Persistent Acute Inflammation at the Implant-Abutment Interface

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J DENT RES 2003 82: 232
DOI: 10.1177/154405910308200316

RESEARCH REPORTS
Biomaterials & Bioengineering

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J Dent Res 82(3):232-237, 2003

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ABSTRACT

The inflammatory response adjacent to implants has not been well-investigated and may influence peri-implant tissue levels. The purpose of this study was to assess, histomorphometrically, (1) the timing of abutment connection and (2) the influence of a microgap. Three implant designs were placed in the mandibles of dogs. Two-piece implants were placed at the alveolar crest and abutments connected either at initial surgery (non-submerged) or three months later (submerged). The third implant was one-piece. Adjacent interstitial tissues were analyzed. Both two-piece implants resulted in a peak of inflammatory cells approximately 0.50 mm coronal to the microgap and consisted primarily of neutrophilic polymorphonuclear leukocytes. For one-piece implants, no such peak was observed. Also, significantly greater bone loss was observed for both two-piece implants compared with one-piece implants. In summary, the absence of an implant-abutment interface (microgap) at the bone crest was associated with reduced peri-implant inflammatory cell accumulation and minimal bone loss.

KEY WORDS: one-piece implant, two-piece implant, peri-implant inflammation, microgap, neutrophil

Received March 22, 2002; Last revision November 1, 2002; Accepted November 25, 2002

INTRODUCTION

Two main dental implant designs have emerged from the original Brånemark and Schroeder studies (Brånemark *et al*., 1969; Schroeder *et al.*, 1981), *i.e.*, the two-piece, submerged implant and the one-piece, non-submerged implant, respectively. In the conventional submerged technique, the top of the implant is placed at the level of the alveolar crest, and abutment connection 3 to 6 months later creates an implant-abutment interface (microgap) at the bone level. In contrast, the conventional non-submerged approach positions the top of the implant above the bone crest and by design requires no secondary surgery. In recent years, clinicians have performed abutment connection to conventionally submerged implants during initial surgery to avoid a second surgical procedure (Ericsson *et al*., 1997; Kupeyan and May, 1998; Roynesdal *et al.*, 1999). However, a microgap still exists at the level of the alveolar crest. In this paper, the nomenclature of "submerged" and "non-submerged" will be used under the context of **surgical technique**, while "one-piece" and "two-piece" will refer to the **implant design**, *i.e.*, two-piece implants have a microgap placed at the alveolar crest level, whereas one-piece implants are continuous at this location.

To date, the central focus of long-term human studies (Cochran, 1996) using either conventionally submerged or non-submerged implants has been *osseointegration* (Brånemark *et al*., 1977), also known as *functional ankylosis* (Schroeder *et al*., 1981). In spite of comparable clinical success rates for hard-tissue integration of various implant designs (Adell *et al*., 1981; Buser *et al*., 1997, 1999; Lekholm *et al*., 1999), the consequences of a microgap at the alveolar bone level are not well-elucidated with respect to peri-implant inflammation, nor is it clear whether the elimination of a second surgical procedure in conventional two-piece, submerged implants renders a different peri-implant soft-tissue response.

Previous studies utilizing two-piece implants have documented that peri-implant soft tissues develop a zone of inflammatory cells in connective tissue below gingival epithelium in the presence of plaque (Berglundh *et al*., 1992; Ericsson *et al.*, 1992); however, the nature and extent of inflammatory cells along the entire implant surface under normal hygiene conditions remain to be established. The purpose of this study was to determine how changes in abutment connection timing (submerged *vs.* non-submerged two-piece implants) or the presence of a microgap (two-piece, non-submerged implants *vs*. one-piece, non-submerged implants) influences the composition of inflammatory cells immediately adjacent to the implant.

MATERIALS & METHODS

Implant Design and Placement

Histologic specimens of three implant designs (two-piece, submerged; two-piece, non-submerged; and one-piece, non-submerged) were procured from a larger study involving 6 different implant types as previously described (Hermann *et al.*, 1997, 2000). In brief, following the approval of the study protocol by the Institutional Animal Care and Use Committee (University of Texas Health Science Center at San Antonio), various experimental implant designs (all sandblasted/acid-etched surfaces) were placed in the edentulous mandibular regions of 5 foxhound dogs. Each implant type was included in duplicate for each animal with random placement in both the left and right mandibles. Mechanical and chemical plaque control were performed 3 times a week by a soft toothbrush and a soft sponge in combination with a 0.2% chlorhexidine gel (PlakOut Gel, Hawe-Neos AG, Bioggio/TI, Switzerland). Abutment connection was performed at the time of initial surgery for two-piece, non-submerged implants and 3 mos after initial implant placement for two-piece, submerged implants. At 4, 8, and 10 wks following second-stage surgery of submerged implants, abutments were loosened and then immediately tightened in *all* two-piece implants to mimic clinical restorative procedures. Six months after initial implant placement, tissues were obtained and histologic specimens were prepared as previously described (Schenk *et al.*, 1984).

Histomorphometric Analyses

Histomorphometry was performed by means of a light microscope (Vanox-T®, Olympus, Tokyo, Japan) connected to a high-resolution video camera (CCD-Iris® Color Video Camera, Sony Corp., Fujisawa, Japan) that was linked to a monitor (Multisync® XV17+, NEC, Itasca, IL) and personal computer. Histomorphometric software (Image-Pro Plus®, Media Cybernetics, Silver Spring, MD, USA) facilitated digital image capture as well as manual counting of individual inflammatory cells and measurement of tissue area.

Sequential peri-implant test fields (0.25 mm x 0.33 mm) along the entire implant surface were evaluated (Fig. 1). Each field (0.0825 mm^2) was digitally captured at high magnification (x 340). The original bone crest was designated as the histological reference point for all implants. For two-piece implants, this reference point corresponded to the implant-abutment interface. For one-piece implants, the same reference point corresponded to 1 mm above the rough/smooth border of the sandblasted/acid-etched (SLA) and machined surfaces. In each 0.0825-mm^2 field, neutrophils and mononuclear cells (lymphocytes, plasma cells, monocytes, and macrophages) were counted. Inflammatory cells within the vessels were excluded. In addition, areas of alveolar bone and gingival or junctional epithelium were eliminated. Thus, only extravascular (interstitial) tissues were assessed. This area of interstitium was then used to calculate cell density, *i.e.*, cells/mm^2, for each field along the implant surface. Linear soft-tissue distances were calculated by summation of the apico-coronal dimension from all captured test fields (0.25 mm). Through this histomorphometric approach, the distance below the microgap for two-piece implants (submerged and non-submerged) confirmed results of a previously published study (Hermann *et al.*, 2000) that evaluated bone loss with the use of these same histological specimens.

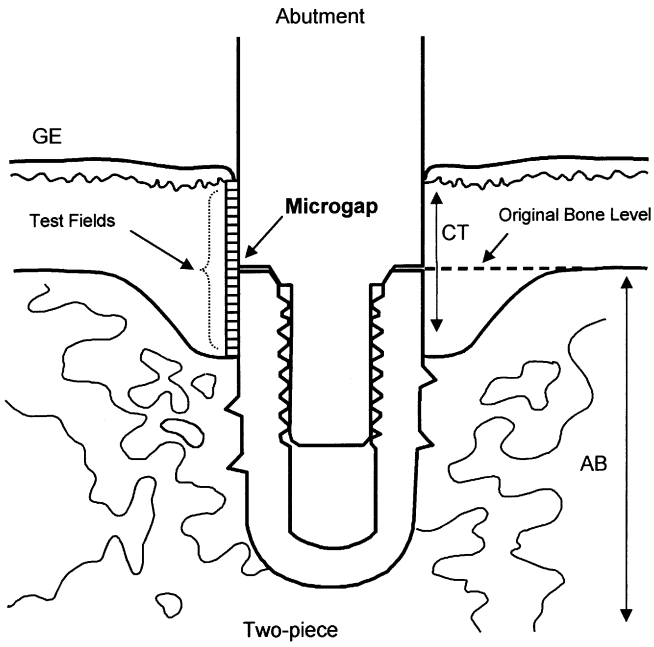

Figure 1. Histomorphometry schematic. Morphometric assessment of peri-implant tissue was confined to connective tissue immediately adjacent to the implant surface and extended from gingival epithelium to alveolar bone. For a given specimen, sequential images of the entire apico-coronal peri-implant soft tissue (0.0825 mm^2; 0.33 x 0.25 mm rectangles) were digitally captured for subsequent evaluation relative to the microgap or original bone crest (for one-piece implants). All interstitial cells were examined; for inflammatory cell quantitation, only neutrophils and mononuclear cells were included. For the latter, lymphocytes, plasma cells, monocytes, and macrophages were collectively considered as a single population of cells. GE = gingival epithelium; CT = connective tissue; AB = alveolar bone.

Data Analysis

Since each implant type was placed in duplicate *per* animal (right and left sides), results from duplicate specimens were averaged. For each variable, these were then used to calculate descriptive statistics among all animals (mean $\pm$ SEM; n = 5). We utilized analysis of variance for repeated measures with Tukey's multiple-comparison test of least-square means to determine whether significant differences existed among implant types. These analyses included adjustments for variability in implant and animal. SAS software (SAS, Cary, NC, USA) was used for all statistical analyses; p values of $\leq$ 0.05 were considered significant.

RESULTS

In peri-implant soft tissues of both two-piece implants, a considerable cellular infiltrate was concentrated at a level immediately coronal to the implant-abutment interface (microgap) and appeared to decrease gradually and progressively in soft tissues toward either bone or gingival epithelium. In contrast, sparse cells were present in the soft tissues adjacent to one-piece implants (data not shown).

Two-piece Implants

The distribution of peri-implant inflammatory cells was analogous for submerged and non-submerged two-piece

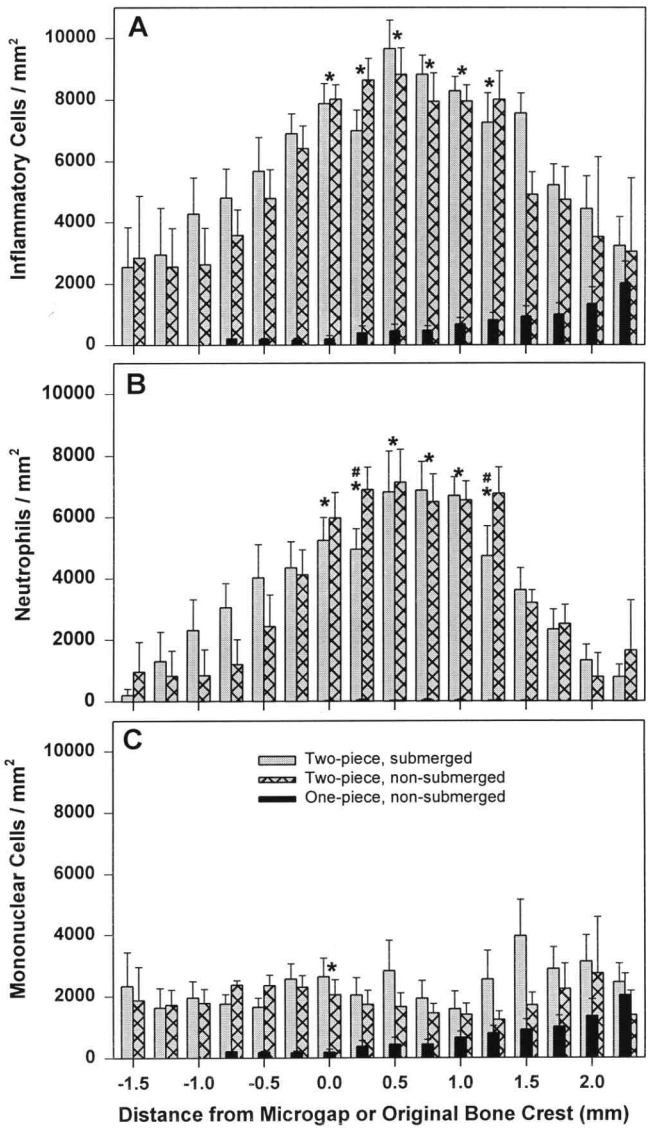

Figure 2. Distribution of peri-implant inflammatory cells among different implant designs. Histomorphometric quantitations of neutrophils and mononuclear cells at specific locations relative to the original alveolar bone were averaged for a given implant type in each animal; these results were then used to calculate the group mean (± SEM; n = 5). Comparisons were made with respect to **(A)** total inflammatory cells, **(B)** neutrophils, and **(C)** mononuclear cells. For corresponding locations relative to the original alveolar bone crest, * indicates significant differences ($p < 0.01$) between non-submerged two-piece and one-piece implant designs, and # indicates significant differences ($p < 0.05$) between submerged and non-submerged two-piece implant designs.

implants (Fig. 2A). In each implant type, the peak density of inflammatory cells occurred 0.5 mm coronal to the microgap (9642 ± 946 cells/mm^2 and 9103 ± 664 cells/mm^2 for submerged and non-submerged, respectively); there were no significant differences in the density of total inflammatory cells at equivalent peri-implant locations. Among individual animals, the maximum density and location of inflammatory cells were comparable (Table 1). The cumulative sum of peri-implant inflammatory cells (1) coronal to the microgap, (2) apical to the microgap, or (3) for the entire peri-implant soft-tissue distance from bone to gingival epithelium revealed no significant differences between these two-piece implant types (Table 2).

Table 1. Effects of Implant Design and Placement on the Maximum Density and Corresponding Location of Peri-implant Cells

	Maximum Cell Density (cells/mm^2)	Distance from Original Bone Crest (mm)[a]
Total cells		
Two-piece, submerged	10,542 ± 847[b,c]	0.48 ± 0.26[c]
Two-piece, non-submerged	10,054 ± 637[c]	0.45 ± 0.12[c]
One-piece, non-submerged	2,680 ± 410	2.08 ± 0.24
Neutrophils		
Two-piece, submerged	8,276 ± 1030[c]	0.35 ± 0.13[c]
Two-piece, non-submerged	8,712 ± 721[c]	0.73 ± 0.13[c]
One-piece, non-submerged	92 ± 47	1.70 ± 0.44
Mononuclear cells		
Two-piece, submerged	5,451 ± 1009[c]	0.25 ± 0.45[c]
Two-piece, non-submerged	3,789 ± 528	(-) 0.15 ± 0.37[c]
One-piece, non-submerged	2,665 ± 415	2.08 ± 0.24

[a] Reference point (original alveolar bone level) corresponded to the implant-abutment interface of two-piece implants and 1 mm above the rough-smooth border of one-piece implants. "(-)" refers to a location apical to this reference point.
[b] Results presented as the mean ± SEM; n = 5 animals/group.
[c] Significantly different from one-piece, non-submerged implants ($p < 0.05$).

For both two-piece implants, the distribution of neutrophils in peri-implant soft tissues closely patterned that of total cells (Fig. 2B). There were no significant differences in the cumulative sum of neutrophils for two-piece implants (Table 2). Thus, the major cell type constituting the inflammatory cell infiltrate for both submerged and non-submerged two-piece implants was the neutrophil (Table 2). Mononuclear cells were evenly distributed along implant surfaces for both two-piece implants (Fig. 2C). Further, the cumulative numbers and density of mononuclear cells were not significantly different between either two-piece design (Tables 1, 2). Finally, there were no significant differences between submerged and non-submerged two-piece designs regarding (1) the entire soft-tissue distance between alveolar bone and gingival epithelium; (2) the soft-tissue distance above the microgap; and (3) bone loss as measured from the microgap to alveolar bone level (Table 2).

One-piece *vs.* Two-piece Implants

There was no discrete peak of inflammatory cells adjacent to one-piece implants (Fig. 2A). Moreover, there were significant differences in the accumulation of total inflammatory cells

between two-piece and one-piece implant types (Fig. 2A). Indeed, inflammatory cell infiltration for all corresponding locations was significantly greater for two-piece implants as compared with one-piece implants. Correspondingly, the cumulative numbers of peri-implant inflammatory cells were significantly greater for two-piece, non-submerged implants as compared with one-piece, non-submerged implants (Table 2).

In parallel with the above, there was no selective neutrophil accumulation adjacent to one-piece implants (Fig. 2B; Table 2), and mononuclear cells were significantly reduced (Fig. 2C). Thus, the cumulative sum of mononuclear cells in two-piece implants was significantly increased as compared with one-piece implants (Table 2). Although the accumulation of mononuclear cells apical to the original bone crest was greater in two-piece implants, the cumulative sum coronal to this reference point was not significantly different between implants (Table 2).

The connective tissue distance adjacent to one-piece implants was significantly less than for two-piece implants (Table 2). Bone loss, *i.e.*, connective tissue distance below the original bone crest, was significantly greater for two-piece implants as compared with that of one-piece implants. Conversely, the soft-tissue distance above the original bone crest was significantly greater for one-piece implants as compared with two-piece implants, in spite of a smaller overall connective tissue distance for the one-piece, non-submerged design.

Table 2. Effects of Implant Design and Placement on the Cumulative Sum of Peri-implant Inflammatory Cells and Apico-Coronal Connective Tissue Distance

	Entire Distance (n = 5)	Above the Original Bone Crest[a] (n = 5)	Below the Original Bone Crest[a] (n = 5)
Total cells			
Two-piece, submerged	4677 ± 654[b,c]	2676 ± 474[c]	2001 ± 338[c]
Two-piece, non-submerged	4238 ± 492[c]	2347 ± 230[c]	1890 ± 386[c]
One-piece, non-submerged	583 ± 118	564 ± 118	25 ± 8
Neutrophils			
Two-piece, submerged	2968 ± 280[c] (63 ± 4)[d,e]	1795 ± 259[c] (64 ± 7)[e,f]	1173 ± 198[c] (58 ± 5)[e]
Two-piece, non-submerged	2826 ± 346[c] (67 ± 4)[e]	1865 ± 232[c] (79 ± 5)[e]	961 ± 240[c] (52 ± 4)[e]
One-piece, non-submerged	11 ± 7 (1 ± 1)	10 ± 7 (1 ± 1)	1 ± 1 (2 ± 2)
Mononuclear cells			
Two-piece, submerged	1709 ± 471[c] (37 ± 4)[e]	881 ± 318 (36 ± 7)[e]	828 ± 190[c] (42 ± 5)[e]
Two-piece, non-submerged	1412 ± 285[c] (33 ± 4)[e]	482 ± 130 (21 ± 5)[e]	929 ± 169[c] (48 ± 4)[e]
One-piece, non-submerged	572 ± 111 (99 ± 1)	554 ± 111 (99 ± 1)	24 ± 7 (98 ± 2)
Connective tissue distance (mm)			
Two-piece, submerged	3.45 ± 0.26[g]	1.85 ± 0.24[g]	1.60 ± 0.17[g]
Two-piece, non-submerged	3.48 ± 0.30[g]	1.78 ± 0.20[g]	1.70 ± 0.14[g]
One-piece, non-submerged	2.88 ± 0.20	2.40 ± 0.23	0.48 ± 0.13

[a] Reference point (original alveolar bone level) corresponded to the implant-abutment interface of two-piece implants and 1 mm above the rough-smooth border of one-piece implants.
[b] Results from duplicate implants in each animal were averaged. These results were then used to calculate a group average and are presented as the mean ± SEM; n = 5 animals/group.
[c] Significantly different from one-piece, non-submerged implants ($p < 0.02$).
[d] Number in parentheses represents results expressed as a percentage of total cells.
[e] Significantly different from one-piece, non-submerged implants ($p < 0.0001$).
[f] Significantly different from two-piece, non-submerged implants ($p < 0.04$).
[g] Significantly different from one-piece, non-submerged implants ($p < 0.01$).

DISCUSSION

This study has provided histomorphometric evidence that a unique pattern of inflammatory cell infiltrate develops adjacent to implants and varies on the basis of implant design. In brief, an intense inflammatory cell infiltrate (predominantly neutrophils) and significant bone loss were associated with the presence of a microgap at the bone crest, regardless of surgical technique, *i.e.*, submerged or non-submerged. Conversely, minimal inflammatory cell infiltrate (predominantly mononuclear cells) and minimal bone loss were observed adjacent to one-piece implants. Thus, the presence of a microgap at the level of alveolar bone was associated with persistent inflammation and increased alveolar bone loss.

The association of neutrophils with the implant-abutment interface of two-piece implants suggests that this physical attribute of implant design contributes to the recruitment of these cells when located at alveolar bone. Significant and comparable inflammatory cell infiltrates were associated with the presence of a microgap at the bone crest regardless of the timing of abutment connection (immediately or delayed) but were not observed in the absence of a microgap. The differential pattern of peri-implant neutrophil accumulation suggests that a chemotactic stimulus originating at or near the microgap of two-piece implants initiates and sustains recruitment of inflammatory cells. Since the presence of microbes at the internal aspects of implant components (Quirynen and van Steenberghe, 1993; Persson *et al.*, 1996; O'Mahony *et al.*, 2000) as well as the phenomenon of microbial and/or fluid leakage through the implant-abutment interface (Quirynen *et al.*, 1994; Jansen *et al.*, 1997; Gross *et al.*, 1999) have been previously described, it is conceivable that microbes from the oral cavity could gain access and provide such a stimulus. Consequently, activation of the host defense system (*e.g.*, antibody, complement, and cytokines) would initiate a

differential host response and result in a gradient of inflammatory cells around the microgap. This scenario, together with inherently restricted access of host defense mechanisms to the microgap, could perpetuate an acute inflammatory process and be further exacerbated by limited access for effective oral hygiene. Thus, this aspect of implant design may create a reservoir of bacteria and possibly facilitate the development of peri-implant inflammation. It is unknown whether different implant-abutment connections, such as an internal cone, would yield a different distribution or intensity of inflammatory cell recruitment as compared with the flat, butt-joint interface used in the present study. However, since microleakage is unavoidable among current implant systems, regardless of the connection type or interface size (Jansen *et al*., 1997; Gross *et al*., 1999), it would appear that one could expect a similar inflammatory response for any interface located at alveolar bone.

In addition to an intense inflammatory process, significantly greater bone loss was observed around two-piece implants as compared with one-piece implants. A relationship between inflammatory events and bone loss seems likely (Assuma *et al*., 1998; Graves *et al*., 1998). In this regard, we noted a positive trend between the accumulation of inflammatory cells apical to the microgap and the degree of alveolar bone loss, although significance could not be established given the relatively small number of animals in this study.

These results from the present study may have important clinical implications in aspects of optimal implant placement and design. While longitudinal descriptive studies (Adell *et al*., 1981; Apse *et al*., 1991; Nevins and Langer, 1993) have indicated that implants may remain functional for extended intervals, conventional success criteria for acceptable bone loss have included 1.5 mm during the first year of function (after prosthesis connection) and < 0.2 mm every year thereafter (Adell *et a*l., 1981; Albrektsson *et al*., 1986). This criterion had evolved from consistent observations of such bone loss around submerged implants with an implant-abutment interface placed at the level of bone (Brånemark *et al*., 1969). This phenomenon around two-piece implants was also demonstrated in later studies (Ericsson *et al*., 1996; Hermann *et al*., 1997) and confirmed by the present study. Although remaining functional, the possible sequelae of bone loss may not meet subjective and exacting esthetic goals. For example, it has been shown that peri-implant bone loss may lead to proportional soft-tissue recession (Hermann *et al*., 2001), as indicated in a study of natural dentition in patients which found less predictability of papilla height with greater distances between the contact point and crest of bone (Tarnow, 1992). Indeed, our own results demonstrated that, with a higher bone level maintained adjacent to one-piece implants, the soft-tissue distance above the original bone crest was significantly greater as compared with two-piece implants. These findings may motivate a clinician to place an implant shoulder above the alveolar crest or to utilize a one-piece implant to minimize potential inflammation and/or possible hard- or soft-tissue loss.

Based on the findings of the current study, we hypothesize that the creation of a microgap at the bone level leads to microbial leakage and a persistent bacterial presence at this peri-implant location. The chemotactic stimuli originating from the microgap then promote sustained neutrophil accumulation. In parallel, mononuclear cells are recruited to the implant surface. The combined and sustained activation of inflammatory cells promotes osteoclast formation/growth and activation to result in alveolar bone loss.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the statistical assistance kindly provided by Dr. Thomas Prihoda and the graphic services rendered by Nancy Place (University of Texas Health Science Center, San Antonio, TX, USA). We also are very grateful to Karen Lucas for excellent assistance in the preparation of this manuscript. This project was supported by grant #02-94/061 from the International Team for Oral Implantology Foundation. Experimental implants were provided by the Institut Straumann AG.

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