
A Quantitative Comparison of Machined Commercially Pure Titanium and Titanium-Aluminum-Vanadium Implants in Rabbit Bone

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Screw-shaped implants made from rods of commercially pure titanium (grade 1) and titanium-aluminum-vanadium (grade 5) were machined, and the implant surface structures were numerically described before being placed in rabbit tibiae for healing periods of 1 month, 6 months, and 12 months. Quantitative comparisons of the removal torque (Ncm) necessary to loosen the implants from the bone bed were performed. Short-term (1 month) observations revealed no significant differences between the two tested materials. However, after 6 and 12 months, the commercially pure titanium implants were significantly more stable in the bone bed, as compared to the alloy samples. After 6 months, the commercially pure titanium had a mean removal torque of 29 Ncm versus 23 for the alloy ($P = .01$), and after 12 months, the mean removal torque was 38 Ncm for commercially pure titanium as compared to 35 Ncm for the alloy ($P = .01$). Quantifications of the bone tissue response to the materials did not show any significant differences; however, the commercially pure titanium showed a tendency to have a higher percentage of bone in contact with the implant as compared to the alloy screws. Bone volumes in the threads were similar. The absence of any quantitative light microscopic difference after 1 month following placement may relate to the fact that there was a sparse amount of bone, since the tissue was in the organization/granulation phase. After 6 and 12 months of follow-up, substantial bone formation had occurred, resulting in significantly increased removal torques for the commercially pure titanium samples. (INT J ORAL MAXILLOFAC IMPLANTS 1998;13:315-321)

Key words: bone, commercially pure titanium, histomorphometry, implants, removal torque, titanium alloy

There are many orthopedic and oral implant systems available commercially today. Biomaterials such as commercially pure (CP) titanium and its alloy, titanium-aluminum-vanadium (Ti-6Al-4V), are commonly used for manufacturing such implants.

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Clinical studies of oral implant systems reveal that CP titanium has the advantage of documented long-term clinical success. Ti-6Al-4V has been used less frequently as a material for oral implants; some designs have resulted in high failure rates,^{1,2} whereas others have indicated a positive clinical outcome at 1 to 4 years of follow-up.³ However, to the knowledge of the present authors, no controlled studies have compared the outcome of CP titanium and Ti-6Al-4V implants in a clinical setting. From a corrosion perspective, CP titanium has been reported to be the more stable material.⁴ Ti-6Al-4V is the stronger of the two materials, and therefore is frequently used for load-bearing orthopedic implants. It cannot be assumed that the two materials will show precisely the same tissue reactions. Any significant differences in bone tissue response could be of clinical relevance, particularly in compromised bone beds.

The aim of the present investigation was to perform several quantitative and qualitative experiments

with machined screw-shaped titanium implants made of commercially pure titanium and titanium-aluminum-vanadium: (1) to numerically describe the implant surface structure; (2) to biomechanically investigate the stability of the bone-implant interface by loosening the implants after various times of follow-up, using a newly developed removal torque unit; and (3) to conduct light microscopic quantitative histomorphometric and qualitative analyses of the bone adjacent to the implants.

Materials and Methods

Animals, Anesthesia, and Surgical Technique.

Thirty adult (average 10 months old) rabbits were used in this study. Group I consisted of 9 rabbits, group II of 11 rabbits, and group III of 10 rabbits. The rabbits were anesthetized with intramuscular injections of fentanyl and fluanison (Hypnorm Veterinary, Janssen Farmaceutica, Beerse, Belgium) at a dose of 0.5 mL/kg body weight and with intraperitoneal injections of diazepam (Valium, Roche, Basel, France) at a dose of 2.5 mg per animal. Local anesthesia with 1.0 mL of 5% Xylocaine (Astra, Södertälje, Sweden) was administered to the tibial metaphyses where the implants were to be placed under aseptic conditions. Prior to surgery, the shaved skin was carefully washed with a mixture of iodine and 70% ethanol. Animal sacrifice involved intravenous injections of a mixture (1:4) of saline and barbiturates (Mebumal Veterinary, 60 mg/mL, Nord Vaccin AB, Malmö, Sweden).

A total of 66 CP titanium implants and 66 Ti-6Al-4V implants, manufactured by machining, were used. Four implants were placed under aseptic conditions in each rabbit tibia: two made of CP titanium were placed in one leg, and two made of Ti-6Al-4V in the contralateral tibial metaphysis, resulting in equal numbers of CP titanium and Ti-6Al-4V implants in each group (group I, $n = 18$; group II, $n = 22$; group III, $n = 20$). A careful surgical technique using low rotary drill speeds (not exceeding 2,000 rpm) and profuse cooling with saline was followed. The implants were placed at a distance of 5 mm apart. Two threads were left visible above the cortex, and the implants were allowed to penetrate the first cortical layer only. Immediately after surgery, the animals were allowed to be fully weight-bearing. Implants were examined after 1 month, 6 months, and 12 months. This study was approved by the institutional review board and the animal ethical committee at the University of Göteborg, Sweden.

Implants and Surface Roughness Tests.

Screw-shaped implants made of commercially pure titanium (grade 1) and the alloy titanium-aluminum-

vanadium (grade 5) were manufactured manually by turning in an identical manner ($n = 66$ of each material). The total length of the implants was 8 mm (threaded 6 mm, with a distance of 600 μm between the thread peaks), the outer diameter was 3.75 mm, and the implants' square top (2 mm) was fitted to a specially constructed pin that could be connected to a removal-torque measuring instrument. The implants were cleaned in trichlorethylene and rinsed in absolute ethanol in an ultrasonic bath, and finally sterilized in an autoclave. Investigations of the microsurface structure were performed on two implants of each material from each group with an optical laser scanning for three-dimensional surface roughness measurements (Top Scan 3D, Heidelberg, Germany). All implant measurements were performed on three tops, three bottoms, and three flanks on a $245 \times 245 \mu\text{m}$ area. For numerical description of the surface topography, the following height-descriptive surface roughness parameters were used: Sa, to describe the average height deviation from the mean plane; Scx, for the peak distance in spatial direction; and Sdr, to describe the surface developed ratio.⁵

Torque Measurement and Preparation of Specimens.

On the day of animal sacrifice, all of the rabbits were anesthetized as described above and the skin and fasciae were opened. All correspondingly placed implants were subjected to removal torque tests. In one case only (one rabbit in the 6-month group) all of the implants were tested for removal torque. All of the biomechanical tests were performed using newly developed strain gauge electronic removal torque equipment (Detektor AB, Göteborg, Sweden) that enables direct readings of the necessary loosening torque in newton centimeters (Ncm). This equipment has been developed to achieve a high reproducibility with minimal influence of handling by the operator compared to previously used manual techniques.⁶

Group I (1 month follow-up) consisted of 9 samples of each material for removal torque tests. In group II (6 months follow-up), 12 implants of each material were torque tested, since both the distal as well as the proximal samples were tested in one animal. In group III (12 months follow-up), 10 implants of each material were torque tested.

The proximal implants were removed en bloc for later histomorphometric and qualitative light microscopic analysis. Each implant with surrounding bone was fixed in 4% neutral buffered formaldehyde (pH 7.0) and further processed to be embedded in light-curing resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). Undecalcified ground sections of a final thickness of 10 μm were obtained using an

Exakt sawing machine and grinding equipment (Exakt Apparatebau, Norderstedt, Germany).⁷ The sections were routinely stained with a mixture of toluidine blue and pyronin G prior to qualitative and quantitative light microscopic investigations. Computer-based (Leitz Microvid equipment connected to a personal computer and a mouse) histomorphometric measurements of the bone-metal contact (bmc) and the bone volume/area surrounding the implant were performed in the eyepiece of a Leitz Aristoplane light microscope using a 10× objective and a zoom of 2.5× (Leitz, Wetzlar, Germany). The histomorphometric investigations were performed around the entire implant as well as in the three best consecutive threads (n = 6 threads on each implant) in the cortical region.⁸

Statistics. Wilcoxon's signed rank test was used for statistical comparisons within the groups.

Results

Surface Roughness. The results from the Top Scan 3D surface structure analysis demonstrated that the CP titanium implants had a more pronounced height deviation as demonstrated by the parameter Sa. The Sa value for the CP titanium samples was 0.74, as compared to 0.58 for the Ti-6Al-4V samples. The CP titanium implants also had a more developed surface area ratio, while the average space between the surface irregularities was very similar. The surface roughness results are summarized in Table 1.

Removal Torque. Comparisons of the removal torque results from the 1 month group (group I) did not reveal any statistically significant differences; the mean removal torque for the CP titanium group was 13 Ncm ± 4.0 (range 5 to 14), versus 14 Ncm ± 1.3 (range 12 to 16) for the alloy samples.

At 6 months follow-up (group II), the mean removal torque was 30 Ncm ± 6.1 (range 20 to 40) for the CP titanium group, versus 24 Ncm ± 6.7 (range 15 to 35) for the alloy. This result was statistically significant ($P = .01$).

After a placement time interval of 12 months (group III), the mean removal torque was 38 Ncm ± 9.4 (range 27 to 57) for CP titanium, as compared to 35 Ncm ± 8.4 (range 26 to 51) for the alloy. Also in this case, a statistically significant difference was obtained ($P = .01$). The removal torque results are summarized in Fig 1.

Histomorphometric Data. Histomorphometric quantifications of bone tissue response to the materials did not show any significant differences; however, there was a tendency for CP titanium to have a higher percentage of bone in contact with the implant as compared to the Ti-6Al-4V implants.

Table 1 Mean Surface Roughness (μm) at Nine Sites on Two Implants From Each Group

Biomaterial	Surface roughness parameters (SD)*		
	Sa	Scx	Sdr
CP titanium	0.74 (0.21)	8.73 (1.21)	1.22 (0.07)
Ti-6Al-4V	0.58 (0.20)	8.48 (0.83)	1.16 (0.07)

*All measurements performed with a Top Scan 3D instrument. Sa describes the height deviation from the mean plane. Scx is the average distance between the surface irregularities in spatial direction. Sdr describes the surface developed ratio.

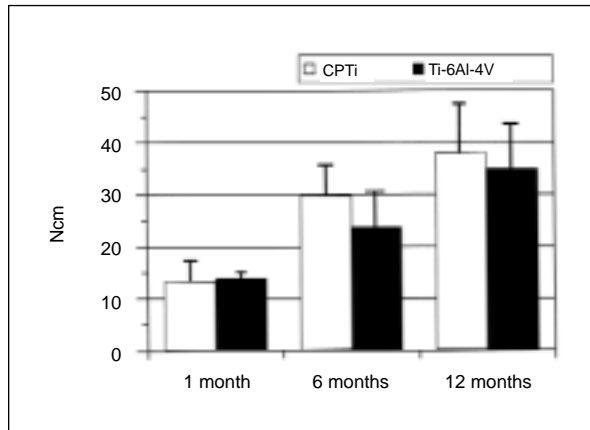


Fig 1 Mean removal torque for implants in group I (1 month after placement), group II (6 months after placement), and group III (12 months after placement).

Bone-Metal Contact. Group I. The 1-month samples demonstrated a mean percentage of bone-metal contact around the entire CP titanium implant to be 10 ± 5.9 (range 5 to 23), compared to 7 ± 4.5 (range 2 to 17) for the alloy samples. The bone-metal contact, as deduced from the three best consecutive threads in the cortical region, demonstrated a mean percentage of 20 ± 10 (range 12 to 43) for CP titanium, compared to 17 ± 8.6 (range 5 to 35) for the alloy.

Group II. The corresponding numbers for the total/entire bone-metal contact in the 6-month group were 27 ± 6.2 (range 17 to 35) for CP titanium and 24 ± 7.8 (range 15 to 37) for the alloy samples. The results from the three best consecutive threads in the cortical region in group II demonstrated a mean percentage of 42 ± 8.1 (range 27 to 53) for the CP titanium samples versus a mean percentage of 39 ± 11.3 (range 24 to 59) for alloy implants.

Group III. The group followed for 12 months revealed a mean percentage of bony contact around the entire CP titanium implant of 31 ± 6.3 (range 22 to 43), compared to a mean percentage of 22 ± 4.9

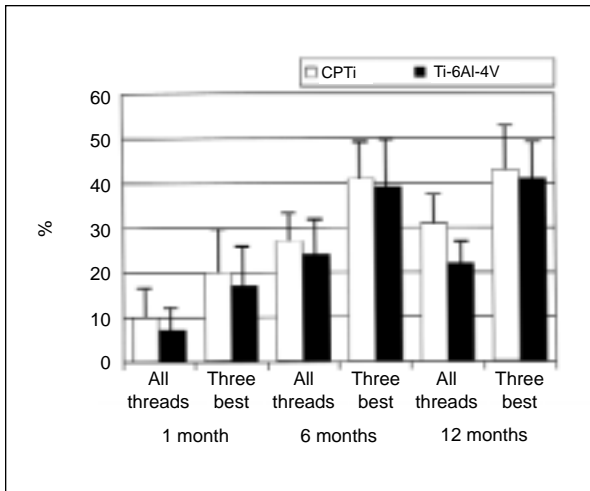


Fig 2 Mean bone-metal contact around the entire implant and in the three best consecutive threads in the cortical region. There were no statistically significant differences between the materials at any time of follow-up.

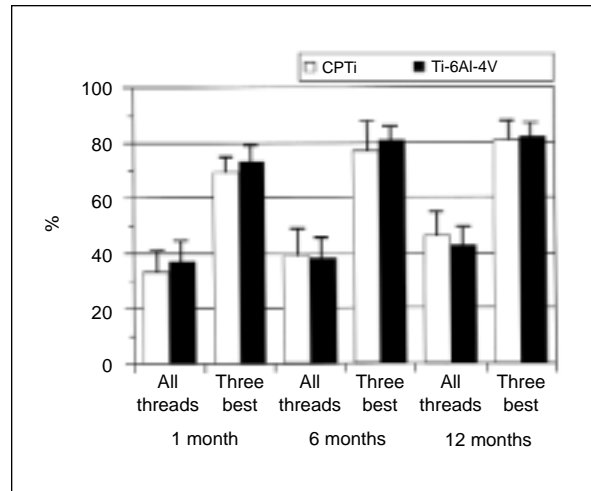


Fig 3 Mean percentage of bone volume in the threads around the entire implant and in the three best consecutive threads in the cortical region. There were no statistically significant differences between the materials at any time of follow-up.

(range 16 to 32) for the alloy samples. The mean percentage of the bone-metal contact in the three best consecutive threads in the cortical region was 43 ± 9.5 (range 30 to 57) for CP titanium, compared to 41 ± 8.5 (range 25 to 57) for the alloy samples.

The histomorphometric results from bone-metal contact measurements are presented in Fig 2.

Bone Area. Group I. Comparison of the entire bone volume after 1 month from placement demonstrated a mean percentage of 33 ± 6.8 (range 24 to 42) for CP titanium and 37 ± 7.2 (range 27 to 46) for the alloy implants. Corresponding numbers at the three best consecutive threads in the cortical region were 69 ± 4.8 (range 60 to 74) for CP titanium and 73 ± 5.7 (range 64 to 82) for the alloy samples.

Group II. The mean percentage of bone volume after 6 months from placement around CP titanium screws was 39 ± 10 (range 19 to 50), compared to 38 ± 7.7 (range 24 to 52) for the alloy screws. Corresponding numbers at the three best consecutive threads in the cortical region were 77 ± 10.2 (range 54 to 87) for CP titanium, compared to 81 ± 4.7 (range 72 to 89) for alloy samples.

Group III. The mean percentage of bone volume in the 12-month group around the entire CP titanium implants was 46 ± 8.5 (range 35 to 63), compared to 43 ± 7.2 (range 32 to 56) for the alloy. Results from the measurements at the three best consecutive threads in the cortical region were 81 ± 6.7 (range 71 to 92) for CP titanium and 82 ± 5.2 (range 75 to 92) for alloy.

Mirror-image analyses, ie, comparison of the bone volume inside the three best consecutive threads to the bone volumes immediately outside the same threads, did not reveal any statistically significant differences within the material or between the two materials tested.

The histomorphometric results from bone volume measurements are presented in Fig 3.

Light Microscopy. Qualitative light microscopic investigations of the tissue response to the materials did not reveal any differences between CP titanium and alloy implants at similar times of observation.

Group I. The group I samples, followed for 1 month, were immature as compared to the samples followed for 6 and 12 months. The longer the time from placement, the more periosteal and endosteal bone growth was observed. The sections from group I also demonstrated a large callus formation on both the periosteal and endosteal sides (Fig 4). The bone structure was very irregular and woven, and it revealed some relatively acellular zones of tissue in the interface, which was clearly demarcated from the original cortical bone. In other regions of the interface, more damaged areas were observed, as was a loose tissue structure. Cells such as macrophages and giant cells were occasionally observed in this damaged interface tissue.

Group II. In the sections from group II (6 months), a more mature endosteal and periosteal bone formation was observed (Fig 5) compared to the 1-month samples. In some of the sections, espe-

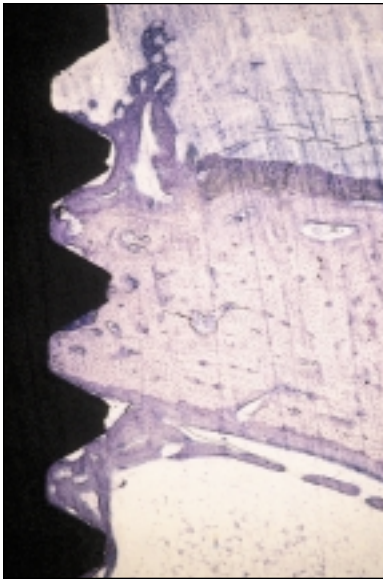


Fig 4 Survey appearance from a ground section representing a titanium implant 1 month after placement in rabbit cortical bone. The diameter of the implant is 3.75 mm and the total length is 8 mm. The distance between the thread peaks is 600 μ m. Periosteal and endosteal new bone formation is clearly visible.

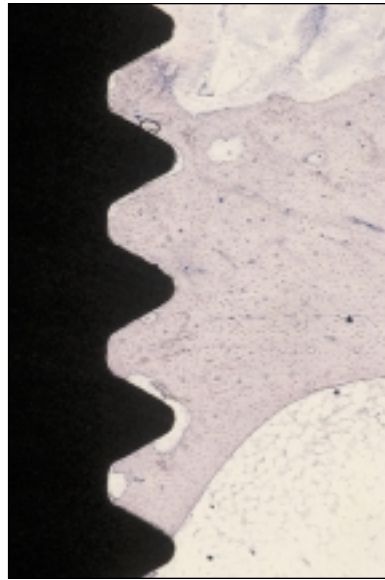


Fig 5 Survey appearance from a ground section representing a titanium implant 6 months after placement in rabbit cortical bone. The diameter of the implant is 3.75 mm and the total length is 8 mm. The distance between the thread peaks is 600 μ m.

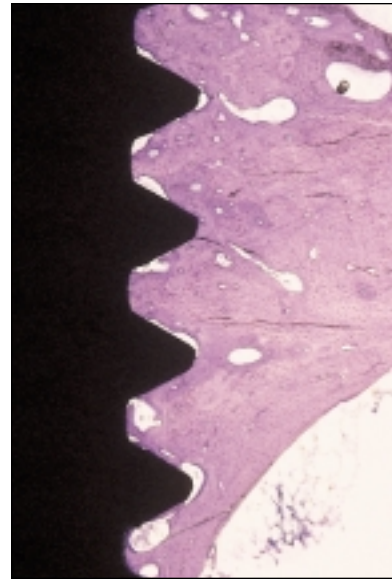


Fig 6 Survey appearance from a ground section representing a titanium implant 12 months after placement in rabbit cortical bone. The diameter of the implant is 3.75 mm and the total length is 8 mm. The distance between the thread peaks is 600 μ m.

cially in the endosteal region, formation of new bone was visible as thin shells inside the threads around almost the entire implant. Evidence of bone formation and resorption was observed in the interface. In these areas, multinucleated giant cells and osteoclasts were visible on bone surfaces.

Group III. The sections from group III (12 months) demonstrated a larger volume of mature bone around the implants (Fig 6) and in the interface compared to the 6-month group. Haversian systems were observed close to the implant surface (Fig 7). In some interface areas, bone remodeling was evident.

Discussion

The present study has demonstrated a correlation between length of placement time and implant stability. In this study, implants were placed for 1, 6, and 12 months. The longer the time of incorporation, the higher the removal torque when detaching the implants from the bone bed. These results concur with those from an earlier study, performed on CP titanium implants only, where the implants were followed for 3 weeks and for 1, 3, 6, and 12 months.⁹

The data from the present study as well as from earlier ones⁹ related to short-term investigations (up to 1 month) did not demonstrate quantitative

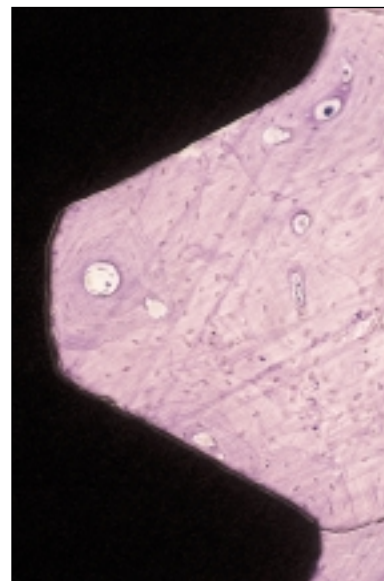


Fig 7 Haversian systems were observed close to the implant surfaces 12 months after the time of placement. The distance between the thread peaks is 600 μ m.

differences between CP titanium and the alloy. This result probably reflects the situation at the interface in the injury and granulation phase,^{10,11} ie, the tissue had only started to remodel after the primary drilling of the bone.

In the present study, the commercially pure titanium implants were significantly more stable in the bone bed after 6 and 12 months than were the Ti-6Al-4V implants. In an earlier study¹² in which similar implants were followed up to 3 months, CP titanium screws were significantly more stable in the bone bed than the alloy implants. Furthermore, data confirming a stronger bone response to CP titanium compared to Ti-6Al-4V implants of slightly altered designs have been reported in yet other studies.¹³ These findings indicate that removal torque tests may be more sensitive for evaluating implant stability than light microscopic quantifications with the cutting and grinding technique. The latter examinations are performed on a single section, that is, in one plane, whereas the torque tests represent the three-dimensional *in vivo* situation, thus perhaps providing a more accurate picture with respect to the real integration of the implant in bone.

To investigate why there are different responses to CP titanium and alloy samples *in vivo*, the possibility of ionic leakage phenomena occurring from the implants into the surrounding tissues has been investigated. For this purpose, special ground sections of the implants with surrounding bone have been studied, enabling proton induced x-ray emission (PIXE) analysis. Recent results indicate similar amounts of titanium ions outside both types of implants, decreasing with increasing distance from the screws. Vanadium ions have also been detected in these experiments.¹⁴ These results are in agreement with those from a 1992 study,¹² in which ionic leakage, including aluminum, outside similar implants followed for 3 months was reported with the secondary ion mass spectrometry (SIMS) technique.

Several reports concerning inhibition of bone formation caused by various substances associated with implants may be found in the literature. Recent reports from *in vitro* studies¹⁵ indicate that cells may be "damaged" when placed in contact with particles of, for example, titanium alloy. Thompson and Puleo^{16,17} demonstrated in an *in vitro* study that the metal ions Ti⁴⁺ and Al³⁺ (ions associated with Ti-6Al-4V) may inhibit the normal differentiation of bone marrow stromal cells to mature osteoblasts. The authors suggested that these ions, when released *in vivo*, may "contribute to implant failure by impairing normal bone deposition." Other *in vitro* studies claim that titanium, aluminum, and vanadium ions may inhibit hydroxyapatite formation, ie, disturbing the bone

mineralization.^{18,19} According to the *in vitro* study by Haynes et al,²⁰ titanium-aluminum-vanadium particles cause release of more inflammatory mediators involved in bone resorption and osteolysis compared to cobalt-chromium particles. The latter particles were found to be very toxic to macrophages, whereas the former titanium alloy particles were "only minimally toxic."

Summary

Screw-shaped implants made of commercially pure titanium and titanium-aluminum-vanadium have demonstrated different biomechanical results after 1, 6, and 12 months following placement in rabbit bone. Titanium was significantly stronger in its attachment to the tissue than was the titanium alloy, when tested by removal torque. Histomorphometric results revealed a higher percentage of bone in contact with CP titanium compared to the alloy implants. One important cause for these differences may be ionic leakage phenomena. However, it must be remembered that results of animal experiments need not necessarily reflect the human clinical reality.

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