A Morphologic Comparison of the Periodontal Ligament of Normal Versus Accelerated Erupting Teeth in Rats and Guinea Pigs

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A MORPHOLOGIC COMPARISON OF THE PERIODONTAL LIGAMENT OF NORMAL VERSUS ACCELERATED ERECTING TEETH IN RATS AND GUINEA PIGS

by

ANDREW P. TRAPANI, D.D.S.

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF LOYOLA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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CHAPTER I
INTRODUCTION AND STATEMENT OF PURPOSE

A controversy over the occurrence of an "intermediate plexus" within the periodontal ligament has existed for a number of years.

Sicher (1923, 1942, 1954, 1959, and 1962) repeatedly alluded to this "intermediate plexus" in his papers. The plexus (he stated) provided the necessary adaptation of drift, attrition, and rapid growth by an unsplicing and resplicing of the principal fibers of the periodontal ligament. These changes were said to occur without a total loss of old fibers, as well as their replacement by new fibers that would involve intensive remodeling of bone and cementum.

Ciancio (1967) stated this intermediate plexus was merely an artifact created by histologic sections which were not cut parallel to the direction of the principal fibers. A number of other researchers have disputed the existence of the intermediate plexus (Trott, 1962; Eccles, 1959; Zwargch and Quigley, 1965; and Crumley, 1964).

In an attempt to resolve some of this controversy, we have performed a number of experiments. The purpose of this paper is threefold:
(1) The histologic structure of the periodontal ligaments of continuously erupting teeth (rat incisor, guinea pig incisor, and guinea pig molar) will be described and compared with those of non-continuously erupting teeth (rat molar).

(2) All teeth on the right side of each animal's mouth will be ground out of occlusion. The reduced teeth will subsequently erupt at an accelerated rate as has been shown by a number of investigators. The periodontal ligaments of normal versus reduced teeth will be studied and compared at the microscopic level.

(3) The results of the above investigations will be evaluated and correlated with current knowledge of the periodontal ligament with emphasis on the intermediate plexus.
CHAPTER II
REVIEW OF THE LITERATURE

A. Principal Fibers of the PDL

Many biologists have histologically studied the principal fibers of the periodontal ligament. Flagg (1839) recorded the first histologic concept of the "Ligamentum Dentis". He thought the periodontal ligament to be a specialized type of periosteum.

Ingersoll (1886) raised the question of the periodontal ligament being a single or double ligament. G.V. Black (1887, 1899) gave the first detailed description of the periodontal ligament. He classified the principal fibers into six distinct groups: (1) the free gingival fibers, (2) the transseptal group, (3) the alveolar crest fibers, (4) the horizontal fibers, (5) the oblique fibers, and (6) the apical group. He also described blood vessels, lymph channels, nerves, and cellular elements within the periodontium.

The periodontal ligament was described as a double ligament by Cupit (1904). He showed the presence of alveolar periosteum coupled to pericementum.

Tomes (1923) showed that periodontal ligament fiber bundles next to the cementum were markedly different than those
next to the alveolar bone. He reported seeing no break in the continuity of the fibers as they stretched from bone to cementum.

Sicher (1923, 1954, 1959, 1962) used the term "intermediate plexus" to describe the splicing of the dental fibers with the alveolar fibers as seen in the middle zone of the periodontal ligament. He demonstrated the intermediate plexus in the rapidly growing teeth of the rat and the guinea pig (Sicher, 1923). The plexus (he stated) provided the necessary adaptation of drift, attrition, and rapid growth by an unsplicing and re-splicing of the fibers. He later added that the plexus is also necessary in man to allow for the constant rearrangement of fibers due to continual eruption and mesial drift of teeth (Sicher, 1959).

A study of silver-impregnated sections through human teeth shows that no single principal fiber can be traced from bone to cementum. Instead, the fibers all seem to end or to begin somewhere in the middle zone of the periodontal membrane. The single fiber bundles consist of shorter fibers that seem to be "spliced" in the middle. The human periodontal ligaments consist of alveolar fibers, dentin fibers, and an intermediate plexus. The possibility of adjustment in this intermediate plexus permits a functional reorientation of the principal fibers and their lengthening or shortening by the activity of fibroblasts in this zone. These changes occur without total loss of old fibers and their replacement by new fibers that would involve intensive rebuilding of bone and cementum. (Sicher, 1959)

Hunt (1959) and Goldman (1962) added support to Sicher's concept of the intermediate plexus. Bernick (1962) described the plexus in the marmoset but could not demonstrate the same
in rat molars (1960).

The existence of the intermediate plexus was disputed by Trott (1962) in the rat, Eccles (1959) in the rat, Zwargch and Quigley (1965) in the mouse, Crumley (1964) in the rabbit, and Ciancio (1967) in the rabbit.

Ciancio (1967) showed that "immediately upon leaving their attachment to bone, the fibers formed an anastomosing network which attached to the cementum by means of numerous fiber bundles." The fiber bundles attached to the cementum were greater in number and finer in appearance than the alveolar bundles. The plexus was only observed in areas where the periodontal fibers were sectioned at an angle to their direction. Therefore, he felt that the intermediate plexus was an artifact due to sections which were not prepared parallel to the direction of the principal fibers.

Weinmann (1955) separated the principal fibers of the periodontal ligament into three groups: (1) gingival fibers, (2) interdental(transseptal), and (3) alveolodental. He reported that the fiber bundles ran as a unit from bone to tooth but not the single fibers. It was necessary, in his view, that the fibers be interrupted in order to allow for the adaptive changes of the erupting, functioning tooth.

Nowhere do the single fibers stretch from bone to cementum. Instead, they are woven together about midway between tooth and socket by a rather cellular layer of
connective tissue. The periodontal ligament can be divided; therefore, into alveolar fibers, dental fibers, and the intermediate plexus which, in a manner of speaking, splices the fiber bundles together. (Weinmann, 1955)

Orban (1957) described the same three zones (alveolar, cemental, and intermediate plexus) within the periodontal ligament. He, too, emphasized that the "bundles are 'spliced' together in an intermediate plexus midway between cementum and bone".

Smukler and Dreyer (1969) studied the direction of the principal fibers of the periodontium in monkeys. They described gingival fibers which had connections with adjacent facial musculature.

In a recent study of the human periodontal ligament utilizing the electron microscope, Bevelander and Nakahara (1969) showed that the arrangement of the principal fibers is more complex than previously described and further, that many of the various cells observed in the periodontal ligament are apparently in an active functional state. The fibers were found to be collagen arranged in bundles with a periodicity of 640 Å. No intermediate plexus was observed.

Wasserman (1959), in another electron microscope study of the intermediate plexus in guinea pigs, showed that there is a continuous breaking down of fibers into fibrils and regrouping into new fibers.
In a study using polarized light, Hindle (1967) demonstrated that the fibers of the middle zone of the rat periodontal ligament are less mature than in the outer zones. He concluded that fiber readjustment occurs in this region.

B. Cellular Elements of the PDL

Schour (1960) described the cellular elements present in the periodontal ligament.

1. Fibroblasts are found throughout the periodontal ligament. They form the collagen fibers. They are spindle-shaped cells with a flattened nucleus and a granular cytoplasm. They stain with hematoxylin.

2. Histiocytes are found in the loose connective tissue between the fibers.

3. Cementoblasts are seen to cover the root between the fibers. They have a flattened, scale-like, irregular outline. Their nucleus is generally oval or slightly flattened.

4. Osteoblasts cover the bone surfaces and are located between the fiber bundles. They are oval-shaped cells and very basophilic.

5. Osteoclasts are the typical multinucleated cells seen in areas of active bone resorption.

6. Epithelial rests, remnants of Hertwig's epithelial sheath, are usually found near the cementum. The major-
ity opinion holds that fibroblasts are differentiated cells which do not give rise to other types of free cells of the connective tissue. There is good evidence, however, that they can develop into bone cells, and some indication that they become slightly phagocytic on intense stimulation. (Bloom and Fawcett, 1962)

C. Development of the PDL

The periodontal ligament is derived from the dental follicle. Around the tooth germ, three zones can be seen: an outer zone containing fibers related to the bone; an inner zone of fibers adjacent to the tooth; and an intermediate zone of unorientated fibers between the other two. During the formation of cementum, fibers of the inner zone are attached to the surface of the root. As the tooth moves toward the oral cavity, a gradual functional orientation of fibers takes place. Instead of loose and irregularly arranged fibers, fiber bundles extend from the bone to the tooth. (Orban, 1957)

Although the bundles run directly from bone to cementum, the single fibers do not span the entire distance. The bundles are "spliced" together from shorter fibers in an intermediate plexus midway between cementum and bone. (Orban, 1957)

Noyes and Schour (1955) demonstrated the presence of the intermediate plexus in the developing tooth. Eccles (1959, 1961) studied the development of the periodontal ligament in the rat molar. Initially, the fibroblasts are unorientated
but soon line up in an oblique direction to the root. Next, collagen is seen between and parallel to the fibroblasts. The collagen subsequently becomes arranged into bundles. The bundles appear to be thicker on the alveolar side. At this time the fibers from cementum and bone appear to be unraveled. Four to five days prior to the tooth's eruption into the oral cavity, the cemental and alveolar fibers meet. "No bundle or fiber runs directly from bone to tooth." (Noyes and Schour, 1955) In the fully erupted tooth the fibers do appear to run directly from bone to tooth.

Bernick (1960) duplicated Eccles' study and the results corresponded. In addition, Bernick pointed out that the gingival and transseptal fibers did not appear in the forming periodontal ligament until the rat molar was fully erupted.

In another study of the developing periodontal ligament, Furstrom and Bernick (1965) demonstrated that the periodontal ligament begins its development simultaneously with the organization of the cementum on the surface of the root. He also noted that the fibers do not run through the plexus but rather, that this plexus intermedius gradually closes as the tooth erupts. Prior to eruption, only obliquely orientated fibers are seen. With eruption, the oblique fibers at the alveolar crest become transseptal and horizontal groups. The transseptal fibers do not organize until the opposing teeth are in occlusion. Complete organization of fibers is not achieved until the tooth
reaches functional occlusion.

D. Functional Alterations of the PDL

1. Eruption.

Kronfield (1931) demonstrated that the dense collagen fiber bundles of the periodontal ligament correspond to the direction of force in a functioning tooth; while a non-functioning tooth exhibits a poorly developed periodontal ligament consisting of loose connective tissue without any true fiber orientation. Weiss (1939), Sicher (1942, 1949), and Orban (1957) all agree that the fibers of the periodontal ligament become functionally orientated.

The eruptive movements of the tooth vertically and horizontally need continual changes in the supporting tissues: alveolodental, interdental, and gingival ligaments. All adjustments occur in the middle zone—intermediate plexus—of these ligaments. (Sicher, 1964)

The dimensions of the intermediate plexus are correlated to the speed of eruptive movements. When teeth erupt fast (i.e., rodent incisors), the intermediate plexus occupies more than half of the periodontal space. Where teeth erupt slowly (i.e., man), the intermediate plexus is inconspicuous and may be overlooked. (Sicher, 1964)

Melcher (1967) reported that as the tooth erupts, the fibers embedded in the cementum travel with it, while those in the bone remain behind.
"It is widely believed that the adaptation of periodontal fibers, which is necessary during eruption as the tooth moves in relation to the bone, takes place in the intermediate plexus." (Weinmann, 1955)

This plexus is believed to be present in all mammalian teeth, being conspicuous or inconspicuous according to the rate of the eruptive movements. (Orban, 1957)

2. Functioning Versus Non-functioning Teeth

If a tooth is taken out of occlusion, its rate of eruption is accelerated. In the instance of the rodent incisor, its eruption rate is more than doubled until occlusal contact is restored. A molar tooth with no antagonist also erupts at a faster rate. (Schour and Massler, 1949)

If a tooth lacks an antagonist (hypofunction), the width of the periodontal ligament space is reduced from the normal thickness of .20-.25 mm. to .10-.15 mm. The fiber bundles atrophy and are replaced by loose, irregular connective tissue with no definite orientation. (Schour, 1960)

Schour also showed that if an excessive force is applied to a tooth (hyperfunction), the width of the periodontal membrane increases from .20-.25 mm. to .28-.35 mm. The fiber bundles become stronger and the quantity of loose connective tissue is reduced.

Stallard (1963) devised an interesting study in which
he reduced the occlusal surfaces of all molar teeth in rats except the first molars. Thus, he was able to study the effects of hypofunction and hyperfunction utilizing different teeth of the same animal. The effects of hypofunction were seen as a slight increase in the Radioactive Index of the periodontal membrane fibroblasts. This cellular activity reached a peak in eight days and dropped back to normal by twelve days.

The effects of hyperfunction were best reflected by a great decrease in the Bone Formation Index and an increase in osteoclastic activity. These values again returned to a normal range within a twelve day period.

Eccles (1965) was successful in showing morphologic differences between normal, hypofunctional, and non-erupting rat incisors. The non-functioning teeth had a marked atrophy of the alveolar bone and alveolar fibers. Therefore, it seems evident that function is necessary for the maintenance of the alveolar part of the periodontal ligament. The cemental fibers were unchanged.

In the animals with non-erupting incisors (gold crowns were placed to stop attrition), he reported a gradual disappearance of the palisading of the fibroblasts and the adjacent cell-free zone which are commonly found at the junction of the alveolar fibers and the intermediate plexus of the normally erupting tooth. This suggests that the cell-free zone may form a well-
defined border to the movement which takes place in the intermediate plexus during eruption. The fibers of the plexus organized into thick bundles due to a loss of continual changes.

It was shown by Cohn (1966) that a particular molar in the mouse can be made to supraerupt if function is selectively removed. Anneroth and Stockholm (1967) reproduced the experiment using monkeys and showed a slight disorientation of the periodontal membrane of the supraerupted teeth. The root cementum was also seen to increase in thickness especially near the apex.

Richardson (1967) found that the thickness of the periodontal space does not differ between the functioning and non-functioning teeth. The previous concept of an increased thickness seen in the periodontal ligament of the non-functioning tooth, he continues, is probably due to an elongation of the tooth in its socket.

3. Age Changes

Kronfield (1936) stated that aging had no noticeable effect on the thickness of the periodontal membrane space. In contrast, Coolidge (1937) indicated a reduction in the width of the space associated with aging.

Dummett (1958) demonstrated a reduction in the prominence of the principal fibers consequent to a reduction in function.
Haim (1965) found no major alteration of the collagen fibers associated with age changes.

Schour (1955) described a decrease in the number of fibroblasts with increasing age.

E. Collagen Formation

The following is a brief review of the available information concerning fibrogenesis according to Jackson (1967). The collagen molecule consists of three distinct polypeptide chains, each having a repeating pattern of units comprising three amino acids (glycine, proline, and hydroxyproline). The amino acids are joined together via strong covalent peptide bonds.

Each polypeptide chain is wound in a left-hand helix, while the whole group of three chains -- forming the molecule (tropocollagen) -- twists slowly in a right-handed helix to give a kind of double coil.

The length of the collagen molecule is approximately 3000 Å but the molecules are aligned to form a 640 Å repeating structure, each molecule running through four periods. Side to side cross-linking takes place.

The fibroblast is the all-important cell in collagen synthesis. The current feeling is that "parts" of the collagen fibers are produced within the fibroblasts and these "parts" are assembled extracellularly into mature collagen units.
The system of aggregated macro-molecules subsequently becomes stabilized by covalent cross-linkages. The number of these cross-linkages increases with time (i.e., a maturation process).

The collagen fibers are flexible, but offer great resistance to a pulling force. The breaking point of human collagenous fibers (tendon) is several hundred kg./cm.², and their elongation at this point is only a few per cent.

F. Fiber Rearrangement Within the PDL

Stallard (1963) using tritiated proline has shown that there is more than one area of the periodontal ligament in which a high degree of collagen fiber formation is occurring. His work supports the concept that the fibers of the periodontal ligament are dynamic throughout their length, are constantly undergoing change, and that tooth eruption can occur due to this rearrangement.

Crumley (1964) found that different rates of collagen formation exist in different areas of the normal periodontium. Bone collagen appears to be forming more rapidly than the collagen in the periodontal membrane. Cementum collagen is forming at the slowest rate.

Using tritiated proline and glycine, Carneiro and de Moraes (1965) were able to show a constant renewal of collagen within the periodontal ligament. Carneiro (1965) demon-
strated that much of the labeled amino acids are incorporated into collagen rather than into other proteins. He also showed that the fibroblasts secrete the collagen.

An even distribution of H\textsuperscript{3}-proline in the periodontal ligament of guinea pig molars led Ramos (1967) to support the concept of a uniform synthesis and breakdown of collagen fibers within the ligament.

G. Cellular Activity Within the Periodontal Ligament

In an experiment utilizing tritiated thymidine in monkeys, Mc Hugh and Zander (1965) demonstrated that the connective tissue cells showed the highest incidence of labeling in the middle one-third of the periodontal ligament with less labeling in the root and bone areas of erupting and erupted teeth.

Macapanpan, Meyer, and Weinmann (1954) reported similar findings and confirmed that the high cellular activity in the plexus intermedius is the occurrence of numerous mitosis in this zone. Other ligaments in the body do not possess this high activity.

H. The Normal Rat Incisor

The rat incisor develops from an epithelial organ which is different on its labial and lingual aspects. On the labial (and slightly on the lateral surfaces), the formative
base represents the human enamel organ. On the lingual and remainder of the lateral surfaces, it resembles Hertwig's epithelial sheath.

The incisor is a continuously erupting tooth and, because cellular activity is greater on the labial than the lingual surface, the tooth is curved. The rate of eruption is 2-3 mm. per week in the upper incisor.

Cementum is laid down along the lingual, mesial, and distal surfaces at a slow rate. It is normally very thin, reaching a maximum thickness of 3 to 4 microns at the incisal end. (Schour, 1960)

The periodontal ligament is clearly arranged into three distinct zones. (Eccles, 1965)

(1) The alveolar fibers are thick, dense, slightly wavy bundles of collagen fibers extending about halfway across the periodontal space. Fibroblasts are aligned parallel to the bundles, and blood vessels pass between them. The orientation of the fibers at the apical and incisal regions are perpendicular to the bone. In the middle of the root the fibers are oblique.

(2) The fibers of the intermediate plexus are arranged in a loose mesh. The fibers, which are mainly oblique, run almost parallel to the root surface. Again, the fibroblasts are arranged with their long axes parallel
to the fibers. The nuclei of these cells appear larger, oval, and less densely stained.

(3) The cemental zone of fibers are characteristically short, thin bundles running perpendicular to the cementum. Cementoblasts surround these fibers and provide for a firm attachment to the tooth.

I. The Normal Rat Molar

Schour (1960) describes the rat dentition as monophyodont (one set of teeth). It consists of one incisor and three molars in each quadrant of the mouth. The first molar is the largest tooth.

The rat molars are of limited growth and similar to those of the human except for the enamel-free areas at the cusps.

The proliferation of the formative organs of the dental tissues and the histo-differentiation of the cellular elements are similar to that seen in human molars.

Secondary cementum forms in relation to functional stresses on the teeth and is added continuously throughout life. The adult rat molar root may owe one-third of its length to cementum alone. The roots are three to five in number.

The eruption of the molars is continuous throughout life but occurs at an extremely slow rate. "Prior to the occlusion of the teeth, the rate of eruption is very rapid; but as
soon as antagonism is established, the rate is markedly and mechanically retarded. Eruption is accelerated following the loss of an antagonist." (Schour and Massler, 1949)

Physiologic attrition is present throughout life and is compensated by the continuous eruption of the teeth and the formation of secondary cementum.

The periodontal tissues closely approximate the human periodontium. Thick transseptal bundles run from tooth to tooth. Just apical to these interdental fibers are found the alveolar crest group of fibers which travel from the cementum to the alveolar bone below. Next is a group of horizontal fibers.

The major portion of the periodontal ligament is formed by the oblique fibers, running from the bone above to the cementum below. The oblique arrangement is primarily due to the fibers of the middle zone, as the alveolar and cemental fibers are arranged at almost right angles to the long axis of the root. (Schour, 1960)

J. The Normal Guinea Pig Incisor

The description of the guinea pig incisor is essentially the same as that of the rat incisor.

K. The Normal Guinea Pig Molar

The molar teeth of the guinea pig grow continuously.
Each tooth is comprised of a core of dentin covered by enamel on all axial surfaces except the lingual of the lower and the buccal of the upper. When viewed from the occlusal surface, two folds can be seen entering the tooth. These folds are made up of a thin enamel lining and are filled with cartilage. (Hunt, 1959)

The apical two-thirds of the teeth are embedded in bone. Periodontal ligament fibers course from the alveolar bone to either enamel, dentin, or cartilage. Transseptal fibers are also present.

The method by which the periodontal ligament attaches to the enamel surface deserves special attention. Fibroblasts, in a spear-shaped aggregation, wedge between the ameloblasts to eventually reach the underlying enamel surface. As the fibers mature, they become calcified and the final outcome is a "cemental pearl."

The periodontal ligament attachment in areas of dentin is via non-cellular cementum.

In areas of cartilage, the alveolar fibers continue into the perpendicular bundles. From the perpendicular bundles, isolated fibers extend at right angles to the tooth surface, branch out, and enter the cartilage.

Active collagen fiber formation is present at the following sites (Hunt, 1959):
(1) Recently maturing enamel (apical one-half)—at the point where the cement pearls form, collagen fibers begin to bridge the gap between enamel and bone.

(2) Middle zone of the periodontal ligament—where the fiber bundles slant up from enamel and down from the bone to intermingle with a central perpendicular bundle of fibers.

(3) A zone just above the crest of the interalveolar bone septum.

(4) Along the axial surface of the alveolus—where bone is being remodeled (slow collagen turnover). Bone resorption is normally seen on the alveolar bone mesial to the first molars and distal to the second, third, and fourth molars. Numerous osteoclasts underlie each apex. The interdental fibers are produced in an area immediately occlusal to the crest of the interdental septum. They are carried occlusally with the teeth and disappear just below the oral epithelium.
CHAPTER III
MATERIALS AND METHODS

A. Animals Employed

Nine adult albino rats and nine adult male guinea pigs were obtained from a commercial supply house. The animals were housed in the animal laboratory at the Loyola University School of Dentistry and placed on the standard animal diets used at the University. All environmental stimuli were kept at a minimum.

B. The Experiment

The animals were anesthetized with ether administered through inhalation. All of the teeth on the right side of the jaws were ground down to the level of the gingiva. A conventional dental handpiece with a green stone was employed to carry out this procedure. Care was taken not to expose the pulpal tissues.

The teeth on the right side were ground "out of occlusion" on seven rats and seven guinea pigs. The remaining four animals, two rats and two guinea pigs, were used as experimental controls.

The animals were returned to the animal rooms and no alterations in diet or environment were made.

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On the nineteenth day the rats were sacrificed. At that time the incisor teeth had fully erupted, but the molars on the test side were still out of occlusion.

Guinea pigs were sacrificed on the ninth day. It appeared that the molars had fully erupted but the incisors had not.

Four more guinea pigs were procured and their teeth ground down as before. This group was sacrificed on the fifth day. Both the molars and the incisors on the test side appeared to be slightly out of occlusion at the time of death.

C. Preparation of the Specimens

All twenty-two animals were killed by an overdose of ether and were immediately decapitated, skinned, and cut into blocks. Six blocks were made from each animal. The blocks were: (1) maxillary right molars, (2) maxillary left molars, (3) maxillary right incisor, (4) maxillary left incisor, (5) mandibular right molars, and (6) mandibular left molars.

The blocks were fixed in neutral formalin for a minimum of forty-eight hours.

The blocks were then decalcified in equal volumes of 20% sodium nitrate and 50% formic acid.

The specimens were washed in clear water (six hours) and then dehydrated in alcohols -- 50%, 70%, 80%, 96% and finally, absolute alcohol. The specimens were blocked in fresh paraffin.
Sections were cut on the microtome twelve microns in thickness. Both longitudinal and cross-sectional cuts were made. Cross-sections were made of the incisal third of the teeth.

Successive slides were stained with either: (1) Hematoxylin and Eosin, (2) Mallory's Connective Tissue Stain (Tri-chrome), (3) Gomori's Silver Stain, or (4) Alcian Blue.

It can be said generally that cellular histology and overall appearance are descriptions of slides stained with H & E, collagen fiber orientation and organization are best seen with either the Mallory or Gomori stains, and the distribution of the mucopolysaccharides is demonstrated by the Alcian Blue stain.

The slides were studied under the light microscope at 100x, 250x, and 400x.
CHAPTER IV
EXPERIMENTAL RESULTS

The following description is a summation of a study of several hundred microscopic slides. No marked differences were observed between the periodontal ligaments of the control animals and those of the untreated sides of the test animals.

The morphology of the periodontal ligament of the maxillary teeth was identical to that of the mandibular teeth and, therefore, no differentiation was necessary in the discussion.

Only the first and second molars of the rat and first, second and third molars of the guinea pigs were used in this study because it was very difficult to completely grind the most distal teeth out of occlusion. This problem in technique would make the most distal molars unreliable and, thus, unacceptable to the study.

A. The Rat Incisor

1. Normal

   a. Longitudinal section -- In the incisal one-third of the tooth, the principal fibers of the periodontal ligament are arranged in three distinct zones. Fine, evenly distributed
fibers leave the cementum and enter a zone of intermeshed fibers (the intermediate plexus) which run parallel to the long axis of the root. The fibers regroup on the alveolar side into long thick bundles of collagen which enter the bone at separated sites. The fibers do not appear to be continuous across the span of the periodontal membrane space. (Fig. 1)

The cemental group of fibers pierces the cementum at nearly a right angle to the root surface. A generous number of spindle-shaped fibroblasts can be observed lying among the collagen fibers. The long axes of the cells are aligned parallel to the general fiber direction. A narrow layer of dark staining cementoblasts can be seen just adjacent to the cementum.

The fibroblasts of the middle zone appear slightly more numerous and larger in size than those seen in the outer zones. They, too, are aligned with the general direction of the fibers, but they are a little more haphazard in arrangement. Small blood vessels are seen in this middle zone.

The alveolar fibers are arranged at nearly right angles to the root but in some areas slope apically from the bone to the cementum. These fibers are grouped into thick bundles which enter the alveolar bone at widely spaced intervals. The spaces between the bundles are filled by large, engorged blood vessels.

The fibroblasts have the classical spindle shape and their long axes are lined up parallel to the fiber bundles. A
layer of osteoblasts (oval, deeply basophilic cells) is closely adapted to the edge of the alveolar bone between the collagen bundles.

There is a marked difference in the histology of the periodontal ligament in the incisal end as compared with the apical end of the tooth. The periodontal ligament found further toward the apex is much more disorganized in appearance. Fewer alveolar bundles are found. The fibers of the plexus are loosely interwoven, fewer in number, and finer in appearance. More ground substance is evident in the middle zone.

b. Cross sections -- The fibers appear to radiate outward from the root surface in a swirl-like pattern. A group of fine, uniform fibers leaves the cementum and after a short course becomes interwoven with the plexus fibers.

The middle group of fibers forms a lacy meshwork running in the same general direction as the cemental and alveolar fibers but have a wavy appearance. (Fig. 3)

At the alveolar side of the ligament the fibers regroup into dense bundles of collagen. Many blood vessels lie between these alveolar bundles. Areas of bone deposition and resorption are seen.

2. Reduced (Nineteen Days)

These teeth were ground out of occlusion nineteen days before the animals were sacrificed. The incisors of all the
treated rats had regained normal occlusal contact at the time of sacrifice.

a. **Longitudinal sections** -- In comparison with the normal, very little difference can be found in the periodontal ligaments of the treated incisors. In general, it can be stated that the periodontal ligaments of the reduced teeth have a greater number of cellular elements (osteoblasts, fibroblasts, and cementoblasts). The fibers of the periodontal ligament appear to be finer. This is especially true of the more lacy intermediate plexus. Vascularity in the alveolar zone is increased. (Fig. 2)

b. **Cross sections** -- The lace-like collagen fibers of the intermediate plexus are readily demonstrated. The fibroblasts of the plexus are large and ovoid in shape. The alveolar fiber bundles are fewer in number and further apart. (Fig. 4) The alveolar zone is more vascular. Deposition of bone is more prevalent than resorption.

B. **The Rat Molar**

1. **Normal**

   a. **Longitudinal sections** -- The transseptal fibers are continuous, densely stained bundles of collagen which run uninterrupted from cementum to cementum. Long spindle-shaped fibroblasts are equally distributed throughout the transseptal ligaments.
The alveolar crest fibers run obliquely from the cementum incisally to the bone more apically. The typical three zones of the periodontal ligament are present but are much less obvious. The alveolar fibers are more grouped into bundles than the cemental fibers. (Fig. 5)

Just apical to the alveolar crest is a group of horizontally directed fibers.

The majority of the fibers associated with the root are obliquely arranged, running from the bone to a more apical insertion on the root surface. (Fig. 5) The angulation of these fibers varies from $60^\circ$--$90^\circ$ to the long axis of the root. These fibers cannot be traced across the periodontal ligament without a break in continuity.

The oblique arrangement is primarily due to fiber orientation in the middle zone. The cemental and alveolar fibers are arranged at more of a right angle to the root and bone surfaces.

Fibroblasts are distributed throughout the entire periodontal membrane space and are normally found with their long axes parallel to the fibers.

Capillaries are noted in both the middle and alveolar zones but appear more numerous in the latter zone.

The physiologic distal drifting of the molars is vividly demonstrated. Bundle bone is overlaid by a layer of osteoblasts on the mesial of the roots, and resorbing bone (and osteoclasts)
is present on the distal side. The collagen fibers have a more orderly arrangement on the tension side.

Hypercementosis of the apical one-third of the molar roots is a normal phenomenon.

b. Cross sections -- The fibers radiate from the cemental surface. Capillaries are seen in the middle and alveolar zones. In the latter group the blood vessels run between dense fiber bundles. Fine fibers leave the cementum, group together and intermingle in the middle zone, and regroup into thick bundles which enter the alveolar bone. (Fig. 7)

A number of cemental tears and areas of root resorption can be demonstrated.

2. Reduced (Nineteen Days)

a. Longitudinal sections -- The transseptal fibers have the same appearance as in the unoperated animals. Thick fiber bundles run continuously from tooth to tooth. Spindle-shaped fibroblasts are evenly distributed throughout the length of the interdental ligament.

Although the alveolar crest fibers have the normal orientation, the number of fibroblasts seems to have increased. The osteoblasts are more numerous and form a layer two or three cells thick along the crestal bone. (Fig. 9) A wide zone of newly formed bone is present over the alveolar crest. Similar changes are seen in the interradicular bone.
Some changes can be demonstrated in the oblique fibers. The angle of the fiber orientation appears to be more oblique. (Fig. 6) The alveolar bundles are more widely separated and fewer in number. The intermediate plexus shows a slight disorientation. Vascularity of the periodontal ligament has increased.

b. Cross sections -- The general scheme is one of fine cemental fibers linking together to form thicker, interlacing collagen fibers which traverse the middle zone. At the alveolar side, the fibers group into dense cords which enter the bone at widely separated intervals. (Fig. 8)

An increase in the number of fibroblasts, as well as osteoblasts, is noted in the area of the alveolar crest as compared to normal.

C. The Guinea Pig Incisor

1. Normal

   a. Longitudinal sections -- The general scheme of the periodontal ligament is the same as that of the rat incisor. Thin, regularly arranged fibers run out of the cementum at a right angle to the long axis of the root surface. After a short distance they turn upward and enter the wide intermediate plexus zone. In this zone the overall orientation of the fibers is parallel to the root surface. The plexus is a network of inter-
meshed, branched collagen fibers. (Fig. 10)

Near the alveolar bone the fibers regroup into thick alveolar bundles separated by large vascular channels. The closer to the apex, the more widely separated are the alveolar bundles. Vascularity is increased near the apex and the fibers of the intermediate plexus are finer, less organized, and shorter.

Cells are uniformly distributed throughout the periodontal ligament and generally parallel the fiber orientation. Spindle-shaped fibroblasts are the predominant cells. At the apical end the fibroblasts appear larger, more ovoid, and less differentiated.

b. Cross sections -- The zones of the periodontal ligament are still evident but the general direction of the fibers is different. The fibers radiate outward from the root surface and swirl gently in a wavelike arrangement to their insertion into the bone. (Fig. 12)

2. Reduced (Five Days)

The periodontal ligament has the same general appearance as the normal. The only notable difference is that the collagen fibers located in the intermediate plexus zone are shorter and finer.

3. Reduced (Nine Days)

The intermediate plexus is lacy. Fewer collagen fibers are seen. The alveolar bundles are replaced by thin, short
fibers of collagen. (Figs. 11 and 13) The cemental zone of fibers is less orderly arranged. Fibroblasts appear larger and more ovoid. They seem to be present in greater quantity.

D. The Guinea Pig Molar

The guinea pig molar teeth grow continuously. Each tooth is comprised of a core of dentin covered by enamel on all axial surfaces except the lingual surfaces of the lower teeth and the buccal surfaces of the upper teeth. When viewed from the occlusal, cartilage can be observed filling in the gaps of the "Z"-shaped teeth.

The periodontal ligament of the guinea pig molar is a complicated structure because it attaches alveolar bone to three types of tissue: enamel (via cemental pearls), dentin, and cartilage. Longitudinal sections will show cemental pearls and transseptal components of the periodontal ligament. In addition, cross sections will contain periodontal ligament attachments to cartilage and dentin.

1. Normal

a. Longitudinal sections -- The odontogenic organ is well demonstrated in the area of the cervical loop. The inner odontogenic epithelium differentiates into high columnar ameloblasts with their nuclei well polarized. The stratum intermedium is made up of several layers of flattened cells, which show a gradual transition into the cells of the stellate
reticulum. The cells of this zone assume a stellate appearance and the cellular network resembles embryonic connective tissue. The stellate reticulum is avascular. The outer odontogenic epithelium is a layer of flattened cells.

In an area of cartilage, no outer odontogenic epithelium is observed. Instead, the ameloblast layers of the adjacent teeth join at the basal end with the stellate reticulum interposed. The stellate reticulum is avascular. (Fig. 15) More occlusally a zone of connective tissue replaces the stellate reticulum and islands of cartilage are seen. Near the occlusal surface the cartilage is avascular and the ground substance appears amorphous, typical of mature cartilage.

It is extremely difficult to demonstrate the basal end of the periodontal ligament because it is irregular, loose connective tissue.

Collagen fibers can be seen running parallel to the root surface in the apical area of the ligament. A few fibers can be traced to the alveolar bone but none can be followed to the enamel.

As the ameloblasts are traced occlusally, they become more cuboidal in shape. In this area aggregates of fibroblasts wedge between the ameloblasts to reach the enamel surface. Collagen fibers are deposited among the fibroblasts and the ground substance becomes calcified. A "cemental pearl" is the result. (Fig. 14) Three zones of collagen fibers are easily demonstrated
in this area. Thick collagen bundles radiate from cemental pearls at right angles to the root. (Fig. 18) These fibers radiate into a middle zone in which loosely arranged collagen fibers run generally parallel to the long axis of the root. Fibers can be traced from one cemental pearl to adjacent pearls above and below, but no fibers can be traced through the intermediate plexus. On the alveolar side of the periodontal ligament, the collagen fibers group into moderately thick bundles which slant occlusally to enter the alveolar bone. These bundles are much less organized than those seen in the rat molar and more closely resemble alveolar bundles of a rodent incisor.

The alveolar bone shows resorption adjacent to the mesial surface of the first molars and the distal surfaces of the second, third, and fourth molars. A thin layer of newly formed bone overlays the crest of the alveolar bone.

Just occlusal to the alveolar crest, a wide zone of loosely arranged collagen fibers fills in the interdental space. As these fibers migrate occlusally with the erupting teeth, they mature and organize into typical transseptal fibers. (Fig. 16) These dense collagen fibers run from one cemental pearl to another on the adjacent tooth. Spindle-shaped fibroblasts lie among the fibers.

The spaces between the cemental pearls are occupied by remnants of the odontogenic epithelium, resembling stratified
squamous epithelium. Large vascular channels course between the transseptal bundles. The interdental fibers (transseptal) are carried occlusally with the teeth and disappear just below the oral epithelium.

b. Cross sections -- In these sections the periodontal fibers appear to radiate from cemental pearls. The fibers radiate outward and intermingle with a network of fibers in the middle zone. Fibers can be traced from one cement pearl to another, but it is impossible to trace continuous fibers from the cemental pearls to the alveolar bone. (Fig. 18)

Vascular channels run between both the alveolar bundles and the bundles of the cemental group.

The periodontal ligament on the lingual surface of the lower molars and the buccal surface of the upper molars runs from alveolar bone to cementum. In these areas the alveolar bundles enter a lace-like intermediate plexus. On the cemental side, fibers are embedded in non-cellular cementum. No true cemental zone of fibers can be distinguished from the intermediate plexus. Blood vessels are seen only in the alveolar part of the ligament.

The area of the periodontal ligament between the alveolar bone and the cartilage is extremely vascular and no true periodontal ligament exists in this area. A few collagen fibers can be traced from the bone to the cartilage and the loose arrangement of the connective tissue indicates a poor dental
support.

2. Reduced (Five Days)
   a. **Longitudinal sections** -- The ameloblastic layer of the odontogenic organ is poorly organized. The cells are not orderly arranged and are not well polarized. The same general scheme exists in the periodontal ligament. It appears that the same histology can be seen as that of the normal animal but at a more occlusal position. For example, the cemental pearls are not as highly organized as in the normal periodontal ligament and the interdental fibers never become as mature in appearance.

   b. **Cross sections** -- The ameloblast layer is poorly developed and immature. Fiber arrangement is basically the same but appears less dense and more loosely arranged, especially in the middle zone. The whole periodontal ligament is less well organized.

3. Reduced (Nine Days)
   a. **Longitudinal sections** -- The ameloblasts are not well polarized or aligned, and the collagen fibers of the periodontal ligament are first seen at a more occlusal position. The most apical periodontal fibers are thin, run parallel to the root surface, and have no apparent connection to either tooth or bone.

   At a more occlusal position, the fibers of the periodontal ligament group together and pierce the odontogenic epi-
theilium to form cemental pearls. Fibers from these cemental pearls sweep up into the middle zone and appear to stop. The bulk of the fibers of the plexus run in a direction parallel to the axial walls. These fibers are short and wavy.

The fibers which run from the middle zone into the alveolar bone are sparsely distributed. The alveolar fibers slant occlusally to the bone and are deeply embedded in the bone. The fibers do not seem to enter the middle zone of the periodontium but rather, stop just short of the intermediate plexus.

Near the crest of the alveolus, the periodontium is very disorganized. The cemental pearls are fewer in number, less calcified, and are not made up of the normally thick collagen bundles. The fibers of the middle zone are wavy and slightly darker. The alveolar bundles are less in number and only loosely attached to the plexus.

The alveolar crest, itself, is in a state of rapid bone deposition. The height of the alveolar bone has increased.

Mature transseptal fibers never form. The fibers from the cemental pearls on either side of the interdental space run into the periodontal space a short distance and stop. The bulk of the periodontium is formed by the middle zone of fibers. These thin, wavy fibers run across the interdental space and are loosely attached to the cemental pearls. The transseptal
fibers never reach the maturity of the dense, highly organized bundles seen in the normal guinea pig. (Fig. 17)

A number of cemental pearls (still attached to the reduced enamel epithelium) can be traced into the oral cavity.

b. Cross sections -- The periodontal ligament looks finer, contains fewer fibers, and is narrower (reduced from .22 mm. to .17 mm.). The cemental pearls seem to be organized but lack calcification. The fibers of the plexus are finer, shorter, and less regularly arranged. The alveolar fibers are sparse and loosely woven to the plexus. (Fig. 19)

Vascularity on the alveolar side of the periodontium has increased markedly. The cellularity of the alveolar crest region has increased.
A. Comparison of PDL -- Teeth of Continuous Versus Non-continuous Eruption

The most striking difference noted in the periodontal ligaments between teeth of continuous and non-continuous eruption occurs in the principal fibers. Three distinct zones are clearly seen in the periodontal ligaments of continuously erupting teeth throughout life. These same three zones (alveolar fibers, intermediate plexus, and cemental fibers) are present in teeth of non-continuous eruption only during the period of rapid eruption. Eccles (1965) states that once the tooth is in occlusion, these separate zones are no longer visible.

Our observations revealed that the three zones of the periodontal ligament are clearly seen in rapidly erupting teeth. Once the non-continuously erupting teeth reach occlusion, the three zones become less evident, but they still exist. It is impossible to trace collagen fibers across the periodontal ligament without a break in continuity. There is always a marked branching of the plexus fibers.
Certainly there is a great difference between the intermediate plexus of the rat molar and the guinea pig molar. There has to be a means of adjustment in the guinea pig molar to allow function to continue as the tooth moves upward through the stationary alveolar bone. This adjustment is reflected in the plexus fibers which are arranged nearly parallel to the root surface.

In the rat molar the need for rapid adjustments between the alveolar and cemental fiber bundles is no longer necessary. However, these teeth are continually moving throughout life and some adjustments are needed.

Beertsen and Snijdir (1969) compared the periodontal ligaments of continuously erupting teeth (adult rabbits and guinea pigs) to non-continuously erupting teeth (adult dogs, rats and humans). Their study had the following conclusions:

1. In all animals, the fibers of the periodontal ligament form a meshwork and no fiber extends the width of the space.

2. The periodontal ligaments of continuously erupting teeth are more cellular. The fibroblasts appear more active (due to appearance of nuclei) than in non-continuously erupting teeth.

3. The angulation of collagen fibers is more oblique in teeth which erupt continuously. The steeper the
course of the fibers, the longer they may persist without losing their mechanical function.

This difference in angulation of the fibers was vividly demonstrated in our study in the molar teeth of the rat and the guinea pig. The principal fibers of the rat molar form an angle of $60^\circ$ to $90^\circ$ with the long axis of the root. The principal fibers of the guinea pig periodontal ligament are angulated at almost $180^\circ$ to the root surface.

B. Comparison of PDL -- Teeth of Normal Versus Accelerated Rates of Eruption

1. Rat and Guinea Pig Incisors

In comparison to teeth of normal eruption, very little difference could be found in the periodontal ligaments of the accelerated erupting teeth. In general, the periodontal ligaments of the reduced incisors had a greater cellularity and vascularity.

One could assume that this increased cellularity and vascularity was a reflection of the greater activity within the periodontal ligament necessary to allow the teeth to erupt at a faster rate. Stallard (1963) found that the mitotic index of periodontal ligament fibroblasts increased in teeth which were taken out of occlusion.

A reduction in the density of collagen fibers was a constant finding. Fibers of the middle zone became less
organized and appeared "lace-like".

Very little change was observed in the organization of the cemental fibers. Thus, it would appear that these cemental fibers are stable and do not have to be destroyed and remade as the tooth erupts. Eccles (1965) showed that the cemental fibers remained relatively unchanged in rat molars taken out of occlusion.

The reduction in the manner and density of collagen fibers within the periodontal ligament of accelerated erupting teeth is in agreement with other studies. Schour (1960) found that in the periodontal ligament of a tooth without occlusion the fiber bundles atrophy and are replaced by loose, irregular connective tissue with no definite orientation.

Eccles (1965) also demonstrated atrophy of the periodontal ligament fibers in teeth which were not in occlusion. He found that the most marked changes were seen in the alveolar fiber bundles.

2. Rat Molars

In the periodontal ligaments of the reduced rat molars, the greatest changes occurred in the alveolar crest and inter-radicular bone. The bone was shown to be rapidly increasing in height. A wide zone of newly formed bone and a greater than normal number of osteoblasts were observed. The vascularity had also increased.
The increase in alveolar bone deposition could be a means by which teeth of limited growth can erupt after the root has completely formed. It would seem that if the alveolar bone were unable to compensate in this manner, the tooth could continue to move occlusally leaving the alveolar process and periodontal support behind.

Some changes were demonstrated in the oblique fibers of the periodontal ligament. The alveolar bundles appeared to be more widely separated and fewer in number. The intermediate plexus was slightly disorganized.

Here again, the reduction in the number of alveolar fibers agrees with the findings of Schour (1960), Eccles (1965), and Anneroth (1967). These authors found that a tooth which lacks an antagonist has a periodontal ligament which resembles loose, irregular connective tissue. They showed a marked atrophy of alveolar fiber bundles.

It was demonstrated by Anneroth and Stockholm (1967) that root cementum increased in thickness in supraerupted teeth. It was impossible to show this in our study because of the short duration these teeth were supraerupting.

3. Guinea Pig Molars

The periodontal ligaments of the accelerated erupting molars of the guinea pig appeared less mature in all areas of the periodontal ligament.
The general impression is that the tooth is erupting so quickly that the periodontal ligament is not able to keep pace. The normal eruptive processes are occurring in the periodontal ligament but at a faster rate. This inability of the periodontal ligament to keep pace with eruption would lend support to the theory that the periodontal ligament is a compensatory mechanism in tooth eruption and not the prime mover.

The ameloblasts, which make up the inner odontogenic epithelium, were not well aligned and showed less polarization of nuclei. The fibers of the periodontal ligament were first formed at a more occlusal position than normally.

Cemental pearls formed in the usual fashion, but never reached the same degree of calcification as in the normal animals. The pearls were fewer in number. Mature, well-organized transseptal fibers never developed.

C. The Intermediate Plexus

Supraeruption of the rat molars after occlusal reduction is reflected mainly by an increase in the height of the alveolar bone.

A mature rat molar root increases in length only by apposition of cementum. The increased apposition of bone at the alveolar crest and interradicular areas was associated with tooth movement in the occlusal direction. Adjustments for this
movement are seen in the periodontal ligament as a disorganization of fiber elements, an increase in vascularity, an increase in cellularity, and an increase in bone formation.

In the periodontal ligaments of the reduced teeth, a loss of fiber orientation was shown. Dummett (1958), Schour (1960), Eccles (1965), and Anneroth and Stockholm (1967) all demonstrated that in the hypofunctioning tooth the periodontal ligament becomes less organized and appears as a loose, irregular type of connective tissue.

The periodontal ligaments of the continuously erupting teeth (rat incisors, guinea pig incisors, and guinea pig molars) are constantly adjusting to allow the teeth to move into the oral cavity while the bone remains relatively stationary. This adjustment appears to take place within the intermediate plexus. Most authors agree that the intermediate plexus is most easily seen in rapidly growing teeth. (Noyes and Schour, 1955; Weinmann, 1955; Orban, 1957; Sicher, 1965; and Furstrum, 1965)

If the concept of a dynamic intermediate plexus were not true, any adjustment between the tooth and alveolus could only be accomplished by the destruction of the bony and/or cemental collagen attachments, and the synthesis of new fiber attachments. Each time new collagen is formed, new bone and cementum would have to be deposited to attach the newly synthesized fibers. This being the case, the root surfaces would
soon be enveloped in an ever thickening cementum sheath. Our results showed no substantial increase in the thickness of cementum.

The cemental fibers are extremely stable and undergo very little change. Melcher (1967) showed that as the tooth erupts the cemental fibers travel with it, while the alveolar fibers remain behind. Therefore, fiber adjustments must be taking place within the periodontal ligament.

The properties of collagen, itself, must be considered. The collagen molecule is extremely stable and resistant to physical and chemical agents. But the binding of fibrils into fibers is a completely different situation.

The fibrils are united by a small amount of cementing substance, presumably a protein, since it is digested by trypsin. The interfibrillar cement dissolves in weak alkalis and the fibers eventually break up into their constituent fibrils. (Blooms and Fawcett, 1962)

Stallard (1963) found an increase in the fibroblast number and activity related to an increase in the eruption rate. The same observation was made in this study.

While new collagen fibers are constantly forming within the periodontal ligament (Stallard, 1963 and Ramos, 1967), it appears that adjustments occur by a reorganization of fibers rather than by formation of new fibers. Wasserman (1951)
showed this continuous breakdown of fibers into fibrils and subsequent regrouping into new fibers. Mc Hugh (1965) and Macapanpan (1954) demonstrated that the fibroblast activity necessary for this reorganization of fibers does occur and, in fact, occurs in the intermediate plexus.

Increased fibroblast activity in the intermediate plexus does not mean that more collagen is being formed in the plexus than in the alveolar and cemental zones. Stallard (1963), Crumley (1964), and Ramos (1967) all demonstrated that new collagen is formed all across the periodontal ligament. There is no "hot spot" of collagen formation in the ligament.

The increased fibroblast activity noted in the plexus could be related to the synthesis and degradation of the protein cements used to bind the fibrils into fibers.

Therefore, adjustment between fibers in the periodontal ligament could be due to a chemical unlocking and rebinding of fibrils. This process may be mediated by the fibroblasts.
CHAPTER VI
SUMMARY AND CONCLUSIONS

A. Summary

This was an investigation to compare the histologic structure of the periodontal ligaments of continuously versus non-continuously erupting teeth. Also, teeth erupting at a normal rate were compared to those erupting at an accelerated rate. A better understanding of the morphologic characteristics of the intermediate plexus of the periodontal ligament was the goal of this study.

Seven rats and eleven guinea pigs had the occlusal surfaces of all teeth on the right side reduced to stimulate the eruption rate. Four animals (two guinea pigs and two rats) were untreated and used for experimental controls.

After a period of time the animals were sacrificed and histologic sections were prepared of the jaws. The sections were stained with either (1) hematoxylin and eosin, (2) Mallory's connective tissue stain, (3) Gomori's silver stain, or (4) alcian blue.

The slides were studied under a light microscope at 100X, 250X, and 400X.
B. Conclusions

The following may be concluded from this study:

(1) The normal histologic structure of the rat and guinea pig periodontal ligaments has been described. This description is in agreement with the dental literature.

(2) Teeth of continuous eruption have a more apparent intermediate plexus, greater cellularity in the periodontal ligament, and a more oblique arrangement of the principal fibers than do teeth of limited eruption.

(3) Supraeruption of the rat molar is accompanied by apposition of alveolar bone, a decrease in number and density of periodontal ligament fibers, increased cellularity within the periodontal ligament, and increased vascularity.

(4) The guinea pig molar, rat incisor, and guinea pig incisor erupt at an accelerated rate if ground out of occlusion. The acceleration in eruption is accompanied by an increase in the number of fibroblasts and a decrease in the bundle size and organization of collagen fibers, especially in the intermediate plexus.

(5) Adjustments occur within the periodontal ligament to allow for a functioning tooth to erupt at a rapid rate. It is likely that this adjustment occurs through-
out the entire width of the periodontal ligament as a breakdown of fibers into fibrils and a regrouping into new fibers.
LITERATURE CITED


PHOTOMICROGRAPHS

Fig. 1.-- The normal rat incisor PDL (long. sect.). Note the three distinct zones within the PDL.
Gomori X 250

Fig. 2.-- The reduced rat incisor PDL (long. sect.). Note the loss of alveolar bundles and disorganization of intermediate plexus fibers. Gomori X 250

KEY

B -- Alveolar Bone
C -- Cementum
D -- Dentin
E -- Enamel

Zone 1 -- Alveolar fiber bundles of PDL
Zone 2 -- Intermediate plexus fibers of PDL
Zone 3 -- Cemental fibers of PDL
Fig. 3.-- The normal rat incisor PDL (cross sect.). Note the branching of the intermediate plexus fibers. Gomori X 125

Fig. 4.-- The reduced rat incisor PDL (cross sect.). Note the loss of alveolar bundles. Gomori X 125
Fig. 5.-- The normal rat molar PDL (long. sect.). Note the dense alveolar bundles. Gomori X 125

Fig. 6.-- The reduced rat molar PDL (long. sect.). Note the increased vascularity and thin alveolar bundles. Gomori X 125
Fig. 7.-- The normal rat molar PDL (cross sect.). Gomori X 160

Fig. 8.-- The reduced rat molar PDL (cross sect.). Note the increased vascularity. Gomori X 160
Fig. 9.— The reduced rat molar interproximal area. (long. sect.). Note the increase in the number of osteoblasts (Os) along the alveolar crest and the newly formed bone (N). H & E X 64
Fig. 10.-- The normal guinea pig incisor (long. sect.).
Gomori X 250

Fig. 11.-- The reduced guinea pig incisor (long. sect.).
Note a decrease in the density of the alveolar fiber bundles and a disorganization of the intermediate plexus. Gomori X 250
Fig. 12. -- The normal guinea pig incisor (cross sect.).
         Gomori X 125

Fig. 13. -- The reduced guinea pig incisor (cross sect.).
         Gomori X 125
Fig. 14. -- The normal guinea pig molar (long. sect.). The fibroblasts form a spear-shaped aggregate which pierce the layer of ameloblasts to attach to the enamel. This mechanism of attachment is called a cemental pearl (P). H & E X 200

Fig. 15. -- The normal guinea pig molar (long. sect.). In an area where cartilage forms part of the tooth, the inner odontogenic epithelium (IOE) runs from one root to the adjacent root. A zone of stellate reticulum (SR) is interposed. H & E X 64
Fig. 16.-- The normal guinea pig molar (long. sect.). Dense transseptal fibers (TS) run from tooth to tooth. Gomori X 64

Fig. 17.-- The reduced guinea pig molar (long. sect.). The cemental pearls (C) are poorly calcified. The transseptal fibers are disorganized. Gomori X 125
Fig. 18.-- The normal guinea pig molar (cross sect.).
Gomori X 125

Fig. 19.-- The reduced guinea pig molar (cross sect.). Note the increased vascularity and the loss of alveolar fiber bundles. Gomori X 125
APPROVAL SHEET

The thesis submitted by Dr. Andrew P. Trapani has been read and approved by members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that all necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 20, 1971
Date

[Signature]
Signature of Advisor