A Comparative Histologic Investigation of the Nerve Endings of the Periodontal Ligament of the Mouse, Rat and Gerbil

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A COMPARATIVE HISTOLOGIC INVESTIGATION OF
THE NERVE ENDINGS OF THE PERIODONTAL
LIGAMENT OF THE MOUSE, RAT AND
GERBIL

BY

B. DOUGLAS AMBERMAN

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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JUNE

1969
DEDICATED TO MY MOTHER AND FATHER
B. Douglas Amberman was born in New York City on
November 10, 1939. He was graduated from the Woodhull Preparatory
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CHAPTER 1
INTRODUCTION AND STATEMENT OF THE PROBLEM

Many studies have been conducted concerning the histologic investigation of the innervation of the periodontal ligament with special emphasis on the nerve terminations there. But in a survey of the literature it is evident that because of the capricious nature of the staining processes, diverse and often conflicting results have been obtained.

In addition, most of the investigators who have conducted research on the nerve endings of the periodontal ligament have confined themselves to just one animal. No comparative study has been performed to show the similarities or differences in phylogenetically related animals.

The present study has been conducted primarily as a comparative endeavor to show what kinds of nerve endings are present in the periodontal ligament of the mouse, rat and gerbil and whether or not there are consistencies in the make-up of these terminations. These three animals were chosen because they are closely related to each other and all of them have non-continuously erupting molar teeth. In
addition their size makes a study of their dentition and the supporting structures conducive to limited laboratory conditions.

The staining technique employed in this investigation, i.e., the Powers' Silver Nitrate Method, is one that has proved to be consistent in its results, thereby reducing somewhat the difficulty encountered when trying to distinguish nerve tissue from other surrounding cellular elements.

Some sections were also stained using the technique of Dr. R. Dmytruk. This is a modification of the protargol method, Bodian, Powers, Rasmussen and Clark method and Pearson's silver gelatin method. This technique was attempted as a comparison of the Power's process, and also, because it is a new approach, to see if the results would be more stable and superior.
CHAPTER II

REVIEW OF THE LITERATURE

For many years investigators have been studying the innervation of the periodontal ligament with specific emphasis on the various types of nerve endings found there. Dependorf in 1913 was one of the first to conduct such an investigation. He found that in the human, the nerve bundles in the periodontal ligament form both coarse and fine networks which terminate as fine pointed processes in the cementoblastic layer.

Kadnoff (1928) in his study of the human did not find nerve endings that entered the cementum of the tooth. He saw nerve fibers arising at the apical area extending the length of the periodontal ligament all the way to the gingival margin. These fibers were joined at different levels by other nerve bundles from the alveolar bone. The endings of the nerves from both of these sources formed a fine network in the ligament proper. Kadnoff found no evidence of any encapsulated nerve endings in this study.

Lewinsky and Stewart in their investigation of man (1936) and
the cat (1937) found that the configuration of the nerve endings in both subjects was similar. Their findings showed that there were two types of termination. Both the nerves of apical origin that made their way gingivally, and the alveolar nerve fibers branched either peripherally or went into the deeper layers of the ligament. Those that were confined to the outer layers were coarse fibers and terminated as specific end organs which were spindle shaped. Those that passed into the deeper layers of the ligament were fine fibers and broke up into fine aborizations without terminal end organs.

Bradlaw (1936) observed that in the periodontal ligament of the monkey the main nerves which were accompanied by the blood vessels gave off side branches at different levels. These fibers terminated as loops and coils supplying both the alveolar bone and the cementum.

Van der Sprenkel (1936) in his study of the mouse observed that the nerve fibers enter the periodontal ligament through foramina in the alveolar bone and from the bottom of the alveolus in the periapical region. He found the following nerve endings: 1.) Small end-rings surrounded completely by peri-terminal reticulum, close to the alveolar bone, lying on bundles of collagen fibrils (these end-rings
were not seen in any other place). 2.) Terminal reticula which surrounded nuclei of the connective tissue and were found only in the peripheral layers of the periodontal ligament. 3.) Nerves that were more centrally located in the ligament sent out branches that helped to form a reticulum that was wide-meshed in a radial direction. Fibrils from this network were seen to penetrate into the cementoblastic layer and then into the dentin of the tooth.

Bernick (1952) in his study of young monkeys found that the nerve supply to the periodontal ligament came from the dental and interalveolar branches of the alveolar nerves. In the molar region there was an additional branch, the interradicular, that supplied the ligament lying between the roots of the teeth. He observed that the fibers from the dental nerve supply the apical region and pass gingivally to form a network with branches arising at different levels from the inter-alveolar nerve. Fibers then branch off in all directions supplying all surfaces. The nerves ultimately terminate as free nerve endings in the cementoblastic layer, the cementum proper, and the connective tissue of the periodontal ligament.

Dockrill in the human (1954) observed two types of nerve term-
lations, looping coils and free nerve endings. He also found knob-like swellings that contained neuro-fibrils and these swellings immediately preceded free nerve endings. He thought that perhaps the knob-like endings seen by other investigators might be the swellings cut at different angles during histologic preparation. Other researchers have seen these swellings along the course of the nerve fiber but as yet their significance has not been ascertained.

Bernick (1956) in his study of the rat employed a staining process that differentially stained the nerve tissue and the collagen. Since both of these tissue elements are argyrophilic it can be difficult at times to distinguish one from the other. Through enzymatic hydrolysis of the collagen the affinity of that tissue for silver is decreased, but the staining of the nerve tissue remains the same. Using this method, Bernick found that there were two types of nerve endings in the periodontal ligament of the rat. One was a knob-like structure and the other was a free nerve ending. Both of these terminations were found throughout the stroma of the ligament. The author found no specialized nerve endings.

Rapp, Kirstine, and Avery (1959) while studying the human, found
two different types of nerve endings. Highly organized neural terminations which were ovoid in shape were seen throughout the periodontal ligament. These structures contained interweaving medulated and non-medulated fibrils and they were surrounded by connective tissue fibers. The other type of termination that they observed was a free nerve ending which was also seen throughout the ligament.

Orban in the human (1962) stated that the nerves of the periodontal ligament follow the path of the blood vessels and that a rich plexus of nerves is formed from fibers originating from the periapical area and the interdental and interradicular regions. Three types of nerve endings were found: one, terminating in a knob-like swelling, another forming loops or rings around bundles of the principal nerve fibers and the third type which was a free nerve ending. All the terminal branches were free of myelin sheath.

Kerebel (1964) in his study of the innervation of the periodontal ligament of the albino rat, found both ring-like structures as well as free nerve endings which appeared throughout the ligament. In his investigation the author found no specialized end organs.
Bernick (1966) in his investigation of the guinea pig observed nerve fasciculi originating from the alveolar nerves entering the interseptal bone and passing a short distance in the bone. Above the region of the enamel organ the nerves perforated the alveolar bone at different levels and entered the periodontal ligament. They coursed occlusally for a short distance giving off branches that supplied the stroma of the ligament. Two types of endings were demonstrable, a fine, free nerve ending and an encapsulated, spindle-like structure that is probably a receptor for touch and pressure.

Simpson (1966) employed the apoxestic technique in his study of the periodontal ligament of the human. In this approach the periodontal tissue is carefully dissected from the roots of extracted teeth and its subsequent treatment is that of a normal section. Utilizing this method the investigator can view a larger and thicker section than usual. Simpson found both free nerve endings and knob-like endings but he saw no evidence of the presence of specialized end organs.

Kizior, Cuozzo, and Bowman (1968) studied the innervation of the periodontal ligament of the cat. Two sources of nerves were found, one from the apical region and the other from the alveolar bone.
The apical fibers extended gingivally following a course in the central portion of the ligament. The alveolar fibers, after entering the ligament diverged in either an apical or gingival direction. The majority of the apical fibers were larger than the alveolar fibers and they demonstrated little branching. The alveolar fibers broke up into finer branches and went in all directions, whereas the apical fibers remained in the center of the ligament closely associated with the blood vessels. Two types of nerve endings were seen, one was a highly organized termination which was ovoid in shape consisting of myelinated and non-myelinated inter-weaving fibers, the other was a free nerve ending. The ovoid type ending was surrounded by a delicate connective tissue capsule and was usually associated with larger size nerve fibers. These endings were seen only in the apical one-third of the ligament. The terminal portions of the small nerve fibers comprised the free nerve endings and these were located throughout the ligament.
CHAPTER III

METHODS AND MATERIALS

In this investigation eighteen animals were used; six albino mice, six albino rats and six mongolian gerbiles. The animals varied in age but were all sexually mature and they all exhibited non-continuously erupting molar teeth.

After they were sacrificed their mandibles were dissected out and both halves were immediately fixed in Formalin chloral hydrate solution. (Formaldehyde 37%, 10 c.c., Chloral hydrate 10 grams, and distilled water 90 c.c.) The specimens were cut mesial to the first molar and distal to the third molar.

After proper fixation, the tissue specimens were trimmed of any extraneous elements and put into a decalcifying solution. (5% Trichloracetic acid in 50% alcohol) The tissue was then washed and dehydrated and embedded in paraffin. Sections were then made of the specimens at eight microns and at fifteen microns, i.e., some mandibles were cut at eight microns and some at fifteen microns.

The sections were then stained using the Powers' modified Silver Nitrate method for nerve tissue. Using this stain, the nerves appear
dark brown to black against a background that varies from almost colorless to a yellow or light brown.

Some sections were also stained with the Dmytruk method.

The sections were viewed through a binocular Zeiss Photomicroscope. Those slides with tissue cut at eight microns were studied serially so that any nervous elements could be followed along their entire course. Those sections cut at fifteen microns were also studied serially but for the most part they were studied for their depth, i.e., more structures could be viewed for a greater distance on a single slide.

Each slide was scanned and examined in detail at 100 X, 250 X, and 400 X magnification and photomicrographs were taken at these different levels of the specific nerves and nerve endings throughout the periodontal ligament. The Zeiss Photomicroscope is equipped with a 35 mm. camera and only the sections of fifteen microns thickness were photographed. The reason for this being that more structures and greater nerve distances could be seen in one photomicrograph.
CHAPTER IV

FINDINGS

A. Mouse:

Upon histologic examination of the sections made at eight and fifteen microns it was observed, using the Powers' technique, that the nerves and the nerve endings of the periodontal ligament stained dark brown to black. The background varied from light yellow to light brown according to the slide being studied. Therefore, the danger of confusing any of the connective tissue or other cellular or fibrous elements with the nerve tissue were greatly reduced. Pre-collagen fibrils which, like nerve fibers are argyrophilic, did not pick up the silver nitrate to any degree as strongly as the nerve elements. In addition, the connective tissue was in a more orderly arrangement with the fibers running in a different direction from the nerves. In contrast the nerves seemed to take a freer more eradic course.

The innervation of the periodontal ligament came by way of the inferior alveolar nerve which sent branches to the periapical region and to the interseptal bone. After the nerve fibers enter
the alveolus near the apex of the tooth they course through the ligament in a gingival direction. For the most part they remain in the central portion of the ligament and ultimately terminate in the area of the gingiva. As they course through the ligament smaller branches and twigs are sent out in all directions throughout the stroma.

The nerve fibers that enter the ligament via foramina at all levels in the interseptal bone run both apically and gingivally. These nerves also send smaller branches and twigs in all directions throughout the ligament. In contrast to the nerves of apical origin the interseptal nerves send a greater supply to the periphery of the ligament. In addition, the interseptal fibers are smaller in diameter than the apical fibers. Blood vessels were seen to accompany the apical fibers in the central portion of the stroma of the ligament more often than they were seen in the periphery.

Both of the above mentioned nervous elements were seen to be myelinated but as they branched and became finer, they gradually lost their myelin sheath. The terminations observed were of two types, one was a coiled or spiral ending and the other was a free nerve
ending. They were found at all levels of the periodontal ligament and appeared with equal frequency as the mode of termination of both the nerves of apical origin as well as those of the interseptal alveolar bone.

The coiled or spiral type of ending was seen more often in the region of the periodontal ligament close to the tooth and in the periphery near the bone. The free nerve ending which was also seen in those areas was also found with more frequency in the central portion of the stroma of the ligament.

The nerve endings were observed to terminate by lying on the cells and fibers of the connective tissue of the ligament, i.e., sandwiched between the interweaving collagen fibers.

The free nerve ending was seen more frequently than the coiled or spiral type but both types were seen throughout the ligament, near the alveolar bone and near the cementum of the tooth. No endings were seen entering the cementum.

Using the Dmytruk staining method, the nerve fibers and the surrounding tissues could not be successfully distinguished from each other. In many of the sections the nerve elements as well as the connective tissue elements appeared a dark blue to black.
B. Rat:

The pattern of innervation of the periodontal ligament and the types of nerve endings observed were seen to be identical to those of the mouse.

C. Gerbil:

Again as in the mouse and rat, the innervation of the periodontal ligament was seen to come by way of the apical nerves and the interseptal nerves. Here too the types of terminations seen were both the coiled or spiral ending and the free nerve ending.
FIGURE 1

MOUSE: NERVE FIBER OF APICAL ORIGIN COURSING THROUGH THE CENTRAL PORTION OF THE STROMA OF THE PERIODONTAL LIGAMENT.

SEEN AT 250 X MAGNIFICATION.
FIGURE 2

MOUSE: FREE NERVE ENDINGS SEEN AT 250 X MAGNIFICATION, FOUND WITHIN THE STROMA OF THE PERIODONTAL LIGAMENT.

INTERSEPTAL ALVEOLAR BONE, SEEN AT 250 X MAGNIFICATION.
FIGURE 3

MOUSE: COILED OR SPIRAL TYPE OF NERVE ENDING FOUND WITHIN THE STROMA OF THE PERIODONTAL LIGAMENT ADJACENT TO THE INTERSEPTAL ALVEOLAR BONE.

SEEN AT 250 X MAGNIFICATION.
FIGURE 4

VIEWED AT 100 X MAGNIFICATION.
FIGURE 5

RAT: NERVE FIBERS SEEN ENTERING THE STROMA OF THE PERIODONTAL LIGAMENT FROM FORAMINA OF THE ALVEOLAR BONE.
VIEWED AT 100X MAGNIFICATION.
FIGURE 6

FIGURE 7

FIGURE 8

GERBIL: NERVE FIBERS SEEN ENTERING THE STROMA OF THE PERIODONTAL LIGAMENT FROM THE INTERSEPTAL BONE OF THE ALVEOLUS. VIEWED AT 250 X MAGNIFICATION.
FIGURE 9

FIGURE 10

GERBIL: COILED OR SPIRAL TYPE OF NERVE ENDING FOUND WITHIN THE STROMA OF THE PERIODONTAL LIGAMENT NEAR THE APEX OF THE MOLAR TOOTH. VIEWED AT 250 X MAGNIFICATION.
FIGURE 11

CHAPTER V

DISCUSSION

By using the Powers' Silver Nitrate method of staining the tissue sections this author was able to view the periodontal ligament and the nervous elements within it with a great degree of contrast. The ligament stained a pale yellow to light brown while the nerve fibers and nerve endings appeared dark brown to black. This factor helped to overcome the capricious nature of some of the staining techniques previously employed, i.e., Romanes, Bodian, and Pearson. In addition, some of the problems encountered by other investigators when trying to differentiate between the various tissue and cellular components was greatly reduced.

Having sections cut at eight microns thickness gave the author the advantage of viewing many sections serially. In this way, nerve fibers and their branches were followed from their entrance into the periodontal ligament all along their course, ultimately to their terminations. The sections cut at fifteen microns thickness were valuable in that each slide could be viewed in depth. In this manner nerve fibers and smaller branches as well as nerve endings were
often found on a single slide.

The pattern of innervation, by way of the Inferior Alveolar Nerve and its fibers of apical and interseptal origin with its subsequent branching in all directions is in accord with most researchers. This was reported by Bernick (1956) in the rat, and by Kerebel (1964) in the rat, and by Van der Sprenkel (1936) in the mouse.

The types of nerve termination and their distribution, i.e., coiled or spiral endings and free nerve endings, found throughout the periodontal ligament, in this investigation, supports the observation of Kerebel. In his study of the rat he found coiled or ring-like endings and free nerve endings at all levels of the ligament.

Bernick (1956) found both free nerve endings and knob-like endings in the periodontium of the rat. This author found no evidence of any knob-like structures as nerve terminations. In the course of this investigation swellings were seen at different points along the length of the nerve fibers, but if the sections are cut in a certain way they give a knob-like appearance. This opinion was first offered by Dockrill in 1954. This author also
has observed that viewed serially, the path of a nerve fibers is often convoluted. In addition the nerve will often double back upon itself. If this is viewed in a single section it too gives a knob-like appearance.

The nerve endings seen by Van der Sprenkel (1936) in the mouse were end-rings which may be the coiled or spiral endings observed by this author. But in our study the terminations were found throughout the periodontal ligament while Van der Sprenkel observed them only in close proximity to the alveolar bone, lying on collagen fibrils. The terminal reticula found by Van der Sprenkel could also be analogous to the network of free nerve endings seen by this author, but again Van der Sprenkel saw them only in the peripheral layers of the periodontal ligament whereas in this study they were observed throughout the ligament.

Although no specialized encapsulated end-organs have been observed in studies of the mouse or rat, they have been found in other investigations. Bernick (1966) observed this structure in the guinea pig and Rapp, Kirstine, and Avery (1959) in the human, and Kizior, Cuozzo, and Bowman (1968) in the cat. But after long and
careful investigation this author could find no evidence of any comparable structure in the periodontal ligament of the mouse, rat or gerbil.

The author also observed that the nerve fibers of apical origin were greater in diameter than those of interseptal origin and that for the most part these nerves followed a course through the central portion of the stroma of the ligament. This was also reported by Sicher (1962) in the human and by Kizior, Cuozzo, and Bowman (1968) in the cat. In addition both of the above researchers found that the nerve fibers of apical origin were accompanied by blood vessels. This confirms the findings of this author.

With respect to the finding of this author that the nerve fibers came from the inferior alveolar nerve and entered the ligament via the apical region and foramina along the interseptal bone, the diameter of the fibers gradually decreased as the fibers went from bone to ligament. It was therefore assumed that the nerves were coming from the bone rather than from the ligament to the bone.

Because the connective tissue elements were poorly stained
it was difficult to report any findings on the make up of the periodontal ligament. Therefore, any reinforcement of the findings of Dr. Sicher with respect to the "intermediate plexus" was impossible.

In an attempt to correlate the histologic findings with the functional roles of the nerve endings, Lewinsky and Stewart felt that the specialized end organs that they observed in the human and the cat (1936) and (1937) were responsible for the modality of tactile sensation. The free nerve endings, they conjectured, were associated with pain sensitivity.

Rapp, Kirstine and Avery (1959) in their study of the human felt that when the encapsulated end-organs were stimulated by pressure there was a change in their shape, and this was sufficient stimulus to activate the associated nerve fiber.

Pfaffmann (1939) found that when he applied pressure to a tooth discharges could be recorded from dental nerves. In addition Jerge (1963) observed electrical activity in the main sensory nucleus of the trigeminal nerve and in the mesencephalic nucleus. Since the latter nucleus serves as the proprioceptive center of
the trigeminal nerve it is felt that the periodontal ligament has a proprioceptive function. Kizior, Cuozzo and Bowman (1968) observed in the cat, that the specialized encapsulated end-organs were stimulated by light pressure and they ascribed a proprioceptive function to them. As for the free nerve endings, they felt that they responded to heavier pressure and were responsible for the sensation of pain.

It is the conjecture of this author that perhaps the two types of nerve endings observed in this investigation of the mouse, rat and gerbil may function in the sensation of pain and proprioception. There are instances when free nerve endings serve a proprioceptive function, as is evidenced by the free nerve ending in the tendon. Adequate stimuli probably initiate impulses that travel from the nerve ending along the nerve fiber to both the main sensory nucleus and the mesencephalic nucleus of the trigeminal nerve.

The author feels that the staining technique of Dmytruk, although not successful in this investigation, still has many possibilities to be of value in future studies. Time was an important factor in the present work and many of the variables
found in the Dmytruk staining technique would have taken too great an effort to resolve.
CHAPTER VI

SUMMARY AND CONCLUSIONS

The pattern of innervation and the types of nerve endings were studied in a comparative histological investigation of the periodontal ligament of the mouse, rat and gerbil. The Powers' Silver Nitrate Method of staining the tissue sections was used as well as the Dmytruk method.

Six mice, six rats and six gerbils were used in the study and sections were cut at both eight and fifteen microns thickness. The slides were viewed under 100 X, 250 X, and 400 X magnification and all regions of the periodontal ligament were carefully observed.

The following may be concluded from this investigation:

1. The Powers' Method of staining histologic sections using Silver Nitrate, differentially shows the presence of nerve fibers and nerve endings from the other tissue elements in the periodontal ligament.

2. The nerves and nerve endings in the periodontal ligament of the three animals used in this study
stained dark brown to black against a pale yellow to light brown background.

3. The pattern of innervation of the periodontal ligament of the mouse, rat and gerbil was similar in all respects.

4. The innervation of the periodontal ligament of the three animals was derived from the apical fibers and the interseptal fibers of the inferior Alveolar Nerve. The apical fibers which were greater in diameter than the interseptal fibers coursed gingivally and were observed for the most part in the central portion of the ligament associated with blood vessels. Branches and twigs were given off in all directions. The interseptal fibers entered the ligament via foramina at all levels of the alveolus and sent branches both apically and gingivally. The latter fibers also coursed throughout the ligament in all directions but for the most part their branches supplied the periphery of the ligament.

5. Two types of nerve termination were seen: one, which was a coiled or spiral ending and the other which was
a free nerve ending. Neither type showed the presence of myelin sheath.

6. Both types of nerve ending were seen at all levels of the periodontal ligament with the free nerve ending found in greater frequency. The coiled or spiral ending was found with great frequency near the tooth surface and bone while the free nerve ending was more in abundance in the central portion.

7. The nerve endings were seen to terminate in all areas of the periodontal ligament, i.e., central portion, near the bone and tooth surface, (although not within the cementum). The endings seemed to lie on the collagen fibers.

8. There was no evidence of the presence of any specialized, encapsulated end organs in this investigation. Nor were there any knob-like endings observed in any of the sections.

9. It is the conjecture of the author that the two types of nerve ending function in pain sensation and
proprioception and that these are mediated via the dental nerves to the main sensory and mesencephalic nucleus of the trigeminal nerve.
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APPROVAL SHEET

The thesis submitted by Dr. S. Douglas Amberman has been read and approved by members of the Departments of Anatomy and Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any 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 16, 1969

DATE

[Signature]