Comparison of Different Dentin Deproteinizing Agents on the Shear Bond Strength of Resin-bonded Dentin

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ABSTRACT

Aim: To assess and analyze the resin-bonded dentin in terms of shear bond strength after using deproteinizing agents 5% sodium hypochlorite, 10% sodium hypochlorite, and bromelain.

Materials and methods: Overall 140 permanent and intact human premolars were split into four groups, three experimental groups and one control group. In all four groups, the occlusal surface of the teeth was wet ground to expose superficial dentin. In group I, teeth were etched and deproteinized with 5% sodium hypochlorite. In group II, teeth were etched and deproteinized with 10% sodium hypochlorite. In group III, teeth were etched and bromelain was used to deproteinize. In group IV, teeth were etched and no deproteinization was being performed and simultaneous fulfillment of the resin composite and later inserted into the plastic tube and polymerized with light. Samples were stored at 37°C for 24 hours and the later samples were transferred to the universal testing machines to shear bond strength analysis at a speed of 0.5 mm/minute.

Results: The outcome of the bond strength was significantly influenced by the application of bromelain enzymes. A remarkable difference was observed between the shear bond strength of sodium hypochlorite (5 and 10%) and in the bromelain enzyme-treated group. Group III showed better results than group I and group II.

Conclusion: This study concluded that bromelain shear has the maximum value for shear bond strength. Bond strength improved because of removal of unsupported collagen fiber with bromelain enzyme after acid etching.

Clinical significance: Natural pineapple enzyme, i.e., bromelain improves bond strength by removal of unsupported collagen fiber. Hence, it is completely safe to use.

Keywords: Bromelain, Bond strength, Sodium hypochlorite (5% and 10%).

INTRODUCTION

On the grounds of upsurging interest for the esthetic restorations, restoring posterior teeth with resin-based composite materials was repeatedly attaining fame.1 Firm durable bonds between dental biomaterials and tooth structures are the requisites to accomplish mechanical as well as biologic and esthetic properties. Composite resins are being handled with a high prevalence today and the dentin/ restoration has become a tremendous activity.2

Etching with phosphoric acid into enamel bonding by Buonocore in 1955 ended up in a strong micromechanical bond. The dentin surface was demineralized by acid etching for dentin bonding and thus expose collagen matrix for resin infiltration in the interdiffusion zone. Framing of resin interdiffusion zone has been considered as the fundamental mechanism of dentin bonding. Bonding to dentin was not as direct as bonding to enamel.3

The bonding of resin to enamel is by virtue of the micromechanical bond between the resin-bonding agent and the highly inorganic substrate of enamel, which is enacted by the acid-etching procedure. This bonding of enamel is a comparably simple process without large-scale scientific requirements or difficulties. Differently, bonding to dentin has been cited as a less safe technique due to the deep-seated characteristics of this substrate. Enamel encompasses insufficient protein, and dentin is an aggressive substrate that contains 17% of collagen by volume. It also contains dentinal tubules filled with dentinal fluid.4

Acid etching leads to exposure of collagen matrix which later forms the hybrid layer. This layer has a bleak role in dentin adhesion. In the absence of water, there is a failure of the demineralized collagen network and nod off to prevent resin monomers infiltration. In opposite, i.e., when moisture is there, a hybrid layer containing voids and porosities would be created within the bonding interface.5 Moisture plays a very important role in bond strength. Thus, bonding to etched dentin is a sensitive technique due to difficulty in finding the fair concord of moisture.5

The pessimistic role of collagen fibrils in dentin adhesion can be reduced by eradication of exposed organic matrix would decrease...
the effects. Hence, a combined utilization of phosphoric acid and sodium hypochlorite was used to produce a permeable mineralized surface similar to etched enamel de-proteinizing the demineralized dentin. Theoretically, with less availability of uncoated collagen fibrils, the technical intensity could be knocked out. Some researchers doubt that the decreased bond strength can be due to potential oxide which influenced the NaOCL on polymerization. But most studies showed confident results with the utilization of NaOCL on demineralized dentin. Few studies reported that the higher the concentration of NaOCL, the greater the dentin bond strength until a high level is reached at a concentration of 10%, for an application time of 60 seconds.

Intensification of the dentin bonding can be executed either by bettering the physical estate of the bonding agent or by tweaking the dentin substrate to act as groundwork for the successive applied adhesive restoration. Refitting of the dentin substrate for acid-etching dentin can be carried out by the use of proteolytic factors, known as dentin deproteinization. It is designed in such a way as to eradicate the negative product connected to the organic content of the dentinal substrate.

The work of deproteinizing solutions transforms the dentin surface ultra-morphology by dissolving the exposed collagen fibrils. Their action stops the exposure of dentinal tubules to strengthen dentin tubules, helping the dentin close to etched enamel, which is favorable for adhesion. The dentinal surface has shown numerous irregularities, with a good mechanical holding of the adhesive in a modified dentin foundation.

The diverse deproteinizing agents encompass sodium hypochlorite, collagenase, bromelain, and Nd:YAG laser. Complete removal of a collagen matrix with sodium hypochlorite has a supporting procedure following the etch rinse technique. The deproteinizing effects of NaOCL on acid etch dentin are also time-dependent. The disadvantage created by using sodium hypochlorite to deproteinizing acid-etched dentin and toxicity of the NaOCL, which are produced by the depth of dentin and extreme taste and odor leading up a new and different outlook to deproteinizing dentin.

Bromelain is derived from the pineapple plant. It acts as an enzyme and belongs to proteolytic enzymes (proteases) and expel collagen network. The purpose of proteases is to catalyze the proteins to give amino acids. Bromelain enzyme can reduce nanoleakage after collagen removal as compared to NaOCL.

**Materials and Methods**

The present investigation was supervised in the Department of Conservative Dentistry and Endodontics, ITS Dental College and Research Centre, Greater Noida, in collaboration with the Research Centre at ITS Dental College, Greater Noida.

The study compared the effect of 5% sodium hypochlorite, 10% sodium hypochlorite, and bromelain dentin deproteinizing representative on the shear bond strength of resin-bonded dentin. A universal testing machine was furnished to measure the shear bond strength.

**Study Design**

*In vitro* study.

**Armamentarium (Fig. 1)**

- Specimen selection and preparation:
  - Selection of specimen:
    - Dental operating microscope (Seiler, Chicago).
  - Ultrasonic scaler (PSBooster Suprason).
  - Specimen preparation.
  - Mounting:
    - Custom-made metal molds.
  - Occlusal reduction:
    - Airotor handpiece (API).
    - Flat end straight handpiece fissure diamond bur (MANI).
  - Composite buildup:
    - Teflon molds.
    - Punch (5 mm).
    - Light cure unit (Satlec Acetone, France).
    - Composite filling instrument (Hu-Friedy, India).
    - Micro applicator tips
  - Measurement:
    - Universal testing machine (Zwick/Roell, Z020, Germany).
    - Custom-made jig.
    - Custom-made mandrel.
  - Miscellaneous:
    - Absorbent cotton pellets.
    - Plastic molds.
    - Punch whole.
    - Adhesive tapes.
    - Micro applicator tips.
    - Self-cure acrylic resin powder (DPI-RR, India).
    - Self-cure acrylic resin liquid (DPI-RR, India).
    - Distilled water.
    - 0.1 thymol solution.
    - Cold mold seal.
    - Scissors.
    - Scale.
    - Tweezers.

**Materials Used**

- Preparation of the specimen
- Restorative material

**Fig. 1:** Armamentarium used for the study. (1) Normal saline; (2) Acrylic resin; (3) Acrylic polymer; (4) Bromelain; (5) 5% NaOCL; (6) 10% NaOCL; (7) Tapered fissure diamond bur; (8) Teflon-coated composite filling instrument; (9) Custom-made cylindrical molds; (10) Adhesive tapes; (11) Punch hole; (12) Micro applicator tips; (13) Cold mold seal; (14) Condenser; (15) Scissors; (16) Scale; (17) Tweezers; (18) Airotor hand piece; (19) Etchant; (20) Prime Bond NT; (21) Light-curing unit; (22) Composite (spectrum 360)
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Preparation of Specimen
All teeth were examined under a dental operating microscope at 20x magnification to detect any crack or fracture. All debris and remaining tissue were removed from the teeth with an ultrasonic scaler. The teeth were kept in 0.1 thymol solution 1 week before the experiment.

Methods
140 intact human premolar teeth were taken (Fig. 2A) and kept in 0.1% thymol solution until they were subjected to use.

The root base of each tooth was embedded in cylindrical-shaped metal mold acrylic resin this cervical line parallel to the floor (Fig. 2B).

Samples were wet ground on the occlusal surface using a tapering fissure bur and occlusal reduction of 1 mm below the DEJ was done (Fig. 3A).

Adhesive punch tape was laid down on the finished dentin to differentiate the working area (Fig. 3B).

Transparent composite cylinders with inter diameters of 2.5 cm and height 1.5 cm were filled with spectrum 360 composite to produce composite cylinders for the purpose to check data since it is known that the bond strength values may be affected by adhesives area dimensions.

Teeth were split into four groups, three experimental groups and one control group.

Group I
Surfaces of teeth were etched with 37% phosphoric acid for 15 seconds and later rinsed with water for 10 seconds (Fig. 3C), blot dried, and deproteinized with 5% sodium hypochlorite for a 60-second was done dentin-bonding agent was furnished (Fig. 3D) and light-cured according to the manufacturer’s instruction composite resin was filled in three additions and light-cured for 20 seconds in cylindrical-shaped Teflon mold.

Group II
Surfaces of teeth were etched with 37% phosphoric acid for 15 seconds, washed with water for 10 seconds, blot dried, and deproteinized with 10% sodium hypochlorite for 60 seconds and then. Dentin-bonding agent was furnished and light-cured according to the manufacturer’s instruction composite resin were filled in three increments and light-cured for 20 seconds in cylindrical-shaped Teflon mold.

Group III
Surfaces of teeth were etched with 37% phosphoric acid for a period of 15 seconds and washed with water for 10 seconds. The absorbent pellet was used to absorb excess water and deproteinized with bromelain enzyme (Fig. 4). The bromelain was washed with distilled water dentin-bonding agent was used and light-cured according to the manufacturer’s instruction composite resin was filled in three increments and light-cured for 20 seconds in cylindrical-shaped Teflon mold.

Group IV
Surfaces of teeth were etched and no deproteinization process was carried out. Dentin-bonding agent was applied and light-cured according to the manufacturer’s instruction composite resin was filled in three increments and light-cured for 20 seconds in cylindrical-shaped Teflon mold.

Fifth-generation DBA, Prime bond NT (Dentsply), was brushed according to the manufacturer’s direction. Upon completion of the adhesive procedure, standardized plastic tubes were placed on the dentin surface. The resin composite spectrum 360 (Dentsply, India) was inserted in the plastic tubes (Fig. 4) and light polymerized.

The plastic tubes were removed to expose the resin cylinder. All specimens were moved to the universal testing machine separately and put through to shear bond strength analysis using a universal testing machine (Fig. 5A) at a speed of 0.5 mm/minute.

The shear bond force was applied on the junction and composite and the force was recorded in kilogram force using the software this value was converted into Newton (Fig. 5B).

Shear bond strength was measured by utilizing the formula force (N)/bonded surface area 1 kgf = 9.8 N.

Results
In this study, 140 premolars are selected and divided into three experimental and one control group. $N = 40, N = 20$. Shear bond strength with mean plus standard deviation values of group I is shown in Table 1, Figs 6 and 7.

Shear bond strength with mean plus standard deviation is shown in Table 2, Figs 7 and 8.

Shear bond strength with mean plus standard deviation is shown in Table 3, Figs 7 and 9.

Shear bond strength with mean plus standard deviation is shown in Table 4, Figs 7 and 10.

Tables 5 to 7 reveal the intergroup comparison of shear bond strength done by one-way analysis of variance which showed that an overall statistically significant difference ($p < 0.05$) was found between the shear bond strength of all the four test groups.

Post hoc pairwise comparison was done by using Turkey’s test and it was established that the shear bond strength of group III, i.e., bromelain was found significantly more ($p < 0.05$) than that of the three groups.

The mean score values of shear bond strength for group I 5% sodium hypochlorite was 67.24, group II was 53.48, group III 78.51, and group IV was 43.26.

Group III bromelain had the maximum among all the group has shear bond strength, while group IV control showed the minimum bond strength values.
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Figs 2A and B: (A) Samples selection; (B) Samples mounted in acrylic

Figs 3A to G: (A) Occlusal reduction; (B) Adhesive tape to demarcate the working area; (C) Application of the etchant; (D) Bonding agent applied; (E) Teflon mold for composite; (F) Light-curing has done buildup; (G) Composite buildup

Figs 4A and B: (A) Samples subjected to shear bond analysis; (B) Bromelain powder

Figs 5A and B: (A) Composite buildup of all the samples in group 1 5% sodium hypochlorite; (B) UTM machine
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Table 1: Shear bond strength with mean and standard deviation values of group I (Newton's)

| S. no | KGF  | Newton |
|-------|------|--------|
| 1     | 3.4  | 33.3   |
| 2     | 6.4  | 62.7   |
| 3     | 5.6  | 53.9   |
| 4     | 16.2 | 158.8  |
| 5     | 5.2  | 50.9   |
| 6     | 3.5  | 34.3   |
| 7     | 5.8  | 56.8   |
| 8     | 7.9  | 77.4   |
| 9     | 10   | 98     |
| 10    | 9.8  | 96.1   |
| 11    | 16.8 | 164.7  |
| 12    | 10.6 | 103.9  |
| 13    | 10.7 | 104.9  |
| 14    | 5.2  | 50.9   |
| 15    | 5.4  | 52.9   |
| 16    | 16.6 | 162.7  |
| 17    | 6.4  | 62.7   |
| 18    | 3.5  | 34.3   |
| 19    | 5.5  | 53.9   |
| 20    | 12.8 | 125.5  |
| 21    | 4.7  | 46     |
| 22    | 3.8  | 37.2   |
| 23    | 3.5  | 34.3   |
| 24    | 5.5  | 53.9   |
| 25    | 11   | 107.8  |
| 26    | 3.8  | 37.3   |
| 27    | 2    | 19.5   |
| 28    | 6.8  | 66.6   |
| 29    | 11.4 | 111.7  |
| 30    | 5    | 49     |
| 31    | 0.8  | 7.8    |
| 32    | 4.3  | 44.1   |
| 33    | 6.6  | 64.7   |
| 34    | 6.1  | 59.8   |
| 35    | 4.5  | 44.1   |
| 36    | 5.1  | 50     |
| 37    | 3.8  | 37.2   |
| 38    | 6.4  | 62.7   |
| 39    | 5.2  | 50.01  |
| 40    | 5.1  | 50.9   |
| Mean  |      | 67.24  |
| SD    |      | 38.32  |

According to Tables 5 to 7, the mean shear bond strength in group I (5% NaOCL) is higher than group II 10% NaOCL. The shear bond strength values of group III, i.e., bromelain was found to be significantly more ($p < 0.05$) than the other three deproteinizing agents.

**Statistical Analysis**

Data were calculated using one-way analysis of variance for mean comparison among groups and unpaired $t$-test for mean comparison among bond strength between groups at a significant level of 0.05.

The statistical analysis was performed using SPSS version 12.1.0 for windows.

The bond strength was significantly increased by using the bromelain enzyme.

A statistically significant difference ($p < 0.05$) was found between the shear bond strength of all four groups.

The highest bond strength was seen in the bromelain enzyme.

**Discussion**

These days patient’s outlook for tooth-colored restorations has increased because of an esthetic look. But still, a proper resin dentin bond is considered a challenge. Dentin has a heterogeneous structure with different mineral content. Demineralization of dentin is always limited to the outermost layer and thus exposing the collagen fibers. Underneath that a partly demineralized zone called the hybrid zone is present.

Studies by Van Meerbeek B, Perdigao J, Lambrechts P, Vanherle G (The Clinical Performances of dentin adhesives) using high resolution techniques showed that the bonding agent of current adhesives failed to completely seal the dentin from acid induced porosities. The size of the porosities in dentin was about the size of 10–50 nm. Low viscosity water appropriate monomer mixtures or microscopic restoration particles are intelligent to close the pores. Therefore, dentin troubled in its cohesive support by acid etching will not be fortified. This belt of somewhat demineralized dentin with micro cavities may be treated as a weak point in the attachment. Similar studies were also present that showed enamel produced no sign for the formation of respective porosities or penetration paths.

Dentins have matrix metalloproteinases (MMPs) and cysteine cathepsins. They activated only after acid application which leads to impair the collagen fibrils and resulting in resin–dentin bond failure. Resin–dentin bond degradation may occur by following (i) breakdown of the polymer phase or collagen fibrils in hybrid layer, or (ii) activation of MMPs. During unmasking of dentin to acid from caries break through or acid-etching dissolution of dentinal mineral phase occurred. This deteriorates the organic matrix and collagen fibrils by collagen-degrading proteases initiation and bacterial enzymes. Uncontrolled partially demineralized human dentin substrates, exhibit collagenolytic activity. Combination of resin and collagen hydrolysis can degrade resin–dentin interface and dwindle the physical properties of the resin–dentin bond interface. This results in less bond strength. Acid etching makes an amorphous gel consisting of denatured and fragile collagen over demineralized dentin.

This gel layer inhibits resin infiltration into demineralized dentin. So denuded collagen fibril of hydroxyapatite (HA) and/or resins are vulnerable to hydrolyses.

To avoid these biodegradations, different strategies have been proposed, such as the demineralized collagen removal and the use of MMP inhibitors.
Sodium hypochlorite (NaOCL) is a well-known proteolytic agent for eliminating organic material. The proteolytic action of NaOCL is believed to commit considerable breakdown of long peptide chains and the emergence of N-chloramines with terminal amine groups that further break down to other byproducts, along with both inter- and intra-molecular crosslinks via Schiff base formation. NaOCL-treated dentin is well off with exposed hydroxyapatite crystals and hence results in a more steady interface over time. Relying upon each testing design and distinct composition of each dentin adhesive, the use of NaOCL upon etching may increase and decrease bond strengths.

However, the use of sodium hypochlorite (NaOCL) to deproteinized acid-etched dentin has manifold disadvantages. It designs a fragility zone and is cytotoxic with a bad taste and odor. As the dentinal depth upsurges, the negative impact of NaOCL rises. These limitations have led to the investigation for better means to deproteinized dentin. Therefore, newer approaches for extracting collagen networks include deproteinizing enzymes such as collagenase or bromelain enzyme.

Bromelain pertains to a group of protein-digesting enzymes derived from the fruit itself or stem of pineapple. Its concentration is greatest in pineapple stem, thus allows its extraction, and the stem is a byproduct and thus cost-effective. Hence, “Bromelain” assigns usually to the “stem Bromelain”. Bromelain is a combination of different thiol endopeptidases and other ingredients like phosphatase, glucosidase, peroxidase, cellulase, escharase, and several protease inhibitors. Various studies validate that bromelain exhibits various fibrinolytic, anti-edematous, antithrombotic, and anti-inflammatory activities. Bromelain is appreciably absorbable in the body without losing its proteolytic activity and without producing any sizable side effects. It is also helpful in osteoarthritis, diarrhea, and various cardio vascular disorders. Bromelain also has anti-cancerous activities and promotes apoptotic cell death.

It is a proteolytic enzyme and catalysis the hydrolysis of proteins to give amino acids. According to a study by Dayem and Tameesh, a lower nanoleakage was detected with bromelain enzyme that had led to collagen removal. To date, one study has been done to see its performance in developing the bond strength.

Continuous failure of bond strength of etch-and-rinse adhesives has been most rated in some studies. One of the factors that are responsible for this degradation is partial infiltration of resin monomers into unsupported collagen networks after acid etching along with strong acids which produce a zone of collagen without any backing of either minerals or resins in the base of the hybrid layer.

Of the adhesives system tested, the total-etch Prime and bond NT displayed the maximum shear bond strength, hence, it was considered a gold standard in the study.

Thus, the purpose of this in vitro study was to assess and analyze the shear bond strength of resin-bonded dentin after using dentin deproteinizing agents 5% sodium hypochlorite, 10% sodium hypochlorite, and bromelain.

In a micro-tensile test, the fracture starts at the weakest part of the bond. A disadvantage of these tests is they are highly technique sensitive. In shear bond strength, the fracture does not start at the weakest part of the bond, but always at the point of insertion of the bond. The predilection for the conventional shear test rather than for the micro-tensile shear test is justified because they are easy to perform, require basal equipment and specimen preparation. In this study, bromelain enzyme behaved superiorly which could be...
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by the agency of reduced nanoleakage as shown by the study of Dayem and Tameesh and it has improved performance in removing unsupported collagen matrix as compared to NaOCL, and lower nanoleakage is seen. This could be because of the reduction of collagen from the surface of acid-etched dentin resulting in increased permeability of dentin substrate due to enlargement of dentinal tubules near the outer dentin surface. This adds to the spreading and diffusion of adhesive monomer through the dentin.

**Conclusion**

This present study concluded that bromelain had the maximum value for shear bond strength and thus showed higher resistance...
to shear forces and the removal of unsupported collagen fibers with bromelain enzyme after acid etching results in improved bond strength and the step of deproteinization with bromelain enzyme is very important to get high adhesives quality and should be taken into consideration before applying bonding agent. Bromelain had shown significantly higher bond strength than 10% sodium hypochlorite and 5% sodium hypochlorite, therefore, can be used as a deproteinizing agent and it is a natural enzyme.

Fig. 8: Graphical representation of mean and standard deviation values of all the groups

Fig. 9: Shear bond strength of all samples in group II

Fig. 10: Shear bond strength of all samples in group III
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Clinical Significance
Elimination of collagen fiber with bromelain enzyme which is a natural pineapple enzyme after acid etching improves bond strength and completely safe.

Limitations
The occlusal reduction done to expose the dentin was enough so that it was affected or not by the acid-etching process was not

Table 4: Shear bond strength with mean and standard deviation values of group IV (Newton’s)

| S. no | KGF | Newton |
|-------|-----|--------|
| 1     | 6.2 | 60.8   |
| 2     | 3.5 | 34.4   |
| 3     | 2.4 | 23.5   |
| 4     | 4.3 | 42.1   |
| 5     | 6.5 | 43.65  |
| 6     | 6.8 | 32.48  |
| 7     | 6.8 | 66.6   |
| 8     | 7.5 | 37.45  |
| 9     | 3.6 | 35.3   |
| 10    | 4   | 28.14  |
| 11    | 3.7 | 52.79  |
| 12    | 4.3 | 36.92  |
| 13    | 7.3 | 45     |
| 14    | 7.4 | 69.43  |
| 15    | 8.6 | 38.56  |
| 16    | 7.4 | 39.67  |
| 17    | 4.1 | 26.86  |
| 18    | 6.8 | 66.6   |
| 19    | 4.1 | 40.2   |
| 20    | 8.6 | 48.29  |
| Mean  |     | 43.26  |
| SD    |     | 13.82  |

Table 5: Descriptive of shear bond strength in each group

| Gr    | N     | Mean  | Std. deviation | p value | Post hoc pairwise comparison |
|-------|-------|-------|----------------|---------|-----------------------------|
| I     | 40.00 | 67.24 | 38.23          | 0.01, S | Gr II, IV < Gr III, Gr I < Gr IV |
| II    | 40.00 | 53.48 | 25.05          |         |                             |
| III   | 40.00 | 78.51 | 41.06          |         |                             |
| IV    | 20.00 | 43.26 | 13.82          |         |                             |

Table 6: ANOVA

| Shear bond strength | Sum of squares | Df | Mean square | F        | p value |
|---------------------|----------------|----|-------------|----------|---------|
| Between groups      | 21,748.056     | 3  | 7,249.352   | 6.550    | <0.0001 |
| Within groups       | 149,406.363    | 135| 1,106.714   |          |         |
| Total               | 171,154.419    | 138|              |          |         |

Table 7: Between-group comparison of shear bond strength (Tukey’s TEST), p values of post hoc pairwise comparison by Tukey’s test

| Gr II | Gr III | Gr IV |
|-------|--------|-------|
| Gr I  | 0.260, NS   | 0.437, NS | 0.048, S     |
| Gr II | –       | 0.005, S | 0.677, NS    |
| Gr III| –       | –       | 0.001, S     |

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