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Original Article

Effectiveness of different preventive agents on initial occlusal and proximal caries lesions: A follow-up study

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KEYWORDS
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Abstract *Background/purpose:* Monitoring the effects of different caries-preventive agents on initial caries lesions in orthodontic patients is important. Therefore, the purpose of this study is to investigate the efficacy of different preventive methods in preventing occlusal and proximal incipient lesions (ILs) during fixed orthodontic treatment.
Materials and methods: Forty-eight subjects at the beginning of fixed orthodontic treatment were included. All subjects were well educated and motivated to use the fluoride toothpaste (Colgate Total, 1450 ppm F) three times a day during the study period. Four different groups were created with a split-mouth design: placebo, fluoride gel, fluoride varnish, and chlorhexidine varnish. The occlusal surfaces of the second molar teeth were assessed with DIAGNOdent pen (DD) during the first 12 months (6th and 12th), and the proximal surfaces of each quadrant were monitored using bitewing radiographs until the 24th month (baseline and 24th month).
Results: The mean DD values increased in each group during the first 6 months compared to the baseline, but a significant increment was only obtained in the control and fluoride gel groups ($p < 0.05$). Fluoride and chlorhexidine varnish had significantly more preventive effects than the control and the fluoride gel for occlusal surfaces at the 6th and 12th month and for intact proximal surfaces at the 24th month, but no significant differences were found between the two varnish groups ($p > 0.05$). No significant differences were found between the four methods in terms of caries progression for proximal ILs after 24 months.
Conclusion: Effective toothbrushing with 1450 ppm fluoridated toothpaste and topical fluoride gel application seems to be inadequate for prevention of new proximal ILs during fixed

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orthodontic treatment. Fluoride and chlorhexidine varnish showed more protection in relation to occlusal surfaces.

Introduction

Incipient caries lesions (ILs) around the surfaces of banded or bonded teeth are a common orthodontic problem.[1] The presence of fixed orthodontic appliances further affects oral hygiene and leads to the cleaning of teeth becoming more difficult; hence, accumulation of dental plaque around brackets and bands increases, which causes enamel demineralization.[2] Current literature indicates that this is associated with a fixed orthodontic treatment prevalence rate of 26%—89% for incipient lesions.[3] Although the patients are instructed about efficient oral care processes, IL still remains an actual clinical problem in fixed orthodontic appliances. These lesions cause problems such as poor esthetics, patient dissatisfaction, and legal complications after orthodontic treatment.[4] Factors such as the patient's medical history, dental history, medication history, diet, salivary flow rate, levels of calcium, phosphate, bicarbonate in saliva, fluoride levels, and genetic susceptibility also play an important role in IL.[5] These problems have made it important to determine patient's saliva, oral care condition and caries risk and to evaluate, when necessary, early-preventive implementations before the orthodontic treatment. In order to respond to this problem, many studies have focused on finding solutions to IL that occur during fixed orthodontic treatment.[3,5]

Most studies in the literature have specifically focused on dental materials used for connecting to orthodontic brackets,[6—9] or for sealing the buccal surfaces of teeth.[10,11] Topical fluoride application of toothpastes, gels, rinses, and varnishes was found to be beneficial in patients with fixed appliances.[12] Chlorhexidine varnish treatment was found to be beneficial in inhibiting salivary *Streptococcus mutans* levels and in reducing gingivitis, thereby improving oral hygiene in these patients.[13] Early detection of ILs during orthodontic treatment is of great importance, as it allows clinicians to implement preventive measures to control the demineralization process before lesions progress.[14] On the other hand, most studies in the literature have focused on buccal surface ILs[8—13]; only one study reported information on occlusal caries progression in patients undergoing orthodontic treatment.[15] In general, the susceptibility of different tooth surfaces to caries lesions is markedly different, with the pit and fissure (occlusal) surfaces the most susceptible, and smooth (labial and lingual) surfaces the least prone.[16] The occlusal surfaces of molar regions remain the most frequent sites of attack during childhood and adolescence. In a longitudinal study of adolescents, it was found that occlusal surfaces on molars and premolars accounted for 60% of the total DMFS score.[17]

Monitoring the effects of different caries-preventive agents on initial caries on different teeth surface is, therefore, very important. However, to the best of our knowledge, no study has investigated the effects of different methods on occlusal and proximal ILs during orthodontic treatment. Therefore, the objective of this study was to compare the effectiveness of four different methods (placebo, fluoride gel, fluoride varnish, and chlorhexidine varnish) in preventing ILs during orthodontic treatment with a fixed appliance. The null hypothesis to be tested was that no statistically significant difference exists between the control and treatment groups at 6th, 12th, and 24th months.

Materials and methods

Ethical approval for this study was obtained from the Ethics Committee of the Dentistry School of the University of Selçuk (2012-08/10). Informed consent was obtained from all participants, and their parents were informed about the clinical study. Subjects were evaluated clinically at each 3-month point over the course of two years, but outcomes were obtained only at baseline and at the 6th, 12th, and 24th month thereafter. The primary endpoint measure was laser fluorescence (DIAGNOdent pen) readings of the occlusal surface of the second molars performed at baseline and at the 6th- and 12th-month appointment. The secondary endpoint was a bitewing radiograph (for monitoring proximal enamel lesions in each quadrant, as described below) carried out at baseline and at the 24th month.

Study design and subjects

This study was a split-mouth, blinded, four-parallel-group study and had a follow-up design. A power analysis was established using G*Power software (Ver. 3.0.10; Franz Faul, Universitat Kiel, Germany). A total sample size of 180 teeth (45 teeth per group) would give more than 90% power to detect significant differences with a 0.25 effect size among four groups and at a $p = 0.05$ significance level.

A sample of 48 patients (28 females and 20 males, 13—16 years old, mean age: 14.4 ± 0.9) undergoing fixed orthodontic treatment at the Department of Orthodontics was recruited for this study. All subjects lived in a community with no water fluoridation. The fluoride level of drinking water was less than 0.3 ppm in the city where the subjects lived. The following inclusion criteria were applied: patients were at the bonding stage of fixed appliance therapy, had occlusal restorations of at least two first molars, had four second molars with clinically intact occlusal surfaces and fully erupted, had all premolar teeth (non-extraction treatment) fully erupted and visible at the start of the study, had all posterior teeth (from first premolar to second

molar), had no previous fixed orthodontic treatment, had permanent dentition, had a malocclusion as symmetrical as possible, and brushed at least twice a day. Subjects with a history of previous orthodontic treatment, caries formation on any second molars, smoking habits, systemic problem, common gingival inflammation, orthognathic surgery, enamel hypoplasia, dental fluorosis, intrinsic and extrinsic pigmentation, and extensive caries in any teeth were excluded from the trial.

Proximal regions were evaluated on bitewing radiographs. New bitewing radiographs were taken for proximal enamel lesions if no record could be found in the system. Occlusal surfaces of the study teeth (we included only upper and lower second molar teeth for DD readings) were carefully examined with the aid of a standard loupe (3.0× Keeler Corporation, England) and mirror after drying. The patients were randomized using an Internet-based computer program. A split-mouth design was applied to four groups: placebo (control), fluoride gel, fluoride varnish, and chlorhexidine varnish. Two preventive agents were applied to the right or left teeth of one subject. The paired teeth were then randomly assigned to preventive applications.

Preventive groups

All subjects were well educated and motivated to use the fluoride toothpaste (Colgate Total, 1450 ppm F) three times a day during the study period. For the *control group*, the following procedures were applied at each 3-month appointment over the course of 24 months by the same operator: oral hygiene instructions, professional toothbrushing with fluoridated toothpaste at the occlusal and proximal surfaces for at least 3 min and topical application of a placebo gel (saline solution). *Fluoride gel*: topical application of a 1.23% APF gel (Topex gel, Sultan Dental Products, Englewood, NJ) was conducted according to the manufacturer's instructions at each 3-month follow-up. *Fluoride varnish*: these teeth received Enamel ProVarnish (Premier Dental, PA, USA) applications (5% Sodium Fluoride) just after the baseline measurements and at each 3-month follow-up visit. Varnish was applied to the quadrants as recommended by the manufacturer and was allowed to dry for 5 min. Subjects were warned not to eat/drink or brush/floss for 1 h after the application of the varnish. The following day, subjects resumed their normal oral hygiene. *Chlorhexidine varnish teeth*: these teeth received a Cervitec varnish [1% chlorhexidine diacetate and 1% thymol] (Cervitec, Ivoclar Vivadent, Schaan, Liechtenstein) application just after the baseline and at each 3-month follow-up visit. Products were applied according to the manufacturer's instructions. After the varnish application, the varnish was dispersed with air and was allowed to dry, and the cotton rolls were removed after 30 s.

All patients were treated with 0.018 inch slot MBT fixed orthodontic appliances (Equilibrium® 2, Dentaurum, Germany), and their teeth were bonded with a light-cured composite resin and adhesive (Transbond XT; 3M Unitek, Monrovia, Calif, USA). At the beginning of the treatment, patients were instructed in teeth brushing practice using a plastic tooth model, and patients were asked to practice what they were shown in front of a mirror. Patients were informed that they should brush their teeth three times a day—after breakfast, after lunch and before sleeping at night. They were told to use proxy brushes and to avoid snacking.

Measurements

The status of the occlusal surface of each second molar was assessed using a DIAGNOdent pen (KaVo, Biberach, Germany) at baseline and at the 6th- and 12th-month visit. The same laser fluorescence (LF) pen and the same tip were used during all the trials. Prior to its use, the instrument was calibrated using a ceramic in accordance with the manufacturer's instructions and was recalibrated for each examination session for each subject. All quadrant teeth were cleaned and dried using an air syringe before the DD was used under cotton roll isolation. It should be considered that the LF readings were carried out before the applications and that no remnants of the preventive agents were clinically visible on the occlusal surface at each appointment. The angle of the tip was rotated and scanned over the occlusal surface to record the peak value, which could range from 0 (sound) to 99 (caries). The same examiner (who blinded the preventive agent groups) made all the measurements 3 times to eliminate the operator effect for each occlusal surface and was calibrated before the study for the use of DD in line with the manufacturer's directions. The manufacturer suggested the presence and depth of a caries lesion at the occlusal surfaces by DD using the following scale: 0—13 = initial caries lesion; 14—20 = enamel caries; 21—30 = initial dentine caries; 31—99 = advanced dentine caries (Clinical Guidelines, Kavo; 2002).[18]

The proximal surfaces (n = 384 [48 × 8] surfaces for each group) of the first premolar to second molars (four quadrant) of all subjects were assessed using a bitewing radiograph at baseline and after 24 months (all subjects completed fixed orthodontic treatment; mean duration was 22.0 ± 1.85) as follows: 0, intact; D1, radiolucency in the outer half of the enamel; D2, radiolucency in the inner half of the enamel but not passing the enamel—dentin junction; D3, radiolucency extending into the dentin but not more than half way through to the pulp; D4, radiolucency in the inner half of the dentin. The proximal *caries increment* was described as an intact proximal surface that changed into an enamel or dentin lesion (0→1-4), while proximal *caries progression* was specified as a proximal enamel lesion that changed into a dentin lesion (1→2-3-4). To assess the reproducibility of the diagnostic criteria application, the intra-examiner calibration was performed. An intra-examiner test was conducted by re-examining 20 randomly selected radiographs one week after the first examination. The level of intra-examiner agreement was measured using Cohen's kappa statistics. Intra-examiner agreement for caries detection was good with a Kappa value of 82%.

Statistical analysis

The statistical analysis was completed using the SPSS 17.0 software system (SPSS Inc., Chicago, Illinois, USA). A p-

value of <0.05 was accepted as being statistically significant. Descriptive statistics, including the means, standard deviations, and frequencies (percentages), were calculated. As the results of the Kolmogorov–Smirnov and Shapiro–Wilk tests showed that the data were normally distributed ($p > 0.05$), parametric tests were used for statistical analysis. For the follow-up DD readings, the proximal DS (decay surface) increment and progression were compared at baseline within each group with the aid of two-way repeated-measure ANOVA. The ANOVA tests were then followed by Newman–Keuls multiple comparison tests in order to test pair-wise differences. For all ILs, the progression or stabilization scores were analyzed by means of a proportional odds ordinal logistic regression model. The counts were then analyzed by a logistic regression model.

Results

The occlusal ILs had a mean DD reading at baseline of 8.35 ± 4.01 in the control group, 7.75 ± 4.26 in the fluoride gel group, 8.06 ± 4.19 in the fluoride varnish group, and 7.18 ± 4.06 in the chlorhexidine group, respectively (Table 1). There were no significant differences between the four groups at baseline ($p > 0.05$). The mean DD values increased significantly in the control and gel groups during the 12 months compared to the baseline ($p < 0.05$). Statistically significant differences were found between the mean DD readings of the two varnish groups and the other groups ($p < 0.05$) at 6th and 12th months, but no significant differences were found between the two varnish groups ($p > 0.05$) at these times. The control group showed the highest mean DD increment between the 6th and 12th month. The mean DD reading of the fluoride varnish group had decreased at the 12th month compared to the baseline score.

Table 2 shows the total caries increment on proximal surfaces after 24 months. Statistically, there were no significant differences relating to the proximal *caries progression* between the four methods ($p > 0.05$). A statistically significant prevention rate (*caries increment*) was obtained in two varnish groups compared to the gel and placebo groups at the 24th month. Table 3 shows the distribution of all proximal lesions according to the scores in each study group. The percentage of ILs that were prevented during the study period for all groups are displayed in Table 4. The differences in the increment score distributions between the four treatment groups were statistically significant ($p < 0.05$). According to the results, a lower caries increment was obtained in the two varnish groups during the 24 months, and this was statistically different from the control and fluoride gel groups ($p < 0.05$). There were no statistically significant differences between all the test groups for caries progression scores during the study period ($p > 0.05$).

Discussion

This study was carried out to establish which preventive agent is most successful for preventing occlusal and proximal ILs during orthodontic treatment with a fixed orthodontic appliance. The preventive agents employed were placebo, fluoride gel, fluoride varnish, and chlorhexidine varnish. The efficacy of different methods on the occlusal surfaces of second molar teeth was evaluated using a DIAGNOdent pen up to the 6th- and 12th-month appointment, and the results for the proximal surfaces of all premolar and molar teeth were interpreted using bitewing radiographs at baseline and at 24 months. The null hypothesis was partially rejected; it was found that fluoride and chlorhexidine varnish was more effective for treating occlusal and intact proximal ILs after 12 months, but the same results were not obtained after 24 months when the proximal surface progression was evaluated.

Several preventive agents are used in order to prevent the occurrence of incipient lesions during treatment.[2,6,9,12]

Table 1 Comparison of scores by DD (x ± SD) between groups.

Groups	Mean ± SD (min–max)	Mean ± SD (min–max)	Mean ± SD (min–max)
DD readings	Baseline	6th month	12th month
Control	8.35 ± 4.01[a] (2–15)	11.02 ± 4.11 [b] (3–18)	12.52 ± 3.35[b] (3–18)
Fluoride gel	7.75 ± 4.26[a] (0–16)	10.81 ± 3.93[b] (2–16)	11.60 ± 2.72[b] (6–17)
FL varnish	8.06 ± 4.19[a] (0–17)	8.43 ± 3.63[a] (0–15)	7.58 ± 3.28[a] (0–14)
Chx varnish	7.18 ± 4.06[a] (0–15)	8.20 ± 3.65[a] (1–19)	7.97 ± 3.61[a] (1–16)

Means with the same superscript letter are not statistically different from each other ($p > 0.05$).

Table 2 Mean caries increment and progression at proximal surfaces (total number of lesion scores at proximal surfaces per 48 quadrant for each group defined mean incidence).

Groups	(n-teeth)	Mean ± SD (min–max)	Mean ± SD (min–max)
		Caries increment	Caries progression
Control	47/13	1.35 ± 1.31[a] (0–6)	0.39 ± 0.70[b] (0–3)
Fluoride gel	52/12	1.45 ± 1.39[a] (0–6)	0.33 ± 0.63[b] (0–2)
FL varnish	42/14	1.06 ± 1.09[b] (0–5)	0.35 ± 0.72[b] (0–3)
Chx varnish	37/12	0.97 ± 1.02[b] (0–5)	0.27 ± 0.57[b] (0–2)

Means with the same superscript letter are not statistically different from each other ($p > 0.05$).

Table 3 Distribution of all lesions according to scores in each group (number of surfaces).

Groups	Caries increment						Caries progression			
	0 → 0 (Intact)	0 → 1	0 → 2	0 → 3	0 → 4	Total lesion	1 → 1	1 → 2	1 → 3	Total lesion
Control	288	35	18	10	2	65	12	11	8	19
Fluoride gel	292	36	23	9	2	70	6	12	4	16
FL varnish	308	32	16	3	0	51	8	13	4	17
Chx varnish	315	28	17	2	0	47	9	8	5	13

Table 4 Changing of lesions during study period (stable or increase, progress).

Caries increment	Increase	Stable	OR[a]	95% CI	p
Control	65 (18.5)	288 (81.5)			
Fluoride gel	70 (19.4)	292 (80.6)	1.03	0.82—1.29	0.749
FL varnish	51 (14.3)	308 (85.7)	0.81	0.64—1.03	0.042
Chx varnish	47 (12.8)	315 (87.2)	0.74	0.57—0.94	0.016
Caries progression	**Progress**	**Stable**	**OR[a]**	**95% CI**	**p**
Control	19 (61.2)	12 (38.8)			
Fluoride gel	16 (72.7)	6 (27.3)	1.58	1.39—2.03	0.687
FL varnish	17 (68.0)	8 (32.0)	1.34	1.12—1.61	0.886
Chx varnish	13 (59.0)	9 (41.0)	0.91	0.76—1.09	0.315

[a] The odds of lesion progression with Fluoride gel, Fluoride varnish and Chlorhexidine varnish compared with the odds of lesion progression with control group.

The literature review showed that most studies have dealt with buccal surface lesions.[8—12] On the other hand, these studies have often ignored the ratio of demineralization caused to the occlusal and proximal surfaces as a result of the increased risk of caries during orthodontic treatment. In this clinical trial, we analyzed the occlusal and proximal surfaces. As occlusal surfaces are prone to plaque accumulation because of their morphology, they are under a greater risk from incipient lesions compared to other surfaces.[16,17] Therefore, in our study, occlusal surfaces were monitored for 12 months, whereas proximal surfaces were monitored for 24 months. Another reason why proximal surfaces were monitored for a longer period of time was that bitewing radiograph analysis during fixed orthodontic treatment is rather challenging.

Visual examination, clinical examination, optical non-fluorescence methods, and optical fluorescence methods were used in the diagnosis of ILs.[19] We considered that visual inspection with the aid of loupe after air drying and visual examination with a dental mirror was appropriate for the present study during the first evaluation of the occlusal surfaces. After that, occlusal surfaces were assessed by a DD, and the readings, which estimated the organic content and bacterial metabolites in caries lesions, were used to point out the changes of the ILs. DIAGNOdent had acceptable sensitivity and specificity compared to visual examination.[20] DIAGNOdent had some disadvantages, such as the fact that readings could also be affected by stains, calculus, and plaque and were based on bacterial metabolites. One study reported that DD may be used to monitor the outcome of caries preventive measure in the occlusal surfaces of orthodontic patients.[15] Bitewing radiographs were preferred to monitor the proximal caries lesion status in this trial. It is generally accepted in the literature that bitewing radiographs detect more proximal lesions than Fiber-Optic Transillumination.[21]

Routine twice-daily tooth brushing is recommended by numerous clinicians as an important part of daily oral care and as part of a plaque control program for all fixed orthodontic patients.[7] On the other hand, the supplementary utilization of fluoride and of chlorhexidine gel, mouthwash and varnish can be an efficient way to remineralize demineralized enamel.[22] Scientifically, the caries-preventive effect of fluoride is explained by the fact that fluoride can be integrated into the crystal structure of dental enamel to create a more resistant constitution against acid. Fluoride is applied topically to teeth to avert initial caries lesions in different ways (fluoridated toothpaste, mouth rinse, gel, and varnish) and methods (fluoride-releasing cements and adhesives) during orthodontic therapy.[23] Chlorhexidine varnish applied as a supplement to fluoride treatment has been verified to have demineralization-inhibiting actions in subjects with orthodontic appliances.[13] The literature showed that durations of follow up were insufficient (4—12 weeks) in most studies,[10—13] and only one study took place over 48 weeks.[15] In most studies, the remineralization effects of several preventive agents were reported based on ICDAS criteria, DIAGNOdent, plaque and gingival index, bacteria level, photographic evaluation and Quantitative Light-Induced Fluorescence.[19] It was observed that most of the studies focused on several preventive methods, and there was no study that investigated different methods collectively. Furthermore, most of the studies focused on buccal surface incipient lesions, and there was only one study that investigated the occlusal surfaces with LF.[15]

The results of this study show that a LF reading improvement of ILs when fluoride or chlorhexidine varnishes were applied differed significantly from that found in the fluoride gel and placebo group during 12 months. Fluoride varnish was found to be more efficient than chlorhexidine varnish, but they were not significantly different from each other. After 24 months, no significant differences were found between the four groups in terms of the progression of proximal ILs, but two varnishes were found to be more effective for prevention in relation to intact proximal surfaces (caries increment). Clinical studies of the effectiveness of a fluoride or chlorhexidine program in orthodontic patients have shown conflicting results. One study has suggested that daily use of a 5000-ppm fluoride gel ensured more prevention than standard oral hygiene in relation to buccal incipient lesions.[24] Only one study in the literature evaluated the effect of chlorhexidine varnish on caries progression in the second molar teeth of orthodontic patients.[15] The varnish was found to be more effective than placebo for prevention of occlusal lesions, which showed less increment LF readings. It has been found that the application of a fluoride varnish resulted in a 44.3% decline in enamel demineralization in orthodontic patients.[25] One study evaluated two varnish methods together in one trial and found that chlorhexidine application in the form of a varnish resulted in longer-lasting suppression of *S. mutans* concentrations by chlorhexidine compared with other forms of application.[13] Attin et al. reported that chlorhexidine varnish reduced salivary MS significantly during four weeks compared to baseline values in orthodontic patients.[26]

Perriini reported that periodic administration of fluoride varnish can cause some prevention of incipient lesions on vestibular surfaces but not at a statistically significant level if the patients have excellent oral care.[27] Kirschneck also suggested that a one-time utilization of fluoride varnish at the beginning of orthodontic therapy did not lead to any additional preventive benefit over sufficient dental care with fluoride toothpaste in relation to the formation of incipient lesions and gingivitis in patients with a low to moderate caries risk.[28] He et al. proposed that fluoride varnish may be slightly more efficacious than fluoride film on the vestibular surfaces of anterior teeth.[29] However, they suggested that further similar clinical trials with more subjects were needed to definitively identify which fluoride treatment was most effective. The variation of different results among studies could be attributed to differences in the examination methods (visual or fluorescence), the number of teeth examined (or subjects), the location of the study sample (cultural or socioeconomic differences), the past caries experience, the tooth surface, the treatment or follow-up duration, and preventive materials. On the other hand, similar compliance of all of the subjects, their standardization, and keeping them under control is difficult during clinical trials. We are of the opinion that these factors may affect all the studies' results.

There are several factors that affect incipient caries lesions during orthodontic treatment. Utilization of different preventive applications in individuals undergoing orthodontic treatment would be advisable for clinicians. On the other hand, for subjects undergoing fixed orthodontic treatment, general caries risk evaluation should be implemented before starting the orthodontic therapy, and the subject should be categorized according to caries risk symptoms. In addition, it should be remembered that a majority of patients undergoing fixed orthodontic treatment are from the low-caries-risk level and have little motivation, and an additional preventive application should absolutely be applied in individuals who are assigned as having poor dental care habits. Thus, ILs that may appear at the end of the treatment can still be prevented in the mild phase.

The following results can be drawn from this study: Fluoride and chlorhexidine varnish showed more protection for occlusal surfaces of second molar teeth than placebo and gel applications over 12 months. Fluoridated toothpaste, gel and varnish, and chlorhexidine varnish application showed a similar caries progression rate on the proximal surfaces of posterior teeth after 24 months. Effective toothbrushing with 1450 ppm fluoridated toothpaste and topical fluoride gel application seems to be inadequate for prevention of intact proximal ILs during fixed orthodontic treatment.

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None.

Conflict of interest

The authors declare no conflicts of interest related to this study.

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