Tissue Reaction and Recovery following Experimental Tooth Movement

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INTRODUCTION

Although orthodontic tooth movement has been accomplished for thousands upon thousands of patients, very few fundamental studies of the underlying biological mechanisms have been reported. Such studies have been restricted almost exclusively to histomorphologic evaluations. Early investigators studied tissue reactions to simple tooth movement.[1-3] Later reports were concerned with the effects that different types of forces had upon root structure and alveolar bone.[4-10] As with the earlier investigations, these latter studies were also restricted to histomorphologic evaluation.

The purpose of the current study was to reevaluate histomorphologic changes subsequent to tooth movement, and to characterize chemical alterations in tissue metabolism by utilization of histochemical procedures. Since chemical alterations usually occur prior to structural changes, it was felt that histochemical techniques might provide more sensitive indications of these changes than would be available from evaluations of structural alterations alone. Although the histochemical approach to the problem appears to have great potential, a thorough review of the literature revealed only one comparable study,[11] and two other reports in which the changes in one enzyme subsequent to tooth movement were reported in each paper.[12,13]

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MATERIALS AND METHODS

Male golden hamsters (Cricetus Auratus), approximately two months of age, were used in this study. The animals were divided into five groups as follows:

Group I	Normal, untreated controls (8 animals)
Group II	Elastics inserted for 1 day, then sacrificed (10 animals)
Group III	Elastics inserted for 7 days, then sacrificed (10 animals)
Group IV	Elastics inserted for 14 days, then sacrificed (10 animals)
Group V	Elastics inserted for 14 days, then removed, sacrificed 30 days later (12 animals).

A diet of Purina laboratory chow and water was supplied *ad libitum*. The animals were maintained for a period of five days after delivery to the animal farm in order to acclimate them to their environment prior to initiation of experimental procedures.

The instrument utilized in placing the elastics was designed and fabricated according to the specifications of Moskowitz and Kronman.[11] With this instrument orthodontic latex elastics, 5/16 inch, were lightly lubricated with vaseline and placed between the contacts of the maxillary left first and second molars of the hamsters while they were under ether anesthesia. After suitable placement of the elastics gingival to the contact area, the tension on the elastics was gradually and completely relaxed. The elastics were then cut on

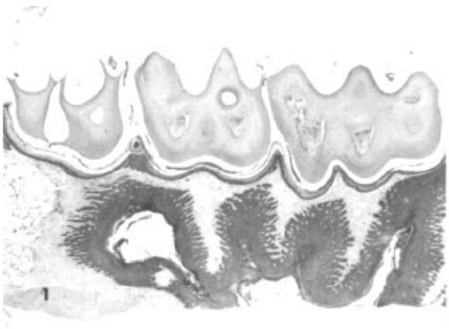

Fig. 1 Sagittal section of the three molar teeth and supporting structures after decalcification in the normal control group. Masson's stain, X25.

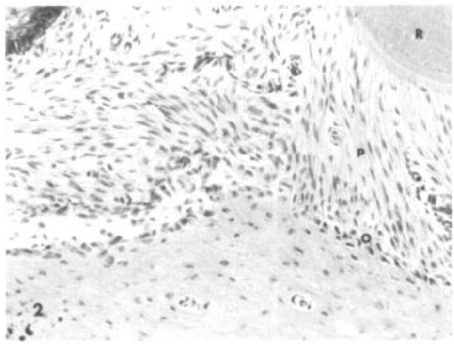

Fig. 2 High power view of osteoblastic activity on the bony surface in Group I. Root (R) is indicated for purposes of orientation. Periodontal membrane (P), in the area indicated, illustrates tension of the fibers and alignment of osteoblasts (O).

the buccal and palatal surfaces, as closely as possible to the surfaces of the teeth.

The animals were decapitated by groups at the appropriate intervals, as listed above. The left half of the hard palate, including the intact alveolar bone and teeth was dissected *en masse*. These tissues were then decalcified in EDTA (ethylenediaminetetraacetic acid) according to the procedure described by Balogh[14] for seven days. This was followed by fixation for three days in neutral buffered formalin for all stains except acid phosphatase. Sections were fixed in cold acetone (4°C) for the latter staining procedure.All sections were then vacuum embedded in paraffin. After suitable orientation on the microtome, 8 micra sections were prepared.

The staining procedures utilized included H & E and Masson's trichromic staining procedures for structural evaluation. The histochemical procedures included the PAS reaction[15,16] for the demonstration of vicinal hydroxyl groups. Control slides were incubated for one hour in a one percent aqueous malt diastase solution at 37°C in order to eliminate the presence of glycogen.[17] Ribonucleic acids and metachromasia were demonstrated by means of the toluidine blue reaction (0.05 per cent) (pH 4.5). Parallel control sections were incubated in a one per cent RNA-ase solution in glass distilled water for one hour at 37°C. Acid phosphatase was demonstrated by the post-coupling technique of Rutenberg and Seligman.[18] The substrate employed was 6-benzoyl-2 naphthyl phosphate dissolved in veronal acetate buffer (pH 6.0). Control preparations were incubated in the buffer without substrate.

Findings

Histomorphology

Examination of the normal control sections (Group I) (Fig. 1) revealed that the periodontal membrane was well defined and was of fairly uniform thickness throughout the field of study. Osteoclastic activity was noted in the alveolar bone adjacent to the distal surfaces of the mesial and distal roots of the first, second, and third maxillary molars with apparent decreased activity as one moved distally. Osteoblastic activity was observed primarily on the mesial aspects of the interproximal bone between the first and second and third molars (Fig. 2). Osteoblasts were observed, however, in all areas examined.

Group II (elastics inserted for one day) was characterized histologically by destruction of the periodontal mem-

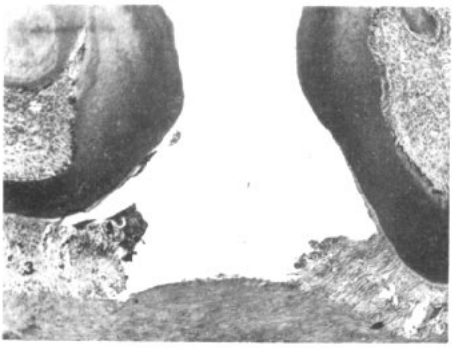

Fig. 3 Destruction of the periodontal membrane at the site of elastic insertion is indicated in Group II.

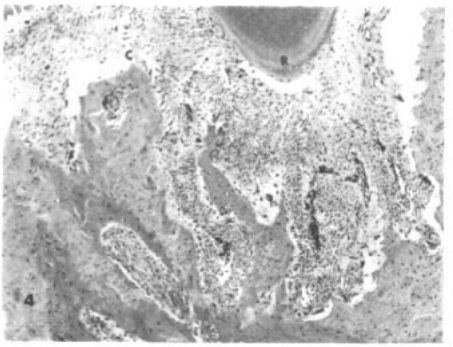

Fig. 4 Dramatic scalloping as a result of extensive bone destruction was observed in Group IV. Root (R) is indicated for orientation. Note the loss in height of bony crest (C) which now approximates the level of the tooth apex.

brane at the site of the elastic insertion (Fig. 3). The periodontal membrane was compressed on the mesial aspect of the second molars. Osteoclastic activity was observed at the sites of compression. Although osteoblastic activity was observed at the sites of tension, this activity was not as uniformly distributed and generalized at the sites of tension as were the osteoclasts at the sites of pressure. Osteoclastic activity was also observed in the interproximal bone between the second and third molars.

Histologic examination of Group III (elastics inserted for one week) revealed almost total loss of the periodontal membrane at the site of insertion. Sites of osteoblastic and osteoclastic activity were comparable to those observed in the preceding group. The most significant change observed was the extensive destruction of supporting bone at the apex and mesial surface of the distal root of the first molar, and at the apex and distal surface of the mesial root of the second molar.

In Group IV (elastics inserted for two weeks) the histologic pattern was comparable to that observed in Group III, but the changes were more pronounced. Dramatic scalloping of bone was observed around the roots of all of the molar teeth (Fig. 4). The alveolar crest of bone between the first and second molars was almost obliterated. Bony repair and osteoblastic organization and activity at the sites of tension were more apparent than in the previous group. In the group which was maintained for one month subsequent to two weeks insertion of elastics (Group V), repair was evident at many sites. The interdental bony crest was partially repaired. The scalloped appearance of the alveolar bone was still evident, but had obviously undergone extensive repair. Osteoblasts were well organized, particularly in the crypts of bone formed by the scalloping. The extensive destruction to the underlying supporting bone had undergone some repair, but was still not restored to the original state (Fig. 5). The periodontal membrane was well organized and almost completely restored to its normal configuration, except at the site of elastic insertion. Here, some epithelial and connective tissue repair had taken place, but a periodontal membrane, as such, was poorly defined.

Histochemistry

Toluidine blue—Those slides treated with toluidine blue were evaluated for staining of osteoblasts and osteoclasts, as well as for metachromasia. Metachromasia was not observed in any area of ground substance involved in the

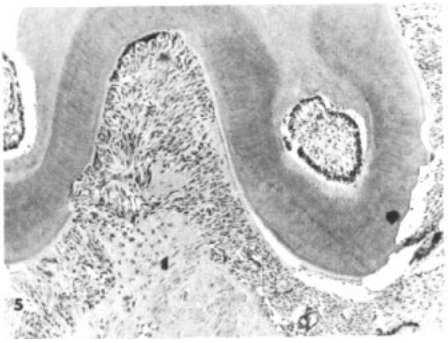

Fig. 5 Extensive repair of the supporting bone was observed in Group V. Contrast the height of the bony crest (C) to the apex. Also compare the smooth outline of the supporting bone with the previous figure. Figure 5 is positioned more occlusally than was Fig. 4 in order to effectively illustrate the altered height of the bony crest.

study. The only metachromatic reaction noted was in mucous secreting minor salivary glands located distally to the third molar. In all other areas the staining was orthochromatic (Fig. 6). In Group II (elastics inserted for 24 hours) the osteoblasts were stained more strongly than were the osteoclasts. This tendency for increased reactivity of osteoblasts in comparison to osteoclasts was consistent in all subsequent groups. The staining itself was primarily moderate rather than strongly or intensely reactive. Examination of

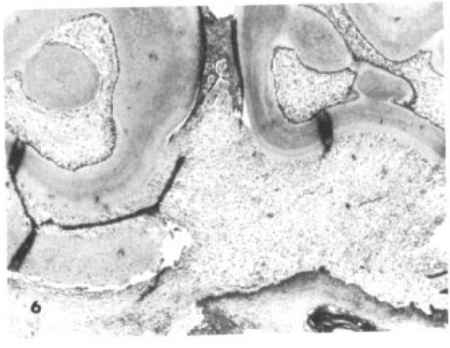

Fig. 6 Toluidine blue stain of second and third molars in Group III. Generalized uptake of the stain is obvious, particularly in areas of cellular concentration.

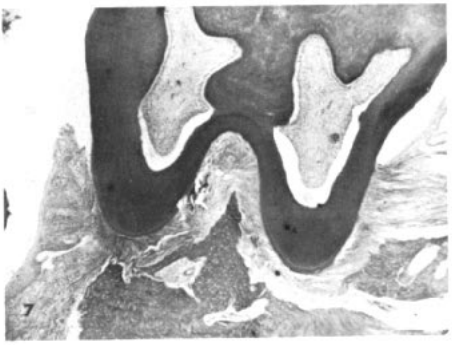

Fig. 7 PAS stain of second molar adjacent to site of elastic insertion in Group III. Note the generalized uptake of the stain with the hard tissues staining more strongly than the soft tissues.

the control slides, incubated in RNA-ase prior to staining with toluidine blue, showed the effective removal of cytoplasmic basophilia. This confirmed the identification of cytoplasmic RNA by toluidine blue staining.

Periodic Acid-Schiff (PAS)—Examination of tissues stained with PAS revealed a comparable pattern of reactivity in all sections and in all groups (Fig. 7). The hard structures stained uniformly and moderately. The periodontal membrane, pulp and epithelial tissue were uniformly, but less strongly, stained. Examination of control sections, incubated in malt diastase prior to staining, showed no alterations in staining reactions.

Acid phosphatase—sections stained to demonstrate this enzyme revealed some moderate reaction in the surface layers of covering epithelium, and moderate reactivity in relationship to the presence of osteoclasts (Fig. 8). Intensity and distribution of this enzyme, therefore, was directly related to osteoclastic activity. Parallel control slides incubated in buffer without substrate were nonreactive.

Discussion

The distribution of osteoclasts on the distal surfaces of the roots, in conjunc-

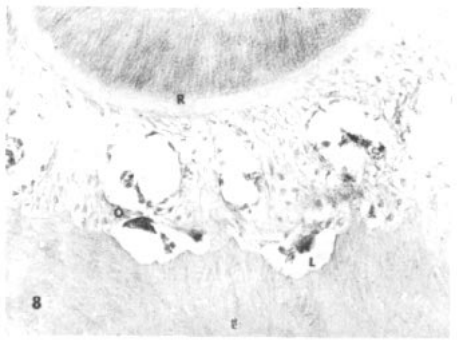

Fig. 8 Acid phosphatase activity localized within osteoclasts (O) of Group III. Root (R) is used for orientation. Lacunae (L) are clearly evident.

tion with osteoblastic activity on the mesial aspects of the molar roots in the normal, untreated animals, is indicative of a normal sequence of distal drift in these teeth. Osteoblastic activity progressively decreased on the medullary surfaces as one progressed distally. This latter finding is also indicative of such a distal drift of the molar teeth. This confirms prior studies in rodents where such drifting had been reported.[11,19] The decreased osteoclastic activity as one progressed distally has also been previously reported, but a complete explanation of this phenomenon has not been offered. Subsequent studies of this type should include the teeth anterior to the molar segment. This would be useful in determining if distal drift occurs in a completely orderly decreasing sequence from the anterior to the posterior segments, or if such drifting is restricted to the posterior buccal segments. Remodeling of alveolar bone,[20,21] as a response to the distal drift of the teeth, successfully maintained the original width of the alveolar bone. The uniform width of the periodontal membrane was not unexpected in the untreated control group.

Prior studies on rodents by Yen and Rothblatt[22] and by Zaki and Van Huysen[23] revealed compression of the periodontal membrane by the fourth to sixth hour after insertion of interproximal elastics. The destruction of the periodontal membrane at the site of elastic insertion, observed after one day, is entirely consistent with these findings. The compression of the periodontal membrane on the mesial aspect of the second molar and the distal aspect of the first molar in Group II, undoubtedly represents a direct reaction of the tissues to the elastic itself, and not to a biologic response by the tooth and supporting structures to the force applied. The appearance of osteoclasts at the sites of pressure would, however, indicate a biologic response to the administered force. Prior studies on rodents revealed cellular and tissue alterations within twelve to twenty-four hours.[24] The histologic pattern apparent in Group II revealed that the distal drift of the first molar had been reversed, or at least interrupted. Increased osteoclastic activity in the region of the interproximal bone between the second and third molars indicated enhanced distal movement in these teeth as a result of the force exerted by the elastic.

Previous investigations of tooth movement by elastic insertion between the teeth of rats[25-27] revealed a nearly maximal tissue response after three days. Undermining resorption and extensive osteoclastic activity, observed at three days, was more pronounced at one week. The findings in the current investigation (Group III) are completely in accord with these earlier reports. The extensive destruction of bone at the distal root of the first molar and mesial root of the second molar reflects a tipping movement of these teeth, which is also in accord with prior investigations.

The histologic effects observed after two weeks (Group IV) revealed that the undermining resorption had progressed to the stage wherein the alveolar bone surrounding all molar teeth was involved. Although bony repair was evi-

dent, destruction of bone was the single most dominant feature of the sections studied in this group.

Thorough examination of the literature revealed no previous studies comparable to the current investigation, in which a period of repair after removal of the orthodontic mechanism was investigated (Group V). Histologic study of sections in Group V clearly indicated that extensive repair had taken place, although the normal condition observed in Group I had not been achieved. The fact that this repair was observed in underlying supporting bone and the periodontal membrane might lead one to speculate that the alterations induced by tooth movement, under the conditions of this study, are reversible. This latter supposition may finally be confirmed by subsequent investigations in which more extensive repair periods following active tooth movement are studied.

Toluidine blue staining was utilized in order to demonstrate RNA and metachromasia. In the current study the presence of RNA in the cytoplasm of osteoblasts and osteoclasts was utilized as an index of protein synthesis and cellular proliferation.[28,29,30] The greater reactivity of osteoblasts as compared to osteoclasts at all stages of the study probably indicates a higher level of protein synthesis in cells concerned with bone formation than in cells related to bone resorption. In the only other similar study encountered in the literature,[11] the results were comparable to those reported here.

A review of the literature revealed reports of the presence of glycogen, mucoproteins and mucopolysaccharides in bone matrix.[31] The positive PAS reactivity in the hard structures examined in this study would tend to confirm these prior reports for mucopolysaccharides and mucoproteins. The fact that the reaction was unchanged after prior incubation in malt diastase would indicate, however, that glycogen was not present. If it were, the diastase incubation would have removed the glycogen and the subsequent reaction would decrease in intensity. Although others have reported increased PAS reactivity in osteogenic cells prior to calcification[32] and in bone matrix adjacent to osteoclasts,[33] differences in staining by group were not noted in the present investigation. The PAS reaction, as observed in the present report, is not essentially in conflict with the prior investigators. The consistency in staining between the various groups, however, would clearly indicate that mucoproteins and/or mucopolysaccharides play little, if any, active role in the biologic adaptability of supporting bone to the movement of teeth in experimental animals.

Acid phosphatase has been well documented as an enzyme which is intimately related to resorption of bone.[34,35] Takimoto and co-workers[13] studied succinic dehydrogenase activity in osteoclasts, subsequent to tooth movement in experimental animals. It was felt that acid phosphatase might also prove to be useful in the study of osteoclastic activity subsequent to experimentally induced tooth movement. The findings obtained relative to the presence and distribution of acid phosphatase in the present study clearly indicated that this enzyme can act as a useful tool in the identification of osteoclastic activity. The author feels that demonstrating acid phosphatase may be preferable to succinic dehydrogenase in future studies, since tissues used to demonstrate acid phosphatase may be embedded in paraffin. This facilitates tissue sectioning of higher quality than can be routinely obtained with tissues prepared to demonstrate succinic dehydrogenase, since the latter must be sectioned in a microtome—cryostat without paraffin embedding.

Summary and Conclusions

Light latex elastics were placed between the contact areas of the maxillary left first and second molars of male golden hamsters in order to achieve tooth movement. The animals were sacrificed at intervals of one, seven, and fourteen days. One additional group was allowed thirty days recovery following fourteen days of elastic insertion. The teeth and supporting tissues were removed, decalcified in EDTA, fixed, embedded, sectioned and stained.

Staining procedures used were H & E and Masson's for histomorphology. The toluidine blue procedure for RNA and metachromasia, and the PAS procedure for 1:2 glycol linkages were also utilized. Acid phosphatase was also demonstrated and localized.

The major conclusions may be listed as follows:

1. Distal drifting of molars in hamsters was confirmed.
2. The concept that osteoblastic activity involves a higher rate of protein synthesis than does osteoclastic activity was substantiated.
3. Maximal tissue damage to the periodontium was at the site of elastic insertion.
4. Maximal damage to the supporting tissues was at the apices of the roots adjacent to the site of elastic insertion.
5. Examination of the group allowed to recover for thirty days indicated a generalized trend toward complete repair.
6. Demonstration of acid phosphatase would be useful in studying osteoclasia in similar future studies.

The current study clearly indicates that future investigation should focus more sharply on the repair mechanisms involved, subsequent to experimental tooth movement. It is also apparent that information obtained from the utilization of histochemical techniques will undoubtedly provide greater insight into the fundamental biologic mechanisms involved in the orthodontic movement of teeth than would be available from histomorphologic studies alone.

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