the small number of teeth studied. The cause of death of the healthy individual of 19 month was a traffic accident, which can not explain the varied values all through the enamel.

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