Odontoclastic Resorption at the Pulpal Surface of Coronal Dentin Prior to the Shedding of Human Deciduous Teeth

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Summary. Histological and histochemical observations of more than 150 extracted human deciduous teeth revealed that, prior to shedding, odontoclastic resorption as a rule takes place at the pulpal surface of coronal dentin. We also found that this phenomenon occurs in all kinds of deciduous teeth. The process of this internal resorption of coronal dentin of deciduous teeth clearly showed time-related histological changes. During the time the roots were actively being resorbed, the pulpal tissue retained its normal structure. However, when root resorption neared completion, inflammatory cells started to gradually infiltrate into the pulp, and odontoblasts began to degenerate. After that, multinucleate odontoclasts appeared, and resorption proceeded from the predentin to the dentin. The odontoclastic activity was initially detected only on the pulpal surface at the bottom areas of the crown. It gradually spread towards the pulpal horn regions along the wall of the pulp chamber. However, this internal resorption of coronal dentin did not continue until the teeth were finally shed. After the elimination of resorption, the resorbed dentin surface was repaired by a cementum-like deposition or covered with fibrous connective tissue.

Histological changes associated with physiological resorption during exfoliation in human deciduous teeth have been studied extensively (Marshall, 1928; Hopewell-Smith, 1930; Kronfeld, 1932; Bernick et al., 1949; Weatherell and Hargreaves, 1966; Boyde and Lester, 1967; Furseth, 1968; Hosokone and Bimstein, 1977; Rölling, 1981; Sasaki et al., 1988); however, there remain many questions regarding this process.

In the dog and cat, odontoclastic resorption occurs on the walls of the root canal and pulp chamber of deciduous teeth from the early stages prior to shedding (Urban, 1931; Kronfeld, 1932; Obersztyn, 1963). Thus, it is believed that, in these animals, internal resorptions takes an active part in shedding the deciduous teeth. As far as human deciduous teeth are concerned, however, there has been much discussion about internal resorption of the pulp chamber wall during the shedding of the teeth. Kronfeld (1932) suggested that internal resorption of the teeth would not occur in the process of shedding, because the pulp retained its normal structure until the tooth had exfoliated. On the other hand, odontoclastic resorption on the pulpal surface of dentin during the process of shedding has been reported by other authors (Bernick et al., 1949; Weatherell and Hargreaves, 1966; Furseth, 1968; Hosokone and Bimstein, 1977; Rölling, 1981). However, descriptions of the histological features of this resorption differed among these authors.

The present study aims to examine whether or not, in all types of human deciduous teeth, odontoclastic resorption occurs on the pulpal surface of coronal dentin during the stage of exfoliation. We also documented the chronology of the histological changes that take place during odontoclastic resorption at the pulpal surface of coronal dentin.

MATERIALS AND METHODS

One hundred and sixty-three extracted sound human deciduous teeth, including 47 incisors, 32 canines and 84 molars, were used in this study. Most were extracted for orthodontic reasons. Radiographs of each tooth were taken before extraction, and the teeth were fixed immediately after extraction in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde in 0.01 M cacodylate buffer, pH 7.3. After a
6 h fixation at room temperature, they were washed for 24 h in same buffer solution, and then decalcified with 10% ethylenediaminetetraacetic acid (EDTA) in 0.01 M cacodylate buffer, pH 7.3, for 3-4 weeks at 4°C. After decalcification, the teeth were washed for 24 h in 0.01 M cacodylate buffer, then cut into halves labio-lingually or medio-distally with a razor. One half was processed for light microscopy, the other for electron microscopy.

**Light microscopy**

Specimens were dehydrated through a series of graded ethanol and embedded in Technovit 7100 (Fa. Kulzer, Germany). Five-micron-thick serial sections were cut and stained with toluidine blue or hematoxylin and eosin. Some sections were stained for tartrate-resistant acid phosphatase (TR-ACP) activity. Staining for TR-ACP was performed according to BURSTONE (1958) by incubation of the sections for 30-60 min. at 37°C in 0.1M acetate buffer, pH 5.0, containing Naphtol AS-BI phosphate (Sigma, USA) as substate and fast red violet LB salt (Sigma, USA) as a stain for the reaction. Tartrate resistance was tested by the addition of 0.01M sodium tartrate to the incubation medium. These specimens were counterstained with hematoxylin.

**Histomorphometric study**

To determine the degree of exfoliation, the root surface length of each teeth was measured. The root surface length was defined as the distance from the cementoenamel junction to the most apical point of the intact cementum surface. For each specimen, the root surface lengths of both sides (minimum and maximum) were measured by means of a micrometer and recorded. Twenty sections of the most central part of each tooth were examined.

**Electron microscopy**

One hundred-micron-thick sections were cut with a microslicer (Dosaka EM, Japan). Then, they were refixed for 1 h in 2.5% glutaraldehyde in 0.01 M cacodylate buffer. After a rinsing with the same buffer solution, the sections were postfixed for 2 h at 4°C in 1% OsO₄. They were dehydrated in graded ethanol solution, and then embedded in Epon resin. Ultrathin sections were doubly stained with lead citrate and uranyl acetate and examined with a LEM-2000 electron microscope (ATB, Japan).

**RESULTS**

**Classification of the four stages of internal resorption prior to shedding**

Light microscopical observations revealed that about 80% of the teeth investigated in the present study showed signs of odontoclastic resorption at the pulpal surface of the coronal dentin. This phenomenon was as a rule observed not only in molars but also in incisors and canines (Table 1). However, the histological features of this internal resorption were considerably different from tooth to tooth. In some teeth, multinucleate odontoclasts were found on the surface of the dentin only at the bottom part of the crown, and the rest of the dentin surface was still covered with odontoblasts (Fig. 1a). In other cases, the odontoblast layer had completely disappeared, and numerous multinucleate odontoclasts were found on the entire surface of the pulp chamber wall (Fig. 1b). Furthermore, in some teeth, many resorption

<table>
<thead>
<tr>
<th>Stage of resorption</th>
<th>Number of deciduous teeth</th>
<th>Root surface length (mean±S. D.)</th>
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<tbody>
<tr>
<td></td>
<td>Incisor</td>
<td>Canine</td>
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<tr>
<td>1. Preresorption</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>2. Early</td>
<td>14</td>
<td>3</td>
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<tr>
<td>3. Later</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>4. Final</td>
<td>6</td>
<td>16</td>
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<tr>
<td>Total</td>
<td>47</td>
<td>32</td>
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*: p<0.01, **: p<0.05
Fig. 1. Various stages of resorption of the coronal dentin of human deciduous teeth in the later stage of exfoliation. 

a. Molar. Odontoclastic resorption is restricted to the bottom surface of the pulp chamber. The rest of the pulpal surface is covered with an odontoblast layer (arrows). Hematoxylin-eosin.

b. Incisor. Entire surface of the pulp chamber has been resorbed from within. Hematoxylin-eosin.

c. Canine. The internally resorbed dentin surface has been repaired by the deposition of a layer of cementum-like tissue. Toluidine blue.

d. High magnification of Figure 1c. The newly formed cementum-like tissue (C) is deposited on the surface of resorption lacunae. Toluidine blue.

D dentin, P pulp. a: × 22, b: × 19, c: × 23, d: × 90
lacunae were detected on the pulpal surface of the dentin, but no multinucleate odontoclasts could be found in the resorption bays where usually a cementum-like tissue had been deposited on the surface (Fig. 1c, d). These variations in histological features between the extracted teeth might be due to time differences in the process of odontoclastic resorption at the pulpal surface. Therefore, in the present study, we have divided the process of this internal resorption into four stages that take place in the pulp chamber.

The classification of the four stages of resorption was based on following histological criteria, respectively.

1. Preresorption stage: The wall of the pulp chamber is covered with an odontoblast layer, and no multinucleate odontoclasts can be found in the pulp chamber.

2. Early resorption stage: Multinucleate odontoclasts are detected on the pulp chamber wall, but the rest of the pulpal surface is still covered with an odontoblast layer (Fig. 1a).

3. Later resorption stage: No odontoblast layer is found, and entire surface of the pulp chamber is lined with multinucleate odontoclasts (Fig. 1b).

4. Final resorption stage: Resorbed dentin surface of the pulp chamber have been repaired partially or totally by the deposition of cementum-like tissue (Fig. 1c, d).

The frequency of occurrence of each stage of internal resorption of coronal dentin in the deciduous teeth observed is shown in Table 1.

Relation between the four stages of resorption and progress of shedding

In order to examine the relation between the progress of internal resorption at the pulpal surface and that of exfoliation, the root surface length of the teeth at each stage of resorption was measured.

Table 1 shows the mean value and the standard deviation of the minimum and maximum root surface length of the teeth at each stage of resorption. The mean value both of the minimum and maximum root surface length was more than 1 mm in the preresorption stage, and showed significant differences between the preresorption and three other resorption stages. This suggested that odontoclastic resorption at the pulpal surface would be initiated when the root surface length fell to less than 1 mm. The mean value of the maximum root surface length in the early and later stages was statistically significantly different between the two stages. While no significant difference could be demonstrated between the latter and final stages, the lowest mean value of the minimum and maximum root surface length of 0.50 mm and 0.16 mm appeared in the final stage. At the final stage in some teeth, intact cementum on the root surface had been totally resorbed. The result of a histomorphometric study demonstrated that the pulp chamber wall was resorbed from the inside with the progress of exfoliation. It also confirmed that the four resorption stages which were classified by histological features actually represented a time sequence of events during the process of internal resorption at the pulpal surface prior to shedding.

Histological and histochemical observations of each stage of resorption

Preresorption stage

While the roots were being actively resorbed, no detectable changes could be found in the pulp tissue, and odontoblasts assumed the columnar shape of those of the normal pulp (Fig. 2a). Therefore, those histological changes that take place in the pulpal tissue prior to the appearance of the multinucleate odontoclasts were examined with special reference to the pulp-dentin interface. When the roots were almost totally resorbed, inflammatory cells such as monocytes, macrophages and lymphocytes gradually infiltrated the pulp chamber. Concomitant with this change, odontoblasts also began to degenerate, becoming atrophied or flattened (Fig. 2b). In some regions, the odontoblasts had completely disappeared from the predentin surface (Fig. 2c). At this stage, large mononuclear cells began to be found in the pulp chamber (Fig. 2d). These large mono-nuclear cells had numer-

Fig. 2. Histological changes in the odontoblast layer region prior to the appearance of multinucleate odontoclasts. Stained with toluidine blue. a. While the roots are being actively resorbed, normal pulp structure is preserved. b. When the roots are totally resorbed, inflammatory cells infiltrate, and odontoblasts become atrophied or flattened. c. In some regions, the odontoblasts have disappeared from the predentin surface. d and e. Large mononuclear cells (arrows) appear in the pulp chamber, and become attached to the predentin surface covered with either degenerated odontoblasts (d) or with fibrous tissues (e). f. Typical multinucleate odontoclasts start to appear on the predentin surface. D dentin, OB odontoblast, OC odontoclast, P pulp, PD predentin. a-f: ×450
Fig. 3. The appearance and location of multinucleate odontoclasts (OC) during the process of resorption at the pulpal surface of coronal dentin. Stained with toluidine blue. 

- **a.** Preresorption stage. No odontoclasts can be found in the pulp chamber, although the roots have been mostly resorbed.
- **b.** Early resorption stage. Odontoclasts are restricted to the bottom area of the pulp chamber wall.
- **c.** Later resorption stage. Odontoclasts begin to appear on the more upper part of the pulp chamber wall. Resorption proceeds from the predentin to the dentin.
- **d.** Later resorption stage. Odontoclasts have reached the dentin surface at the pulpal horn area. 

*D dentin, OB odontoblast, P pulp, PD predentin.* 

*a: ×570, b, c: ×380*
ous TR-ACP-positive granules, probably primary lysosomes (Fig. 5a).

Ultrastructurally, they contained many mitochondria, small vacuoles, and poorly-developed endoplasmic reticulum in their cytoplasm (Fig. 6a). These large mononuclear cells might be precursors of the later-appearing multinucleate odontoclasts. In fact, at subsequent stages, mononuclear cells attached to the predentin surface by their cytoplasmic processes could be observed (Figs. 2e, 5b, c, 6b). The initial contact of these mononuclear cells with the predentin surface did not appear to require any interaction with odontoblasts, since this phenomenon was also found on the predentin surface that was covered with fibrous tissue (Figs. 2b, 5b).

Early resorption stage
As mentioned above, during the time that the roots were being actively resorbed, no multinucleate odontoclasts could be found in the pulp chamber (Fig. 3a). The appearance of multinucleate odontoclasts in the pulp chamber was related to the degree of root resorption. The present histomorphometric study demonstrated that the root surface length of the deciduous tooth in which multinucleate odontoclasts could be found in the pulp chamber was mostly less than 1 mm (Table 1).

Figure 3b shows the beginning of odontoclastic resorption of coronal dentin. Multinucleate odontoclasts were initially found on the surface of the
Fig. 5. Histochemical demonstration of TR-ACP in odontoclasts and their precursors during the process of internal resorption of coronal dentin. 

**a.** TR-ACP-positive mononuclear cells are seen in the pulp chamber in the early resorption stage. 

**b** and **c.** TR-ACP-positive mononuclear cells attach to the predentin surface by their cytoplasmic processes (arrows).  

**d.** TR-ACP-positive multinucleate cells, odontoclasts, on the predentin surface.
Fig. 6. Ultrastructure of multinucleate odontoclasts and their precursors. 

**a.** Mononuclear precursor cell in the pulp chamber. Numerous mitochondria and vacuoles can be observed in its cytoplasm. 

**b.** Mononuclear precursor cell attached to the predentin surface. 

**c.** Multinucleate odontoclast resorbing predentin. This cell has a distinct clear zone (arrows) and ruffled border at the resorption face. 

**d.** Multinucleate odontoclast resorbing dentin. 

D dentin, PD predentin. 

**a, d:** $\times 2,200$, 

**b:** $\times 2,900$, 

**c:** $\times 3,700$

-e. Typical odontoclast resorbing dentin. Staining for TR-ACP is found in granule-like structures, probably lysosomes (arrows). 

-f. Later resorption stage. TR-ACP-positive multinucleate cells away from the resorbed surface can be seen (arrows). 

g. Detached multinucleate cell shows signs of denaturation, containing large vacuoles and diffuse staining for TR-ACP. 

D dentin, P pulp, PD predentin. 

**a-d, g:** $\times 810$, 

**f:** $\times 360$
predentin at the bottom areas of the remaining crown. Then, the number of multinucleate odontoclasts gradually increased, and the cells became detectable on the predentin or dentin surface of the more upper parts of the pulp chamber (Fig. 3c). Therefore, at the later resorption stage, the resorbing cells were found on the dentinal surface of the pulpal horn area (Fig. 3d).

Histochemical staining for TR-ACP, which is a useful marker for identifying odontoclasts and their precursors, clearly demonstrated the sequence of events during the process of resorption at the pulpal surface of coronal dentin. At the early resorption stage, as shown in Figure 4a, only a few TR-ACP-positive cells could be seen in the pulp chamber or on the predentin surface. These TR-ACP-positive cells were mostly mononuclear. Then the number of TR-ACP-positive multinucleate odontoclasts was gradually increased on the predentin surface, and resorption was initiated, starting with the predentin (Figs. 2f, 5d, 6c).

Later resorption stage
In the later resorption stage, odontoblasts had completely disappeared, and numerous TR-ACP-positive multinucleate cells were detected on the entire surface of the wall of the pulp chamber (Fig. 4b). At this stage, the predentin layer was gradually resorbed by odontoclasts, and resorption proceeded to the dentin (Figs. 3c, d, 5e, 6d). Ultrastructural studies demonstrated that the multinucleate odontoclasts on the predentin had the same ultrastructural configuration as those on the dentin being resorbed. They had clear zones and well-developed ruffled borders on the resorption face; numerous mitochondria and free ribosomes were observed in their cytoplasm (Fig. 6c, d).

Final resorption stage
The resorption, which started in the predentin, gradually spread to the dentin. However, this odontoclasts resorption of coronal dentin did not seem to continue until the teeth had exfoliated. At this stage, numerous multinucleate odontoclasts that were away from the resorbed dentin surface could be found in the pulp chamber (Fig. 5f). Most of them had large vacuoles and diffuse TR-ACP stainings, and appeared to degenerate (Fig. 5g). After odontoclasts had disappeared, the resorbed dentin surface was repaired by a cementum-like deposition or covered with fibrous tissue. This conversional phase from resorption to repair of the pulp chamber wall is well demonstrated in Figure 7.

Newly formed cementum-like tissue on the resorbed coronal dentin did not appear to be resorbed until

![Fig. 7. Light micrographs of odontoclastic resorption of coronal dentin in the conversional phase at the final resorption stage. Stained with toluidine blue. a. Pulpal surface of dentin is resorbed by multinucleate odontoclasts (OC), and numerous resorption lacunae can be seen. After the disappearance of odontoclasts from these lacunae, cementum-like tissue is gradually formed on the resorbed surface (arrow). b. Resorption lacunae are coated with newly formed cementum-like tissue (arrow). C cementum-like tissue, D dentin, P pulp. a: ×170, b: ×340](image-url)
the teeth were lost. At this stage, no odontoclastic activity could be detected on the surface of the cementum-like deposition. The sequence of histological events that transpire during the process of odontoclastic resorption at the pulpal surface of coronal dentin of human deciduous teeth in the later stage of exfoliation is summarized in Figure 8.

DISCUSSION

The present study demonstrated that as a rule odontoclastic resorption on the internal surface of coronal dentin takes place in near-exfoliated human teeth. Furthermore, our observations revealed that this resorption showed time-related histological changes. It began from the predentin surface at the bottom areas of the pulp chamber, and gradually proceeded toward the pulpal horn region. After elimination of this resorption, the resorbed pulp chamber wall was repaired by a cementum-like deposition or covered with fibrous tissue.

Some investigators (BERNICK et al., 1949; WEATHERELL and HARGREAVES, 1966; FURSETH, 1968; SOSKOLNE and BIMSTEIN, 1977; RÖLLING, 1981) demonstrated that odontoclastic resorption took place from the inner surface of the pulp chamber during the shedding of human deciduous teeth. However, conflicting results have been reported regarding the histological configuration of this resorption. WEATHERELL and

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**Fig. 8.** Schematic representation of sequence of events of resorption at the pulpal surface of coronal dentin occurring in the later stage of exfoliation in human deciduous teeth. 1 Preresorptin stage, 2 early resorption stage, 3 later resorption stage, 4 final resorption stage.
Hargreaves (1966) mentioned that resorption on the internal surface of coronal dentin was usually observed only in multi-rooted, but not in single-rooted, teeth. Furseth (1968) reported that resorption was found in the root canal wall of teeth with healthy as well as inflamed pulp, and occasionally in the pulp chamber wall. The results of the present study well agree with those of Soskolne and Bimstein (1977) and Rölling (1981), who suggested that conflicting findings for this resorption might be reflections of the different stages of the shedding process. Indeed, in this study, we could find clear time-related histological changes in the process of this resorption, some of them showing the same histological features as previous investigators have reported.

It is suggested that, in the dog and cat, internal resorption takes an active part in resorption during the shedding of deciduous teeth, since numerous odontoclasts appear on the wall of the root canal or pulp chamber and resorb the dentin (Urban, 1931; Kronfeld, 1932; Obersztyrn, 1963). However, as we presently reported, in human deciduous teeth, odontoclastic resorption of coronal dentin occurred prior to shedding, after the roots had been almost totally resorbed. In humans, internal resorption at the pulpal surface seems to be of minor importance in resorption during the process of shedding.

It is difficult to interpret why internal resorption of coronal dentin take place only at a late stage of shedding. However, this phenomenon might be related to inflammatory infiltration in the marginal periodontal tissue. It has been reported that prior to shedding, the epithelium proliferates along the root surface toward the resorbed area, and inflammatory infiltration in the marginal periodontal tissue is commonly observed (Kronfeld, 1932; Bernick et al., 1949, 1951; Soskolne and Bimstein, 1977; Rölling, 1981). In the present study, we also confirmed the infiltration of inflammatory cells into the pulp chamber before the appearance of multinucleate odontoclasts on the pulp chamber wall. Thus, when the roots are completely resorbed, inflammatory cells in the marginal periodontal tissue proliferate into the pulp chamber and cause resorption at pulpal surface of coronal dentin. If this is true, internal resorption of coronal dentin prior to shedding would be "pathological" resorption rather than the "physiological" one that is observed in the root resorption during the process of exfoliation.

Much information and evidence has been reported concerning the cementum-like deposition on the resorbed root surface during the process of shedding (Kronfeld, 1932; Bernick et al., 1949; Furseth, 1968; Soskolne and Bimstein, 1977; Rölling, 1981; Sasaki et al., 1990). To our knowledge, however, our study is the first to report that the resorbed pulp chamber wall is as a rule repaired with a cementum-like deposition prior to shedding. The physiological role of repair of the resorbed pulpal surface is unknown, since the teeth exfoliate a little while after the deposition of cementum-like tissue. However, there is a possibility that this phenomenon plays a role in helping to retain the deciduous teeth until that moment.

From the viewpoint of the mechanism of tooth resorption, resorption from the inner surface of coronal dentin prior to shedding is an interesting and unique phenomenon. The resorption of dental hard tissues is usually initiated at the surface of the cementum and enamel, because the tooth surface is covered by these two hard tissues. The odontoclastic resorption as we reported can give us an opportunity to observe the tooth resorption that begins at the predentin. It is interesting to study the process of tooth resorption initiating from the predentin, since the nonmineralized layers that cover the mineralized tissues, such as the osteoid, precementum, and predentin, are usually resistant to resorption; among these, predentin seems to be the most resistant one (Reitan, 1974; Emslie, 1978; George and Miller, 1986).

It has recently been suggested that osteoclastic resorption in bone tissue begins at the mineralized surface after osteoblasts have removed the unmineralized layer, the osteoid. Osteoblasts secrete collagenase, which breaks down the osteoid, and thus the mineral is exposed. This mineral attracts osteoclasts and stimulates them to resorb the bone (Chambers et al., 1985). It is assumed that osteoblasts have an inter-relationship with osteoclasts and play an active role in bone resorption (Rodan and Martin, 1981). However, for tooth resorption, there has not been any evidence that formative cells such as cementoblasts or odontoblasts have the same ability as osteoblasts (Jones and Boyde, 1988). As far as the present observations are concerned, odontoblasts do not seem to have any interaction with odontoclasts in the process of resorption for the following reasons: 1) the odontoblasts had begun to degenerate before odontoclasts or their precursors appeared in the pulp chamber; 2) odontoclastic resorption was also initiated from the surface of predentin that had no odontoblasts but was covered with fibrous tissue. There might be a different mechanism to initiate tooth resorption, especially resorption from the predentin surface. We are now investigating its possibility.

As summarized in Figure 8, the resorption of the coronal dentin of human deciduous teeth just before
shedding clearly showed time-related histological changes. This phenomenon might be a suitable model to study the sequence of cellular events during the process of tooth resorption as well as cytodifferentiation of odontoclasts.

REFERENCES


