A Histological and Histochemical Study of Tooth Movement in Hamsters*

GARY J. MOSKOWITZ, D.M.D., M.S.

JOSEPH H. KRONMAN, D.D.S., Ph.D.

INTRODUCTION

Although millions of teeth have been subjected to orthodontic movements, studies of the fundamental biologic mechanisms involved have been meager. The histomorphic tissue changes that occur in tooth movement were investigated in the early 1900's.[1,2] Later investigators have examined the effects of different types of forces upon the root structure and alveolar bone, but again these studies were restricted to histomorphologic evaluation.[3-9]

The purpose of this study was to evaluate the histochemical changes induced by experimental tooth movement. Since chemical alterations in tissue metabolism precede structural alterations, it was felt that a histochemical study of the effects of tooth movement on the supporting structures would allow for a better understanding of the biologic mechanisms involved. The need for such study was further indicated when a comprehensive review of the literature revealed only two such prior studies utilizing two enzymes.[10,11]

MATERIALS AND METHODS

Male golden hamsters (Cricetus auratus), approximately ninety days old, were used in this experiment. The animals were divided into five groups thirteen animals per group (eleven treated, two control). A diet of Purina laboratory chow and water was supplied ad libitum. The animals were maintained for a period of three weeks prior to the initiation of the experimental procedures.

The instrument designed to facilitate the placement of orthodontic elastics between the crowns of the maxillary left first and second molars was a ligature tying plier modified by grinding the tips of the instrument to reduce the depth of the slotted area and to reduce the overall dimensions of the beaks. Three lingual buttons were welded to the left shaft at 80, 93, and 103 mm respectively from the tip of the instrument (Fig. 1). In the majority of animals, the button farthest from the tip was utilized in the placement of elastics. In cases of relatively loose contact areas, however, the middle button was used.

With this instrument orthodontic latex elastics, 5/16 inch, were stretched

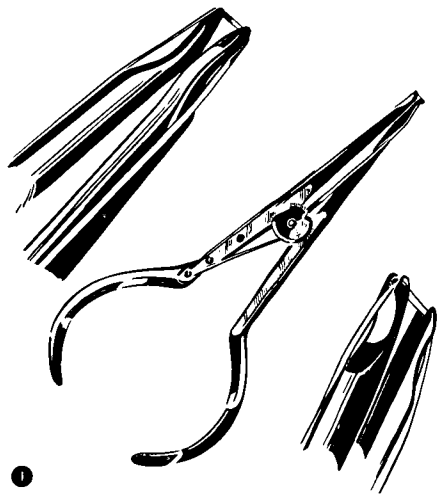

Fig. 1 Diagrammatic sketch of instrument with elastic in place ready for interproximal placement. The two inserts show enlarged views of the beaks.

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over the beaks and around the most distal button, in most instances, and around the circular hooking device. The elastics were lightly lubricated with vaseline and placed between the contacts of the maxillary left first and second molars of hamsters under ether anesthesia. After insertion of the elastic gingival to the contact area, the tension was gradually released and the elastic was cut on the buccal and palatal surfaces as close to the teeth as possible.

The animals were decapitated in groups at the following intervals after elastic insertion: 6, 12, 24, 48 hours, 4 and 7 days. At these intervals, two control animals were killed with each group. The left posterior quadrant of the maxilla was dissected and specimens were placed in 10 percent EDTA (Ethylenediaminetetraacetic acid) solution adjusted to pH 7.0.[12] Tissues were agitated in the cold room at 0-5°C. and solutions were changed every 24 hours. At the end of seven days the tissues were fully decalcified. One half of the specimens were frozen and sections cut at 14-20 microns in an International Microtome Cryostat at —18°C. The following enzymes were demonstrated on the fresh frozen sections: alkaline phosphatase by means of the azo dye coupling method;[13] acid phosphatase by the post-coupling method;[14] lactic dehydrogenase;[15] and succinic dehydrogenase.[11]

The other one half of the tissues, after decalcification in EDTA, were fixed in neutral buffered formalin. Tissues were then vacuum embedded in paraffin and sections were cut at 8 micra. These sections were utilized in the following manner: H & E and Masson's trichromic staining procedures were used 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 1 percent aqueous malt diastase at 37°C. in order to eliminate the presence of glycogen.[17] Acidic mucopolysaccharides were demonstrated by means of the Alcian blue staining procedure (pH 1.9).[18,19] Ribonucleic acids and metachromasia were demonstrated by means of the toluidine blue reaction, 0.05 percent (pH 4.5). Parallel control sections were incubated in a 1 percent RNA-ase solution in glass distilled water for one hour at 37°C.

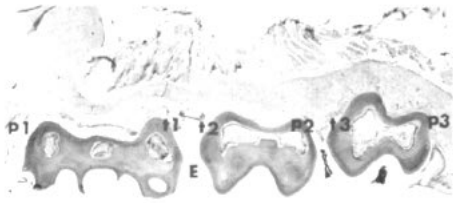

Fig. 2 Sagittal section through the molar roots and crowns from the 6-hour group. Site of insertion is indicated (E). Areas described in the text are labeled. H&E stain, X40.

Findings

In order to tabulate the histologic and histochemical tissue changes, a diagrammatic sketch was made and the areas to be studied were appropriately marked (Fig. 2). Evaluation included the following tissue changes: 1) thickness of the periodontal membrane, 2) osteoclastic and osteoblastic activity.

Histomorphology

On the normal control slides the periodontal membrane was fairly uniform in thickness in all the areas studied. The variations that existed were not significant. Numerous osteoclasts were generally noted in the interproximal alveolar bone just adjacent to the root of the first molar. (T1). To a lesser extent, osteoclasts were observed in the interproximal bone between the second and third molars (P2, T3) and also just mesial to the distal root of the second molar. Osteoblastic cells were arranged in rows in all of the areas investigated. Most of the osteoblastic

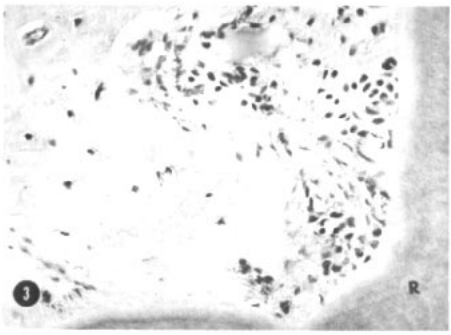

Fig. 3 Higher magnification of Fig. 2, illustrating the mesial aspect of the distal root of the third molar. Osteoclastic activity (c), and the root portion (R) are indicated. H&E stain, X350.

activity appeared on the medullary surfaces and there seemed to be a general decrease in activity progressing distally.

In the treated six-hour group the periodontal membrane appeared to be compressed at all the interproximal areas (Fig. 3). It was thicker mesial to the first molar (P1) and distal to the third molar (P3). Several slides showed complete destruction of the periodontal membrane at the site of elastic insertion. A moderate number of osteoclasts were observed in the interproximal bone between the second and third molars (P2, T3). Other areas of osteoclastic activity included the distal aspect of both roots of the first molar, the distal aspect of the distal root of the second molar, and the mesial aspect of the distal root of the third molar. Increased numbers of osteoblasts were seen in the bone at the site of elastic insertion and to a lesser degree mesial to the first molar (P1) and distal to the third molar (P3). Generally, there was little osteoblastic activity between the roots of the second and third molars (P2, T3).

In the twelve-hour group, destruction of the periodontal membrane at the site of elastic insertion was observed. Some osteoclasts were present in the interproximal bone between the first and second molars (T1, T2). There was also consistent evidence of osteoclasia on the distal aspect of the mesial root of the first molar. This, however, was not observed in all the sections studied. Minimal osteoblastic activity was seen at the area of elastic insertion.

Histologic detail was not well preserved in the treated twenty-four-hour sections. Therefore, no accurate findings could be reported for this group.

In the forty-eight-hour group the periodontal membrane was destroyed between the first and second molars (T1, T2) and was considerably compressed in the area between the second and third molars (P2, T3). An increased number of osteoclasts were located in the interproximal bone between the second and third molars (P2, T3). Increased osteoclastic activity was also seen at the apical area of the mesial root of the first molar. Osteoblasts appeared with a fair degree of uniformity in most sites. There was, however, greater activity in the interproximal bone between the second and third molars (P2, T3). At this site most of the activity appeared at the medullary surfaces. There was also noted, but to a lesser degree, some osteoblasts aligned on the cortical sides.

Sections of the four-day group revealed a periodontal membrane of fairly uniform thickness with complete destruction at the site of elastic insertion. Mesial to the first molar (P1), the periodontal membrane appeared compressed as it approached the apical area. Numerous osteoclasts were observed in the interproximal bone between the second and third molars (P2, T3). In addition to this, many osteoclasts were seen near the apex of the mesial root of the first molar and to a lesser extent along the mesial surface of this root. They were also observed near the apex of the distal root of the

third molar. Strong osteoblastic activity was noted in most areas studied. There was a particularly strong reaction in the interproximal areas of the three molars. As in the previous group, osteoblastic activity was seen in the cortical surfaces between the second and third molars.

In the seven-day group the periodontal membrane appeared to be similar to the findings of the preceding group. Increased osteoclasia was seen in the bone between the second and third molars (P2, T3). There were also numerous osteoclasts along the bone adjacent to the mesial root of the first molar (P1) progressing toward the apex. The mesial root of the third molar also showed osteoclasts adjacent to it apically. Osteoblasts were generally uniformly distributed. It is of interest to note that osteoblasts were now observed around the distal root of the third molar, in contrast to the findings in the previous group.

Histochemistry

In the sections stained with toluidine blue, the findings were compiled according to the staining reaction in the cytoplasm of the osteoblasts and osteoclasts and the metachromasia of the intercellular substance. The metachromatic staining of the ground substance was considered minimal in these sections. In comparing the treated groups with the normals, it was found that in the six-hour group there was an increased staining of osteoblasts. The osteoclasts, on the other hand, demonstrated an increased stain but to a lesser degree. The twelve-hour group revealed only slight increases in staining as compared with the previous group. These slides also showed that the osteoblasts were stained more strongly than were the osteoclasts. In the twenty-four-hour group the osteoclasts seemed to stain slightly darker than did the osteoblasts, although neither stained very

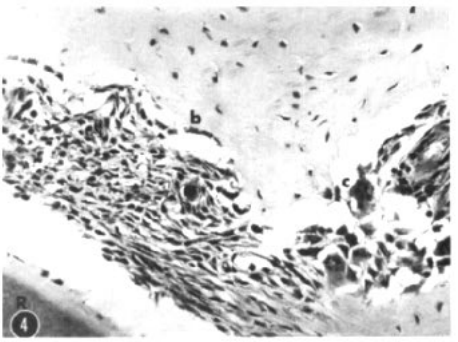

Fig. 4 Distal root of second molar, 4-day treated group, toluidine blue stain. Photograph shows osteoblastic (B), and osteoclastic (c) activity of alveolar bone. Root (R) is indicated for purposes of orientation. X380.

intensely. The findings of the forty-eight-hour group and the four-day group were comparable (Fig. 4). In these sections there was a widespread and intense staining of both osteoblastic and osteoclastic cells. The osteoblasts, however, generally stained with greater intensity in most areas. In the seven-day group the high degree of osteoblastic staining is maintained, while the osteoclasts appeared to diminish in their staining capacity.

Microscopic study of the sections stained with PAS revealed no appreciable uptake of stain by the tissues examined in this study.

The Alcian blue slides, which were specific for acidic mucopolysaccharides, also displayed no conclusive differences in staining in all sections studied.

The results of the enzyme staining were inconclusive and it was felt that these findings should not be included as part of this section. This will be discussed more fully in the following section.

Discussion

In the normal control slides the periodontal membrane was fairly uniform in thickness, as might be expected. The presence of osteoclasia just distal to the

distal root of the first molar (T1) and to a lesser extent between the second and third molars (P2, T3) seems to indicate a distal movement of the molars. This is to be expected as distal drift of posterior teeth in rodents has been reported in the literature.[20,21] It is interesting to note that osteoclasia was progressively reduced as one progressed distally. There is a possibility that the anterior teeth may also drift posteriorly and force the first molar distally. This could explain the tapering of osteoclastic activity toward the posterior. Another interesting finding was the osteoclastic activity observed on the mesial surface of the distal root of the second molar. This can be interpreted as a sign of distal coronal tipping of this tooth following the above pattern. The osteoblastic activity on the medullary surfaces also diminished posteriorly. This seemed to follow the pattern of osteoclasia. There was a remodeling of bone which was greatest in the areas of most active resorption. This concept of alveolar bone having an inherent tendency to maintain its original width is well documented.[22,23]

Sections of the treated six-hour group revealed compression of the periodontal membrane in the interproximal areas. This was the result of the second molar being forced distally by the placement of the elastic. In several slides the periodontal membrane between the first and second molars (T1, T2) was completely destroyed. When there was tissue destruction at the site of elastic insertion, attempts were still made to record tissue changes in adjacent structures. The distribution of osteoclasts was basically similar to the control slides. There was some increase in osteoclastic activity between the second and third molars (P2, T3), although this was not consistent in all slides. There was an increase in the space between the first and second molars caused by the elastic insertion. Therefore, it can be said there was mechanical tooth separation but no true evidence of any increased biologic response. The osteoblastic increase noted at the site of elastic insertion may be considered to be the tissue response to the wedging apart of these two molars.

In the twelve-hour group osteoclasts appeared for the first time in the area of elastic insertion (T1, T2). Osteoblastic activity in this group seemed to be minimal in nature. The findings seem to indicate that osteoblastic activity, as evidenced at T1 and T2 in the six-hour group, may precede osteoclastic activity. In other words, the initial reaction observed at the site of elastic insertion was bone formation followed by some evidence of bone resorption six hours later.

In the forty-eight-hour group the periodontal membrane was completely destroyed at the site of elastic insertion and very much compressed between the second and third molars (P2, T3). This is direct evidence of the distal movement of the second molar. Consistent with this was the increased number of osteoclasts found between the second and third molars (P2, T3). At this site, there was the combination of distal pressure on the second molar causing a compression of the periodontal membrane and the production of osteoclasts and active bone resorption. Osteoclastic activity was also noted around the apex of the mesial root of the first molar indicating tipping of the root of the tooth distally. The osteoblasts were increased in number in the interproximal bone between the second and third molars (P2, T3). Most of this activity was on the medullary surfaces indicating bone buildup in order to maintain the "inherent" bony width of the alveolar crest. Some cortical osteoblastic activity, mainly at T3, was noted for the first time. This is typical of a tension site as the third molar was pushed distally. Therefore, the reactions ob-

served in the tissues between the second and third molars are classical in nature and can be summarized as follows: 1) compression of the periodontal activity; 2) increased osteoclastic activity; 3) increased osteoblastic activity on the medullary surface of the pressure site (P2); and 4) increased osteoblastic activity on the cortical surface of the tension site (T3).

The four-day sections again demonstrated appreciable osteoclasia between the second and third molars (P2, T3). From the apex of the mesial root of the first molar an extension of the osteoclastic activity was seen along the mesial surface of the root. This is an indication of a reversal of the distal tipping of the first molar. Resorption along the mesial of the first molar revealed a tendency toward bodily tooth movement to the mesial. The osteoblastic activity of this group was very pronounced. In all the interproximal areas (T1, T2, and P2, T3) there was a marked increase in the number of osteoblasts. It appears as though an equilibrium may exist with regard to the osteoclastic and osteoblastic reaction.

In the seven-day group there was a continued increase in osteoclasts between the second and third molars (P2, T3). There was also a greater number of osteoclasts along the mesial root of the first molar (P1) indicating an uprighting or bodily movement of this tooth mesially. Osteoblasts were generally uniformly distributed in these sections but seemed to be decreased in numbers. There was, however, an accumulation of osteoblasts around the distal root of the third molar, which area formerly showed osteoclastic activity. Osteoclasts were now evident at the mesial root of this tooth. There was, therefore, a reversal of pressure and tension areas around these roots. This would indicate that initially there was a tipping of the third molar distally. At seven days an uprighting of this molar was observed. This is similar to the reaction of the first molar as previously described. It may be said that when an elastic was inserted between hamster molars the initial reaction was a tipping of teeth away from the site of pressure followed by the eventual uprighting of these teeth.

In the histochemical portion of this investigation, prepared tissues were stained in order to demonstrate substances or enzymes which are involved in tissue metabolism. It was hoped that a pattern of chemical changes might be established and thereby increase knowledge of the biology involved in tooth movement.

Sections stained with toluidine blue were utilized to demonstrate the presence of RNA in the cytoplasm of osteoblasts and osteoclasts. RNA has been used as an index of protein synthesis and has been shown to be related to increased cellular proliferation,[24-26] and to metabolically active cells, particularly osteoblasts.[27,28] In all the groups studied metachromatic reactivity was minimal. Compared with the normal there was an increased staining of the osteoblasts in the six-hour treated animals. Staining intensity diminished in the twelve-and twenty-four-hour groups. In the forty-eight-hour group the osteoblasts stained very intensely and this was maintained in the four- and seven-day sections. This chemical response seems to follow closely the histological response at six hours comparable to the beginning of osteoblastic activity seen in the histomorphic sections. The osteoblasts in the twelve- and twenty-four-hour groups lacked reactivity with regard to RNA. At forty-eight hours there was an increased number of osteoblasts and increased staining with toluidine blue. This was maintained in the succeeding groups. The increase in RNA can be interpreted as an increase in protein synthesis in the cytoplasm. Therefore, the increased protein metabolism closely

follows the numerical increase of osteoblasts as seen by histologic investigation. The staining reaction of the osteoclasts also generally followed the histologic response, although more variations existed. The six-hour group revealed some stain uptake but less than seen in the osteoblastic cells. The twelve-hour group demonstrated less staining, whereas the histologic response of this group revealed an increased number of osteoclasts. It is probable that the metabolism of proteins precedes the numerical increase in cells seen histologically. In the twenty-four-hour slides the RNA stained with greater intensity than that seen in the osteoblastic cells. This again might be interpreted as the increased chemical reaction prior to the increase in osteoclastic cells as seen in the forty-eight histologic sections. The forty-eight-hour and four-day RNA groups demonstrated strong staining of osteoclasts, although generally they stained less intensely than did the osteoblasts. This coincided with the osteoclastic buildup evident in the morphologic slides of the forty-eight-hour and four-day groups. The seven-day RNA slides, however, showed a unique staining pattern. The staining reaction of the osteoclasts diminished dramatically, while the histologic slides revealed a continued high activity of these cells. It is interesting to note that in all groups, with the exception of the twenty-four-hour group, the osteoblasts stained more intensely for RNA than did the osteoclasts. This might indicate that a greater activity of protein metabolism may exist in the formation of new bone than that which occurs in bone resorption.

The literature has several reports of histochemical reactions in bone utilizing PAS staining. Leblond[29] detected the presence of glycogen, mucoproteins and mucopolysaccharides in bone matrix after decalcification. Sognnaes[30] stated glycogen has been demonstrated in osteogenic cells prior to calcification. He also spoke of another PAS-positive component in the cytoplasm of osteoblasts and in the ground substance. Other authors confirmed the presence of glycogen and mucopolysaccharides accompanying areas of high alkaline phosphatase activity.[31-33] Mucopolysaccharides demonstrated with PAS were also found in high concentrations in Haversian canals and only slightly in the matrix.[34-36] Heller-Steinberg[37] demonstrated more metachromasia during bone formation than during bone resorption. Yaeger[38] stated that bone matrix adjacent to osteoclasts was more PAS-positive than resting bone.

In the present study the slides stained with Alcian blue and PAS revealed no conclusive differences between the different groups. Since there were no variations observed in the treated or controls in the sites studied, one may, perhaps, conclude that neutral and acid mucopolysaccharides do not play a significant role in the metabolic response of tissues to the movement of teeth through bone in experimental animals.

The enzymes used in this study were selected in order to provide meaningful information relative to the metabolic cycles which may be involved in the deposition and resorption of bone induced by tooth movement. Alkaline phosphatase has been well established as playing an important role in bone formation. Using decalcification methods, osteoblasts were demonstrated to have high alkaline phosphatase activity.[39,40] Similar conclusions were reached using undecalcified bone.[41-44] It has been reported that alkaline phosphatase activity is associated with early matrix formation, and that phosphatase activity is absent in more highly calcified areas.[30,31,33,45-47]

Acid phosphatase has been shown to play a role in bone resorption. Mori, Takada, and Okamoto[48] found a strik-

ing acid phosphatase reaction in epiphyseal bone trabeculae. Schajowicz and Cabrini[49] used a decalcification method to demonstrate acid phosphatase in osteoclasts located in ossification centers in man, rats, and mice. Burstone[50] and Yoshiki[46,47] also found acid phosphatase activity in the early stages of calcifying matrices. It is obvious that the above two enzymes are of tremendous significance in understanding the movement of teeth through bone.

It was anticipated that glycogen would play a role in providing energy for these reactions. Glycogen and mucopolysaccharides had been demonstrated in osteogenic cells prior to calcification. Bevelander and Johnson[31] concluded that glycogen and mucopolysaccharides are present in osteogenic cells and also in fibers. In studying normal compact bone of dogs, Engfeldt and Hjertquist[34] found PAS-positive material in newly-formed lamella of the Haversian systems. The present study was designed to demonstrate the presence of glycogen (by the PAS reaction). Based upon this supposition, it was felt that some indicator of anaerobic and aerobic glycolysis should also be demonstrated in order to provide a more meaningful interpretation of the anticipated findings. Lactic dehydrogenase was selected as an index of anaerobic glycolysis in the Embden-Myerhoff cycle.[51,52] Succinic dehydrogenase was used as an index of oxidative metabolism in the succin-oxidase system.[46,47,51-53]

In this experiment tissues were decalcified in EDTA[12] prior to sectioning. The literature presents many opinions on the subject of decalcification. Morris and Benton[54] evaluated several acids for decalcification and concluded that hydrochloric acid was best, followed by formic acid. Schajowicz and Cabrini[49] demonstrated acid phosphatase with several decalcifying agents. Their best results, with little enzyme loss, were with sodium citrate and formic acid.

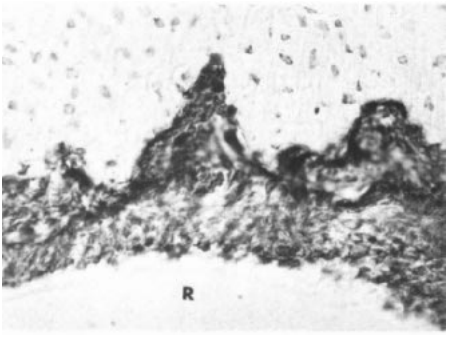

Fig. 5 Lactic dehydrogenase in 24-hour group. This photograph illustrates that enzymes may be demonstrated subsequent to EDTA decalcification. Root (R) is indicated for orientation. X450.

They also stated that Versene destroys or inhibits this enzyme. EDTA decalcification has been used by many investigators.[48,51,55] It was the general opinion of these authors that EDTA was an agent that retained enzymatic activity. Many other investigators have used undecalcified techniques for demonstrating enzymes. Burstone[42,50] indicated that this method is the most accurate for enzyme detection. Other investigators[43,44,56] report better histochemical results when using undecalcified sections.

Experience gained in the present study clearly indicates that, although the method chosen for decalcification and enzyme demonstration may be employed in studies of bone, it was entirely inappropriate for the current investigation (Fig. 5). It was necessary in this study to procure parasagittal sections through the jaws, supporting structures, and teeth in the areas involved. Following the procedures described by others, these tissues were sectioned in the cryostat without prior embedding. This posed several problems. The first of these involved proper orientation of tissues in order to produce the desired plane of section. This was found to be extremely difficult to accomplish. In addition, in many instances teeth were dislodged from their supporting tissues.

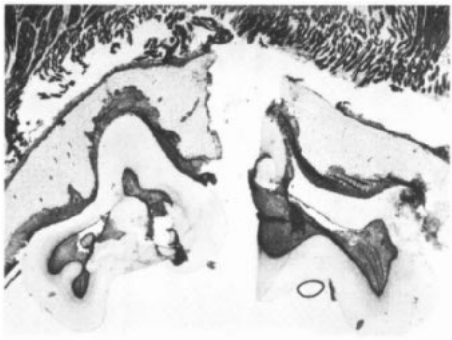

Fig. 6 Lactic dehydrogenase in 24-hour group. This section illustrates the problems encountered in obtaining suitable sections, as described in the text. X100.

A further complicating factor, as one might expect with unembedded tissues of this type, involved severe wrinkling, folding, and induced sectioning artifacts (Fig. 6). It was therefore impossible to recover sufficient numbers of suitable sections from the appropriate groups which would be necessary in making a critical evaluation of the results. It should be stated, however, that the findings in this study confirm the fact that the enzymes employed may be demonstrated subsequent to the decalcification conducted relative to the enzymatic processes involved in the movement of teeth in experimental animals. It is felt that utilization of a microtome that would allow for sectioning of undecalcified material would probably be essential in providing meaningful results necessary for such a study.

Summary

Experimental tooth movement was produced in male golden hamsters by the placement of latex elastics between the contact areas of the maxillary left first and second molars. The animals were sacrificed at intervals of 6, 12, 24, 48 hours, and 4 and 7 days, and the teeth and paradental tissues were decalcified in EDTA and studied for histologic and histochemical changes. Morphology was demonstrated by H and E and Masson's staining. Histochemical procedures included the PAS reaction for the demonstration of glycogen, Alcian blue staining for acidic mucopolysaccharides and the toluidine blue reaction for the presence of RNA and metachromasia. The enzymes investigated in this study were alkaline phosphatase, acid phosphatase, lactic dehydrogenase and succinic dehydrogenase. The conclusions may be summarized as follows:

1. Histologic findings confirmed the normal distal drift of molars in hamsters, as reported previously.
2. The placement of interproximal elastics caused an initial tipping of teeth away from the area of elastic insertion, followed by an uprighting of teeth at approximately seven days.
3. Osteoblastic activity seemed to precede osteoclastic activity in tooth movement.
4. Osteoblasts stained more intensely than did osteoclasts with toluidine blue. This might indicate that a greater activity of protein metabolism may exist in the formation of bone than that which occurs in bone resorption.
5. The increase in RNA seemed to follow the numerical increase of osteoblasts and osteoclasts and, in many instances, preceded the increase of these cells.
6. Slides stained with Alcian blue and PAS revealed no conclusive variations between the control and treated groups.
7. The results of enzyme staining were inconclusive. It was felt that although enzymes may be demonstrated subsequent to decalcification procedures, better results would be obtainable with utilization of a more suitable microtome.

136 Harrison Avenue
Boston, Mass. 02111

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