

Collagenolytic Activity in Traumatized Human Primary Teeth Undergoing Accelerated Resorption

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Abstract

Human periodontal ligament from rapidly resorbing primary teeth which also exhibited periapical radiolucency on radiograph demonstrated collagenolytic activity when incubated on collagen gels. This activity was directly proportional to the size of the explant and length of time of incubation.

Introduction

Normal connective tissue resorption is a universal process that occurs during the exfoliation of the primary teeth. Specific enzymatic activities^{1,2} have been associated with this event and have been related to the resorptive phenomena. Although little information is available as to the actual mechanism of the accelerated resorption seen in traumatically involved primary teeth, it has been shown that periapical pathology superimposed upon the natural resorptive process hastens tooth loss.^{3,4}

Collagenolytic activity from the periodontal ligament has recently been reported to be associated with physiological resorption.⁵ No data, however, exists for this enzyme activity during pathologic root resorption. Since collagen is degraded during this process, the purpose of this study was to demonstrate the presence and relative magnitude of such activity in periapically involved primary teeth.

Methods and Materials

Teeth from a total of sixty children were included in the study; twenty-four females and thirty-six males

ranging in age from 34 to 46 months for those who displayed advanced resorption and from 10 years 1 month to 12 years 3 months for premolar controls. Sixty-three caries-free primary incisors were extracted because of radiographic evidence of root resorption and periapical radiolucency. The etiology of devitalization was previous trauma confirmed by the parents and patient. The pulp chambers and canals were examined under a dissecting microscope after sectioning these teeth according to the method of Engstrom and Ohman.⁶ Visual examination revealed empty chambers and canals except for the presence of slight necrotic debris; the teeth were therefore termed "pulpless". Resorption was judged clinically and radiographically to be between 50 and 75 percent completed, which is accelerated for this age group.³ This measurement was based upon comparison of root lengths from standards obtained from Kramer and Ireland.⁷ The premolar control teeth were removed prior to orthodontic therapy and judged by visual examination to be fully formed.

Using a high speed handpiece with water coolant, 5 mm of the remaining apical third of the root was removed with a diamond disc on all specimens. From the apically sectioned root apex, periodontal ligaments were removed with a scalpel and washed for three hours at 37°C in mammalian Tyrode's solution containing 0.5 mg/ml streptomycin, 1000 units/ml penicillin, 40 ug/ml amphotericin B, and 75 ug/ml gentamycin.

After washing, the tissue was cut into sections ranging in size from 1 to 4 mm² using an ocular micrometer. This range was selected so as to permit comparison to data obtained from the periodontal liga-

ment of normal physiologic resorbing primary teeth.⁵ The bare root fragments were fixed in 10 percent buffered formalin and processed routinely for light microscopy. These tissues were then treated with collagen stains⁸ in order to indicate the resorbing front of the root structure.

The detection of collagenolytic activity was accomplished by incubation of periodontal ligament fragments for seven days on reconstituted collagen gels as described by Gross and Lapiere.⁹ Acid extracted calf skin collagen* dissolved at neutral pH was used to prepare the gels for the substrate culture. These translucent gels were formed from 2 ml of a 0.1 percent solution of collagen in cold mammalian Tyrode's medium by warming at 37°C for three hours in glass culture dishes.

Periodontal ligament cultures were incubated at 37°C in a moist atmosphere containing 90 percent O₂ and 10 percent CO₂, and were observed daily for seven days for lytic activity. Areas of explant and lysis were measured with an ocular micrometer. Resorption products that occurred in the area of lysis were collected with a micropipette and analyzed biochemically for hydroxyproline according to the methods of Stegemann.¹⁰ Control chambers were incubated in the same manner as above, but without tissue explants.

Bacterial contamination occurred infrequently and, when present, was visible as discrete colonies on the gel. Although none of these cases produced observable lysis of the gel, these specimens were discarded. Immediately after incubation, periodontal ligaments were fixed in 10 percent buffered formalin and processed routinely for light microscopy. A second group of ligaments from experimental and control specimens was fixed immediately after removal from the tooth and processed for histologic examination in the same manner described above.

Results

Histology

The periodontal ligaments from the periapically involved teeth displayed an inflammatory infiltrate throughout their architecture (Figure 1). No inflammatory tissue appeared in ligaments from the premolar controls. No osteoclasts were observed in either tissue. Ligaments fixed immediately were identical to those cultured and then fixed in formalin except for a cellular outgrowth in the latter.

Collagen staining of the stripped primary root tips indicated that a resorbing front existed which extended approximately 0.5 mm into the tissue (Figure 2). The loss of collagen stain was directly adjacent to the formerly present periodontal ligament. Once past this resorbing front, the dentinal tissues stained nor-

mally. Premolar roots stained uniformly with no indication of resorptive activity.

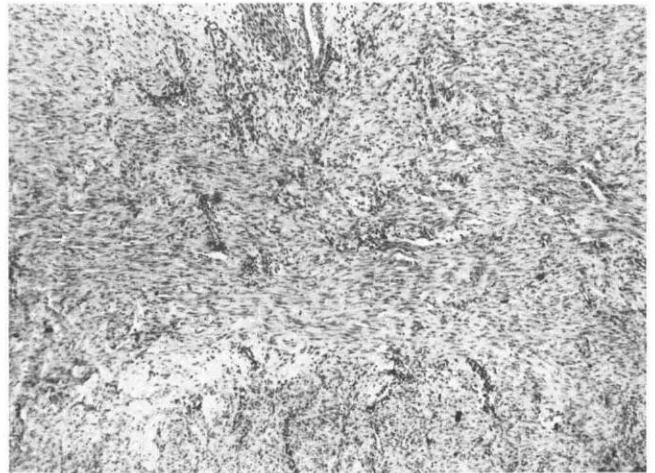


Figure 1. Inflamed periodontal ligament from a periapically involved deciduous central incisor. Hemotoxylin and Eosin [X 63].

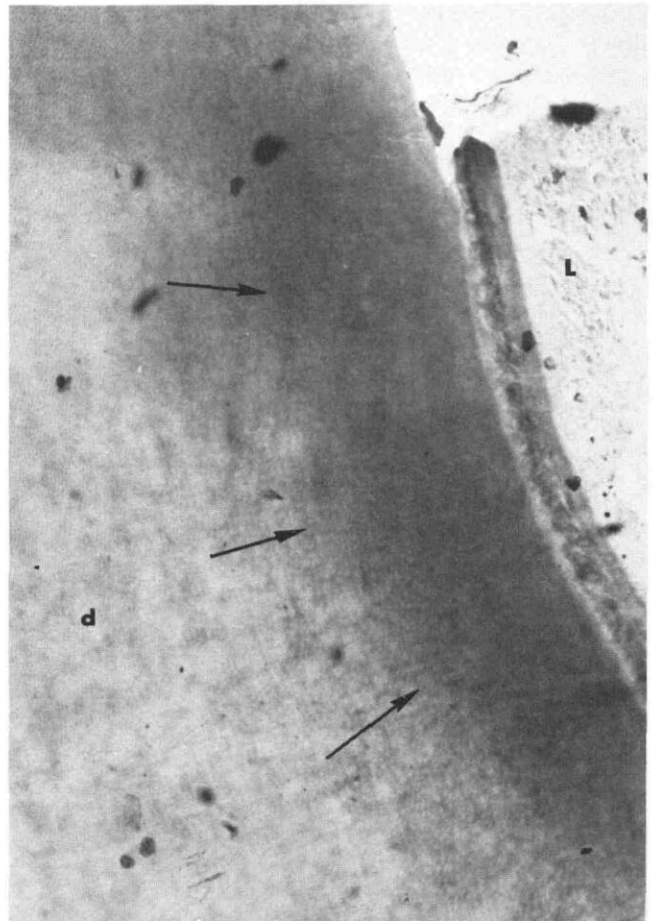


Figure 2. Root tip of a deciduous central incisor partially stripped of its periodontal ligament. Note the difference in staining intensity, indicating a resorptive collagen front (arrows) and normal adjacent dentin (d) and periodontal ligament (l). Masson's Trichrome [X 100].

* Sigma Chemical Company; Saint Louis, Missouri, 63148.

Table 1. Collagenolytic activity of periodontal ligament* in traumatically resorbing and control teeth.

Tooth	Incubation Period (days)						
	1	2	3	4	5	6	7
deciduous incisors (N = 63)	+	++	+++	++++	+++++	++++++	+++++++
First premolars (N = 10)	-	-	-	-	-	-	-

* Area of ligament explants was approximately 2 mm².

- = No lysis of collagen gel.
- + = Lysis of gel beginning beneath the tissue.
- ++ = Lysis of gel between 1 and 2 mm surrounding the tissue.
- +++ = Lysis of gel between 2 and 3 mm surrounding the tissue.
- ++++ = Lysis of gel between 3 and 4 mm surrounding the tissue.
- +++++ = Lysis of gel between 4 and 5 mm surrounding the tissue.
- ++++++ = Lysis of gel greater than 6 mm surrounding the tissue.

The Frequency and Extent of Collagenolytic Activity in Periodontal Ligament

The degree of collagenolytic activity was measured over a period of seven days with 2 mm² tissue explants (Table 1). All periodontal ligament fragments from resorbing teeth showed lysis of collagen gels. Control ligaments displayed no activity (Figure 3).

A 3.3-fold size increase in mean explant area resulted in a 65.1 percent increase in gel lysis (Table 2) and a 91 percent increase in measurable hydroxyproline after seven days of incubation (Table 3).

Discussion

Although the resorption of primary teeth is a physiological process, recent evidence indicates that the

superimposition of inflammatory tissue accelerates this event.⁴ Since the structure of dentin and cementum consists, in part, of collagen,¹¹⁻¹² it follows that an enzyme capable of degrading this component be present during resorption.

Collagenolytic activity has been observed in the periodontal ligament during orthodontic tooth movement¹³ and in inflammatory-free human physiological primary root resorption.⁵ Levels of enzyme

Table 2. Relationship between size of periodontal ligament explant and area of lysis.*

Number of Cultures	Explant Area in mm ²	Mean Explant Area in mm ² **	Mean Diameter of Lysis, mm**
7	0.8 - 1.4	1.1 ± 0.3	5.2 ± 0.4
7	1.9 - 3.1	2.2 ± 0.3	6.5 ± 0.3
7	3.2 - 4.2	3.6 ± 0.3	8.6 ± 0.1

* Incubation period was seven days for all cultures observed.
** Data represents ± the standard deviation.

Table 3. Relationship between explant size and hydroxyproline released from collagen gel.*

Number of Cultures	Explant Area in mm ²	Mean Explant Area in mm ² **	Hydroxyproline (µg)**
7	0.8 - 1.4	1.1 ± 0.3	6.7 ± 0.2
7	1.9 - 3.1	2.2 ± 0.3	9.1 ± 0.3
7	3.2 - 4.2	3.6 ± 0.3	12.8 ± 0.5

* Incubation period was seven days for all cultures observed.
** Data represents ± the standard deviation.

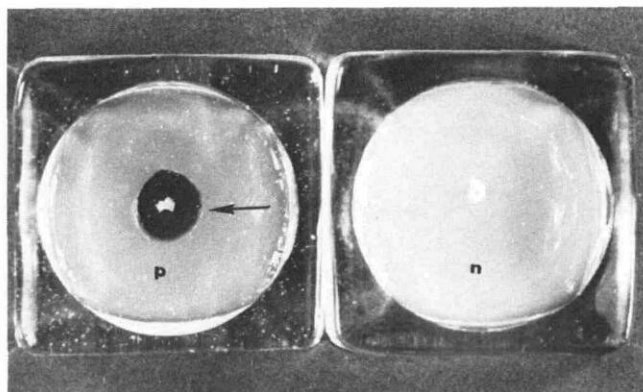


Figure 3. Periodontal ligament from the root of a pathologically resorbing deciduous central incisor (p) and from the root of a normal premolar (n) incubated on a collagen substrate for four days. Note the area of lysis (arrow) surrounding the tissue, indicating collagenolytic activity.

activity as measured by the extent of gel lysis and hydroxyproline released per unit of tissue over time, however, are increased during the resorption of periapically involved primary teeth.

When compared to normal resorption,⁵ such activity was increased between 30 and 40 percent when an inflammatory infiltrate was present. This increase in collagenolytic enzyme activity may account, in part, for the accelerated resorption seen in traumatically involved primary teeth which is analogous to increases in resorptive activities from other tissue components.^{3,4}

Interestingly, a resorptive collagen front was present in the root areas adjacent to the source of enzyme activity — the periodontal ligament. This front may be responsible for the decreases observed in total dentin hydroxyproline during the resorption of primary teeth.¹⁴

Since trauma to the pulpal tissues resulted in the devitalization of the teeth, which in turn led to periapical involvement, such tissue should not be discounted from contributing to root resorption. Pulp studies in human and animal models have indicated its involvement in the process of physiological resorption.¹⁵⁻¹⁸ Participation from healthy and inflamed pulp in the resorptive phenomena is currently under investigation and will be reported shortly.

Although our results indicate an increase in collagenolytic activity in the presence of inflammatory tissue and hence, acceleration in resorption, the importance of the contribution of such cells to the resorptive process may be overemphasized. Phagocytosis of collagen fibrils by the periodontal ligament fibroblasts may also contribute to tissue removal by processes other than enzyme activity.¹⁹ Whether the inflammatory cells stimulate fibroblast activity (enzyme production or phagocytosis) and/or participate directly in tissue removal, still remains to be seen.

Conclusions

Collagenolytic activity has been demonstrated in cultures of human periodontal ligaments of traumatized and rapidly resorbing primary teeth. The superimposition of an inflammatory infiltrate resulted in an increase of 30 to 40 percent in collagenolytic activity when compared to periodontal ligaments from teeth that were in the process of physiological resorption.

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