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A Comparison of Nerve Fibers and Nerve Endings in the Sutures of the Cranial-Facial Complex and the Periodontal Ligament

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A COMPARISON OF NERVE FIBERS AND NERVE

ENDINGS IN THE SUTURES OF THE CRANIAL-FACIAL

COMPLEX AND THE PERIODONTAL LIGAMENT

BY

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LIFE

Roger Craig Nettune was born on March 27, 1943 in Bronxville, New York. He was the second of four children of Louis and Gertrude Nettune.

His grade school years were spent in the parochial school system in Bronxville. In 1954, his family moved to Closter, New Jersey, and it was here that his interest in dentistry began.

Roger was admitted into the Bergen Catholic High School, which is located in Oradell, New Jersey, where he received a diploma in June, 1961.

He completed his undergraduate pre-dental education at Temple University in Philadelphia, Pennsylvania in June, 1966.

After being accepted to the New Jersey College of Dentistry in 1966, he received the degree of Doctor of Medical Dentistry (D.M.D.) in 1970. He received departmental awards in orthodontics and gold foil operators.

He began his graduate studies in oral biology at Loyola University, Maywood, Illinois.

He is married to the former Pamela Randolph Hook of Chester, Pennsylvania and they have one child, Deborah Ann.

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INTRODUCTION

Histologic investigations of the nervous system have produced much worth-
while information of use in trying to better understand the form and function
of living tissues. Early histologists, developing individual techniques for
their particular areas of interest, contributed immeasurably to our under-
standing of the nervous system, and many of the concepts they discovered are
still valid today. With the passage of time, however, new techniques and
materials have been developed which enable more detailed studies into the
anatomy of the nervous system. Well controlled experiments have provided
considerable data regarding the neural control of muscles during locomotion
and in postural mechanisms. Less attention, however, has been directed
toward the sensory nerve components acting within the sutures themselves.

The purpose of this study is to try and demonstrate nerve fibers in the
sutures of the cranio-facial complex. An attempt will also be made to compare
these findings with those nerve fibers found in the periodontal ligament. A
comparison of the periodontal ligament will be made with the sutures histolo-
gically. Nerve fiber diameters will be measured in both the periodontal
ligament and the sutural ligaments and compared statistically. Two staining
techniques will be compared for their reliability, i.e. methylene blue with
intra-vital perfusion and silver impregnated material.

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REVIEW OF LITERATURE

A. Nerve Endings in Dense Connective Tissue

Dense connective tissue structures such as fascia, joints, tendon, liga-
ment and aponeuroses have been studied by numerous investigators.

Ralston, Miller and Kasahara (1960) did perhaps the most comprehensive
study of nerve endings in human fascia, tendon, ligament, periosteum and
joint synovial membrane. Using intravital perfusion with methylene blue on
amputated limbs, they found three major varieties of nerve endings: free,
unencapsulated complex and encapsulated. The free ending could be either an
unbranched, single terminal or have several arborizing branches ending with
tapered tips. They are derived from small myelinated fibers with diameters
of less than three microns. The unencapsulated endings were Ruffini or Golgi
type endings. These were five to twelve microns in diameter, and branches
ended with "spray" type. The final category of nerve endings were found in
the deeper structures and include Golgi-Mazzoni, Paciniform, and small and
large Pacinian corpuscles.

Stillwell's (1956,57) study of monkeys and two human fetuses described
free nerve endings in dense connective tissue and Ruffini type endings. He
also found a few small Pacinian corpuscles and he considered all three a
proprioceptive triad. Stillwell concurred with Ralston, Miller and Kasahara
(1960) that there are morphological variations observed in these complex
unencapsulated endings and that this variation may reflect adaptations to

local anatomical configurations of receptors with similar functions.

Keller (1965) used methylene blue in his study of sensory nerve endings in the temporomandibular joint in monkeys. His findings were consistent with those of Gardner (1963) who maintained that articular nerves resemble cutaneous nerves in their fiber spectrum and that articular receptors are: free, complex non-encapsulated, and complex encapsulated.

B. Innervation of the Periodontal Ligament

The periodontal ligament, in the mandibular arch, is derived mainly from the inferior alveolar branch of the mandibular division of the fifth cranial nerve. In the maxillary arch, the periodontal ligament is innervated by the posterior, middle and anterior superior alveolar branches of the maxillary division. The nerves closely follow the vasculature supply in the periodontal ligament, penetrating the alveolar bone at the apex of the tooth and giving off branches to that tooth and its periodontal ligament. However, there are also interalveolar nerves which branch laterally through foramina in the alveolar bone and into the ligament. The fibers generally course coronally. Many investigators of the periodontal ligament and its innervation have taken credit for these findings but there still remains no total agreement as to the description of the terminal endings. Some of the most prominent research done is also one of the originals done by Dependorf (1913). This work was followed by van der Sprenkel (1936), Kadanoff (1929), Simpson (1964), Lewinsky and Stewart (1936,37), Bernich (1967), Pradlow (1936), Sakai (1968) and Rapp et al (1957). All of the above were fairly similar in their approach with the exception of Simpson's "apxoestic" approach. Apoxestic comes from apoxeo, meaning I scrape off. His approach was quite different but his results were

similar.

Most authors describe both large and small diameter nerve fibers in the periodontal ligament, and imply that the larger myelinated fibers are concerned with touch sensation, whereas the smaller myelinated and nonmyelinated are concerned with pain Kizior et al, (1968), Lewinsky and Stewart (1936).

Dependorf (1913) first found nerve bundles form coarse and fine networks and end in fine, pointed processes. Lewinsky and Stewart (1926-37) investigated the above and with minimal modifications, using Cajal technique, found this to be true. Kadanoff (1929) claimed the nerve fibers given off from the main bundles end in what appeared to be a terminal plexuses, but no actual anastomosing of the terminal fibers. Some of the terminal fibers ended in small knob-like swellings, but no encapsulated nerve endings were found. Lewinsky and Stewart found nerve fibers in the periodontal ligament come from the apical region of the tooth and run towards the gum in longitudinal bundles in company with the blood vessels. These bundles communicate with each other and are reinforced in their course by fasciculi which enter the membrance through formina in the alveolar process. Simpson (1964) introduced the apoxestic microscopy next and found roughly the same as above using the Winkelmann's axoplasm technique. Finally, Sakai (1968) described the elastic tissue component of the periodontal ligament, specifically the oxytalan fiber because of its resistance to acid hydrolysis.

C. <u>Cranial and Facial Sutures</u>.

Pritchard, Scott and Girgis (1956) gave a detailed report of a suture and described five basic layers. This description applies to an actively growing

suture and not to one which is, or is about to be calcified. The five layers are: two cambial layers, two capsular layers, and a middle layer which is entirely united together with the periosteum of the bone. The cambial layer shows fine collagen bundles running in a radial direction from the bone to the fibrous capsule. These are the osteogenetic fibers, some of which will later develop into stout Sharpey's perforating fibers. The cambial layer is very cellular, showing an outer zone of small, rounded pro-osteoblasts and an inner zone next to the bone of larger, pyriform or polygonal definitive osteo-blasts. It is the site of very active osteogenesis. The capsular layer is fibrous and runs parallel to the edge of bone connecting periosteum on the inner and outer surface of the cranial and facial bone. The middle layer lies between the two capsular layers, contains loose connective tissue, and is very vascular. Two fibrous areas connected the edges of the periosteum of the adjoining bone. These are located on the inner and outer aspect of the joint and are considered the main uniting mechanism between the two bones.

The meeting of the cranial vault bones is different from facial sutures in that the approaching bone territories are not separated by loose mesenchyme but are united by the fibrous ectomeninx which is continuous with the perio-steal layers of each bone. Moreover, there are no preformed fibrous capsules as in the facial skeleton. This investigator also points out two differing modes of suture formation in the foetus. In the formation of the facial sutures the cambial and capsular layers are present around the advancing edges of the bones well before they meet, but the uniting layers are not differentiated until the sutural junction is about to be effected. In the skull vault, on the other hand, the edges of the bone are provided with a

cambial layer, but no capsular layer, and the uniting layers are already

present (as the undivided ectomeninx), although not yet delaminated from each

other. In the skull vault it is the capsules which appear as the bones meet.

The middle layer in each case arises from the mesenchyme or ectomeninx which

lies between the bone territories. As age increases, all these layers become

inactive and thinner and eventually extinct one calcification occurs.

Whimpey (1970) was unable to distinguish more than three layers in

humans and monkeys. He did note fibroblasts, osteoblasts and undifferentiated

mesenchymal cells with collagen attachment similar to that in the average

periodontal ligament. The mean width of the suture is 0.08mm to 0.24 mm.

Whimpey also showed that the vascular supply to the sutures was derived pri-

marily from the periosteum surrounding the cranial bones and less from the

cranial bones themselves. It was not recorded to be as an extensive a vascula-

ture supply as that seen in the periodontal ligament.

No mention has been made concerning the existence of nerves in the cran-

ial and facial sutures other than various assumptions that they must be pre-

sent (Pritchard, Scott and Girgis, 1956). This study demonstrates the presence

of nerve endings in the sutures of the maxillo-facial cranial complex using

silver impregnation material and characterizes their caliber spectra.

E. Nerve Fiber Diameters

Nerve fiber diameters have never been measured in the sutures to the

knowledge of the author. This is the purpose of this research. However,

nerve fiber diameters have been measured in periodontal ligament. Bernick

(1959), Kizior (1968), Avery (1959) and Rapp et al (1957), all studied and

measured the nerve fibers in the periodontal ligament. The authors findings

concurred with the above, i.e. a mean diameter of 4 microns in the perio-

dontal ligament. The author is the first to measure nerve fibers in the

sutural ligaments and found the nerve fiber diameter to be one micron.

F. Methods Used to Study Nerve Endings

The demonstration of nerve endings is subject to a number of defects, all

of which are capable of distorting a functional interpretation. Weddell and

Pallie (1954) stated that the ideal method of studying nerve tissue is by the

examination of a transparent tissue, like the cornea, in the living state under

the phase contrast microscope. Unfortunately, this is not possible in the

vast majority of tissues and other methods must be employed. The peripheral

nerve tissue has been demonstrated by a great variety of methods, largely

because of the difficulty of preparing nerve tissue for observation when it is

in a bed of dense connective tissue. Often it is only the originator of a

method who has been able to obtain consistently good results with his tech-

nique. The two best known methods for studying the peripheral nervous system

which offer the most profitable information are impregnation with silver and

gold chloride and intravital staining with methylene blue.

  1. Silver Impregnation Technique

Ramon y Cajal (1928) modified the original technique of silver impregna-

tion used by Golgi for the staining of neurofibrils and peripheral nerve

endings by using alcohol fixation with ammonia. Bielschowsky (1904) used a

lengthly procedure in which he stained tissue with low concentrations of sil-

ver nitrate followed by ammoniacal silver and then reduced in formalin.

Bodian (1936) employed protargol, with hydroquinone as the reducing agents and metallic copper for accelerating the reaction, to demonstrate nerve cells and nerve processes in paraffin sections. Winkelmann et al (1957) demonstrated a simplified method of staining axoplasm with high concentrations of silver nitrate utilizing thick frozen sections. The chemical mechanism involved in silver impregnation as postulated in electron microscope studies by Lund and Westrum (1966) is that reducible silver granules are deposited on the neuro-filaments and their light microscopic correlate, neurofibrils. When the deposited silver is reduced, the tissue neurofilaments will be preferentially stained by contrasting depths of color. Winkelmann (1960) postulated that reduced silver in tissues is probably a silver oxide because of its solubility in hydrogen peroxide. He also pointed out that not only does the silver stain nerve tissue satisfactorily but also collagen fibers, reticular and elastic fibers which accounts for the wide range of possible interpretations. Ber-nick (1957) tried to minimize collagen staining by subjecting the tissue to enzymatic digestion and then impregnating thick sections with silver nitrate. Weddell and Pallie (1954) indicated that gold or silver impregnation may dis-tort or even destroy some tissue elements causing loss of fine detail and contrast because of selective staining of the nerve fiber is not uniform.

2. Methylene Blue

Although Ehrlich (1886) was the first to point out the staining poten-tialities of methylene blue in nerve tissue, it was seven years until Dogiel (1893) published the first work on the innervation of the skin studied by a methylene blue technique. This discovery was a breakthrough in selectively

staining nerve tissue because this technique avoided the artifacts due to silver impregnation and it simplified the tracking of nerve fibers.

Ehrlich maintained that the methylene blue was taken into the axis cylinder in a colorless reduced state and then is oxidized to the dark blue state. He felt that the presence of oxygen and an alkaline pH were two conditions for the success of the methylene blue reaction. There are still diverse opinions as to the actual mechanism of staining with methylene blue.

Conn (1946) maintains that methylene blue is so readily oxidized that it is almost impossible to obtain in the pure state because azures and methylene violet are almost always present. The zinc-free chloride salt of methylene blue is the type of methylene blue most commonly used for biological studies. Once in solution the ionized methylene blue tends to oxidize very readily with alkalinity accelerating the reaction. Ehrlich (1886) postulated that some sort of chemical reaction in the nervous tissue reduces the ionized methylene blue to its leucobase. Baker (1958) recommended that methylene blue be reduced to its leucobase first by acidifying and adding sodium thiosulphate in order to make it enter axons more easily. Harris and Peters (1953) investigated the penetration of the colorless leucobase and they found that as the pH is lowered from alkalinity, the increased permeability of the cellmembrane increases the uptake of methylene blue until pH 5 is reached; from this degree of acidity onwards, the uptake is less, because there is less leucobase. This led to the postulation that the reduction of the ionic molecule may be accomplished outside the nerve fiber, thus allowing it to enter the axis cylinder more easily. Most investigators use an ionized solution of methylene blue with various physiological saline solutions in their experiments.

Numerous methods as well as various dye concentrations of methylene blue are used. One method is to remove tissue and immerse in methylene blue solution for a specified time and then oxidize in atmosphere as shown by Andrew (1954) in his study on the medial ligament of the cat. Another technique as proposed by Weddell and Pallie (1954) was to first inject hyaluronidase to allow spreading of the dye in the subcutaneous tissue and then inject locally with methylene blue. The advantage of the local injection technique is that the tissues are stained in their vital condition. Feindel, Sinclair and Weddell (1947) pointed out that one of the shortcomings of the injection technique is mechanical tissue distortion due to the relatively large volume of dye needed as well as considerable pressure to get the dye into the deeper structures. They felt the method of choice was to do a vascular intravital perfusion of the entire animal or part of the animal being studied, with methylene blue so the tissues, due to their anatomical situation or structure, would stain--which they would not do otherwise. Various concentrations of dyes have been used by most investigators as well as varying time intervals to allow the dye to saturate the tissue.

Once the nerve tissues are saturated with methylene blue it appears somewhat colorless because the dye is reduced to its leucobase. Baker (1958) maintains that abundant oxygen is needed to convert the leucobase to a dye in its ionized state. Ehrlich (1886) simply exposed the tissue to atmosphere until sufficient bluing was obtained while Boyd (1958) oxygenated tissue by placing it in a oxygenated Kreb's solution. O'Leary (1968) postulate that a sufficient oxygen tension is required to obtain adequate color of nerve

tissue. They oxygenated the tissue in a hyperbaric chamber at 45 $lb/in.^2$.

Most methods of fixation have proved inadequate as the stained nerve fibers

often fade in time, especially in light. Bethe (1896) was one of the first

to find that ammonium molybdate not only fixed the tissue but that the ammo-

nium molybdate ion formed an insoluble salt of methylene blue which made it

insoluble in various reagents commonly used in making permount microscopic

preparations. O'Leary devised their high pressure oxygen chamber in such a

way that after the tissue is completely oxygenated, the tissue is then im-

mersed in ammonium molybdate could diffuse into the interior of the tissue.

It is well known that there are more failures with the methylene blue

technique than there are successes. Weddell and Pallie (1954) claim that it

is almost impossible to control the concentration of the dye reaching a given

tissue, to allow selective staining, so other tissue elements may pick up some

stain.

MATERIALS AND METHODS

Animals

Six cats ranging in age from one to eight weeks were utilized in this study. The age of the cats were eight weeks, four weeks, two weeks, and one week old. Four of the animals were studied with Ungewilter's urea silver nitrate technique, whereas, the other two casts used were intravitally perfused with methylene blue as advocated by Boyd (1958).

Specimens were obtained for staining with the silver nitrate technique by surgically removing the soft tissue epithelial covering and exposing the bony structure of the hard palate. The mid-palatal suture was then removed, in an intact unit, and fixed in 10% unbuffered formalin. The coronal and saggittal sutures were also exposed and removed. A mandibular lateral incisor and mandibular canine were also collected and fixed.

Silver Staining

The technique used for silver impregnation consisted of washing the specimens in running tap water overnight (after fixation for three days or longer), decalcifying in equal parts of 20% sodium citrate and 50% formic acid and placing on a shaker. The specimens were returned to running tap water for twenty-four hours and then dehydrated with changes of 30%, 50% and 70% ethyl alcohol for twenty-four hours each. Final dehydration was accomplished with n-butyl alcohol for two to three days. Specimens were embedded in paraffin and sectioned at 10, 15, 20 or 25 microns.

Sections were deparafinized and hydrated in 80% alcohol and then immersed directly from 80% alcohol, for 60 to 90 minutes at 50 to 60°C in the following

solution: 1% aqueous silver nitrate - 100 cc
              urea U.S.P.   - 20 to 30 grams
       1 gram mercuric synaide
       1 gram picric acid in - 1 to 3 drops
         100 ml distilled water

They were then rinsed quickly in two changes of distilled water, reduced for 3-5 minutes at 25 to 30°C in a solution prepared by adding the following reagents in the order indicated:

        distilled water   100 ml
        sodium sulphite anhydrate 10 grams
        hydroquinone   1-2 grams
        urea   20-30 grams

The slides were shaked gently for two minutes, washed thoroughly in 4 to 5 changes of distilled water, and run through graded alcohol to 80% alcohol. If the staining was inadequate, the same process was repeated except that the immersion in alcohol was reduced to 10-15 minutes. Some specimens were stained 2, 3 and 4 times. When staining was completed the sections were dehydrated through 95% and absolute alcohol, cleared in xylol and mounted. With this method, nerve fibers are stained a deep brown to black against an almost color-less background. Nerve endings were photographed after mounting and studying.

Sutures were generally sectioned in the horizontal plane (Figure 1). The plane of section for the periodontal Ligament was longitudinal (Figure 2).

Methylene Blue Technique

The methylene blue technique has many advantages: it stains the nervous tissue in its vital state and various nerves can be traced to their terminal endings. However, this technique has been shown to be a difficult one to

master (Weddell and Pallie, 1954; Bernick, 1957). In light of this problem

material was gathered from the periodontal ligament where nerves have been

demonstrated with different techniques (Dependorf, 1913; Lewinsky and Stewart,

1926-37; Kadanoff, 1929, Simpson, 1964; Sakai, 1968). Hence the validity of

the intravital perfusion with methylene blue technique could be compared with

the more widely used silver impregnation technique. Once this was established,

both techniques were used to study the cranial-facial sutures.

The procedure used for the methylene blue technique was that advocated by

Boyd (1958). Methylene blue stains chiefly the axis-cylinder, but myelinated

fibers may be readily identified by the morphology of the axis cylinder at the

nodes of Ranvier (Feindel, Sinclaire, and Weddell, 1947). The perfusion solu-

tion contained 0.3 grams of methylene blue, 1000 ml of distilled water, 1.0 -

2.0 grams of glucose, 0.5 to 1.25 grams of $MgBr_2$ and 8 grams of NaCl as pro-

posed by Schabadasch, (1935). The solution was adjusted to pH 6.6 by the

addition (Na_2HPO_4 and KH_2PO_4) buffered and maintained at a constant room temp-

erature. The solution was used within 24 hours. Glucose gives the solution a

detailed and selective staining of the nervous tissue, hence, the duration of

the staining can be shortened. The Mg^{++} ions are absorbed by the nervous tis-

sue and increase the selectivity of the staining, especially the finest struc-

tures at the periphery. The distilled water is simply to dilute the methylene

blue so as not to be toxic to the tissues and, hence, fatal.

The animals were anesthetized to a surgical level by intramuscular injec-

tion of pentobarbital sodium (50mg/ml). The animals were secured to a large

animal board for the surgical procedure. Blunt dissection was used to expose

the trachea and the carotid sheaths on both sides. In the first cat, 8 cc of

the methylene blue solution were injected (Figure 3). The jugular veins on

both sides were then clamped to create a back pressure and a second 8 cc were

injected. The injection was done slowly at a rate of about 1/2 cc/minute.

The opposite jugular vein was then exposed and 16 cc of methylene blue solution

were injected while the contralateral jugular vein was clamped (Figure 4).

In the second cat, the same procedure was followed except that 8 cc were used

on either side and 16 cc of solution were injected intercardially. The infer-

ior vena cava was clamped periodically so the solution would be forced into the

smaller vessels (Figure 5). The sclera were examined and a definite "blueing"

effect (Polyak, 1941) was noted (Figure 6). Specimens were then gathered from

the hard palate (Figure 7) and the coronal and sagittal sutures (Figure 8).

They were placed in an oxygen chamber at 45 lbs/inch^2 pressure for a period of

one hour (Figures 9 and 10). Following oxygenation, the specimens were im-

mersed under pressure in a fixative of chilled 8% ammonium molybdate solution

for 12 to 18 hours. The specimens were then fixed, decalcified and washed in

cold running water for 2 to 4 hours. These sections were then removed and

sectioned free hand, with a sharp razor blade under the dissection microscope

(Simpson, 1964-66). The specimens were flattened manually, dehydrated with

Dioxane three times for 45 minutes and cleared with methyl-salicylate for

4 to 8 hours. They were mounted in Permount, coverslipped and studied. Photo-

graphs were taken of nerve endings as soon as possible after mounting because

fading occurs in methylene blue stained tissue.

Hematoxylin and eosin sections were also carried out to obtain a general

orientation of the periodontal ligament and sutures. Sections were cut at

12, 15, 20 and 25 microns. Periodic acid-Schiff sections were prepared at 12, 15, 20 and 25 microns to investigate the direction of the fibers in various regions of sutures and periodontal ligament. These were also studied and photographed.

Nerve Diameter Measurements

Nerve fiber diameters were measured at 450X magnification. A two milli-meter metric slide was used along with a micron ocular eyepiece to set up the micron metric scale needed. Nerve fiber diameters were measured at the apex of periodontal ligament and recorded. Also, measurements of nerves associated with blood vessels in the sutures were measured. Nerves not associated with blood vessels were also measured in the sutures and recorded. All of the above was performed twice on different days and "t" tests were performed to determine the statistical significance of the measuring technique. "T" tests were also carried out to determine the statistical significance between the nerve fibers in the periodontal ligament and sutures; and between nerves asso-ciated with blood vessels and those not associated with blood vessels.

RESULTS

Periodontal Ligament

I. <u>Mandibular Lateral Incisor</u>

    A. One Week Old Cat

Hematoxlin-eosin sections of the mandibular lateral incisor of a one week old cat showed a rounded apex of the alveolar crest. A large number of mitotic figures were observed oriented longitudinally in an apico-coronal direction. In the middle of the periodontal ligament, coronal-apically, the peridontal fibers were more obliquely oriented. The periodontal fibers in this area were more superior at their bony origin and more inferior as they approached the tooth. Periodontal fibers at the alveolar crest were in a more oblique angle. Fibroblasts had large rounded nuclei, the osteoclasts and Howship's lucannae were sparse. An abundance of spongy immature bone and osteoblasts were seen. Numerous blood vessels were observed.

Periodic acid-Schiff sections of the mandibular lateral incisors in a one week old cat showed the direction of the fibers, at the apex to be oriented in a apico-incisal direction and did not appear to be tooth to bone oriented. Those fibers at the middle of the periodontal ligament were positioned in a more oblique direction and are somewhat more tooth to bone orientated. Serial sections of the future alveolar bone proper were observed and they appeared to consist of only bony spicules with minimal fusion of individual spicules.

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Those periodontal fibers in the superior third of the periodontal ligament are oriented in a more oblique direction than those in the middle region of the periodontal ligament (See Figure 11). Only the middle fibers of the periodontal ligament appear to traverse in an oblique direction to the cementum. These findings concur with Wasserman (1956).

Ungewilters silver nitrate staining of a mandibular lateral incisor periodontal ligament in a one week old cat presented numerous nerve fibers to the periodontal ligament. The nerve fibers of the periodontal ligament appear to come from the apical region of the tooth and coarse upward in the central area of the ligament (See Figure 12). Nerve fibers penetrate through the cribiform plate of the alveolar bone at various levels appear to stay close to bone tissue and very few go across the periodontal ligament. Some fibers were seen to appear to end as free nerve endings; others fused with other nerves. Some nerve fibers appeared to end in a knob-like swelling (See Figure 13). These results are in agreement with Dependorf (1913), van der Sprenkel (1936), Kadanoff (1927) and Rapp et al (1957).

This investigator was unable to trace any nerve fibers into the cementum of a tooth. The fibers approaching the cementum looped back away from the cementum. This is in agreement with Kadanoff (1929), Lewinsky and Stewart (1936) and Rapp et al (1957). Most nerve fibers in the periodontal ligament approximated the blood vessels, although, some did not and these were found tortously arranged with the periodontal fibers.

B. Two Week Old and Four Week Old Cats

Hematoxylin-eosin, periodic acid-Schiff, silver nitrate and methylene blue stains were also used to investigate cats two weeks and four weeks old. Mandibular lateral incisors were investigated with no major changes noted, except for an increase in bone trajectories from previous bone spicules, i.e., more organized bone.

C. Eight Week Old Cat

The eight week old cat was observed to be approaching an adult in size and the findings were notably different.

In the eight week old cat, stained with hematoxylin-eosin, the mandibular lateral incisor showed less mitotic activity, more reversal lines, much more widely dilated blood vessels and a decrease in the trajectory of bony processes into the periodontal space. Fibroblasts appeared fewer in number and osteoclasts, with accompanying Howship's lucannae, appeared more frequent than in the younger cats. No spongy immature bone was noted (See Figure 14).

Periodic acid-Schiff stain of the eight week old cat in the mandibular lateral incisor revealed all fibers to be running from the tooth to alveolar bone alone, with some additional fibers running from the tooth to the gingivae.

Silver nitrate staining of the mandibular incisor in the eight week old cat appeared to reveal a decrease in the overall number of nerve fibers, especially free nerve endings. The major nerve supply continued to come from the apical region of the tooth and coarse upward along with the widely dilated blood vessels. Also, the nerves piercing the cribform plate of the alveolar bone at various levels was still observed.

II. Canine

A. One Week Old Cat

The mandibular canine tooth of a one week old cat stained with hematoxylin eosin showed similar findings to the lateral incisor in most respects with the exception of the amount of blood supply.

The mandibular canine tooth of a one week old cat, stained with periodic acid-Schiff, also showed the direction of the fibers to be similar to that seen with the mandibular lateral incisor. The age of the connective tissue fibers could be compared, i.e., the younger connective tissue stains pink and the older connective tissue stains blueish purple.

In comparing a mandibular lateral incisor to the mandibular canine tooth of a one week old cat, stained with silver nitrate, the cuspid appeared to have a greater supply of nerve fibers and nerve endings. The nerve fibers, from the apical region of the tooth can be followed towards the gingivae as longitudinal nerve fiber bundles communicate with each other and are reinforced in their course by fasciculi which enter the membrane through foramina in the alveolar process. As these apical fibers appear to go towards the gingival margin, individual nerve fibers divide dichotomously into small branches. Some of these break nerve fibers up into fine aborizations, and some of these fine nerve fibers end in small rounded bodies.

B. Two Week and Four Week Old Cat

Hematoxylin-eosin, periodic acid-Schiff, silver nitrate and methylene blue stains were also used to investigate cats two weeks and four weeks old. Mandibular lateral canines were investigated with no major changes noted, except for an increase in bone trajectories from previous bone spicules, i.e.,

more organized bone.

C. Eight Week Old Cat

Hematoxylin-eosin staining of the mandibular canine revealed more widely dilated blood vessels than the younger cats. There was also a flattened, more regular bony appearance as opposed to the trajectory of bony processes in the younger kittens. More reversal lines were seen within the alveolar bone and less mitotic activity. Osteoclasts, with accompanying Howship's lucannae, were seen more frequently than in the younger cats.

Periodic acid-Schiff stain of the eight week old cat in the mandibular canine revealed all fibers to be running from tooth to alveolar bone alone, with some additional fibers running from tooth to gingivae.

Silver nitrate staining of mandibular canine of the eight week old cat appeared to reveal a decrease in the overall number of nerve fibers, especially free nerve endings. This is an empirical observation only. The major nerve supply continued to come from the apical region of the tooth and coarse upward along with the widely dilated blood vessels. Also, the nerves piercing the cribform plate of the alveolar bone at various levels was still observed.

III. Nerve Fiber Diameters

Nerve fiber diameters, at the apex of the periodontal ligament, were measured (See Figure 15) on two different days. The mean and standard error of the mean were calculated and plotted on a histogram (See Table 1). "t" tests were then performed to demonstrate no statistical significance at the .05 level, hence the measuring technique proved to be statistically accurate. This was done on all cats from one to eight weeks and the results recorded

(See Table 2).   Nerve fiber diameters were then plotted for all cats on one

histogram (See Table 1).   These results all concurred with those shown by

Kizior et al (1968).

SUTURES

I. Coronal and Sagittal Sutures

A. One Week Old Cat

The coronal and sagittal sutures in a one week old cat, stained with hematoxylin-eosin gave the observation of a multi-laminar surface with clearly defined pre-osteoblastic and osteoblastic zones. Cellular proliferation was indicated by the frequency of mitotic figures in these zones. The outer perimeter of the sutures had old bone with new bone being laid down on its surface. Numerous mitotic figures at its periphery were observed (See Figure 16). Across the suture, to the other side of the bone, the fibrous layers were observed to be at right angles to the bone. Approximating this fibrous layer was the central or middle layer of the suture where blood vessels could be seen (See Figure 7). Adjacent to this central area was another fibrous layer running parallel to the bone and a multicellular layer with mitotic figures, identical to that previously described on the other side of the central area. There was a small amount of new bone with reversal lines seen as apposition on the surface of the old bone. Lucannae were observed with nuclei in the old bone. These findings are in agreement with Pritchard, Scott and Girgis (1956).

The coronal and sagittal sutures of a one week old kitten, stained with periodic acid-Schiff, revealed a distinct separation of various layers in the sutures. There are bony processes of spicules of bone present. Adjacent to

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this, is the cambial layer with fibers running at right angles to the older bone. This cambial layer is quite thick in a one week old kitten. Adjacent to this cambial layer is the fibrous layer which is very thin in a one week old cat and stains a pinkish color. This fibrous layer is seen to run parallel to the bone. A second fibrous layer, camibal layer and bone, is seen on the opposite side of the middle layer, as previously described (See Figure 18).

The coronal and sagittal sutures of a one week old cat, stained with silver nitrate, revealed numerous nerve fibers and endings located around blood vessels in the middle layer of the suture only (See Figure 19). In addition, other nerves were seen, but they were not associated with blood vessel. These nerve fibers, not associated with blood vessels, were throughout the entire length of the suture in minimal quantities (See Figures 20 and 21). Nerves did not appear to traverse through the surrounding bone as in the periodontal ligament although perhaps the staining technique tended to negate this observation.

B. Two Week Old and Four Week Old Cats

Hematoxylin-eosin staining demonstrated a decrease in the multilaminar surface. The periphery of the bone appeared to be beginning to take on the flattened bony processes along the alveolar borders (particularly the four week old cat). There was a decrease in the cellular proliferation when compared to the one week old cat. The cambial layer of the suture was generally thinner than that observed in the one week old cat. The fibrous layer was thicker than that observed in the one week old cat. This is what was reported by Pritchard, Scott and Girgis (1956). The middle layer appeared to remain

approximately the same width, although, no measuring technique was employed to prove this.

Periodic acid-Schiff revealed a distinct separation of various layers in the sutures. The bony processes were increased in surface area as opposed to that seen in the one week old cat. The cambial layer again, revealed fibers running at right angles to the older bone. The fibrous layer was seen to run parallel to the middle layer, i.e. parallel to the bone.

Silver nitrate staining revealed numerous nerve fibers and endings located around blood vessels in the middle layer of the suture only. Other nerves, not associated with blood vessels, were seen but were sparse. Nerves did not appear to traverse through the surrounding bone as in the periodontal ligament, although, perhaps the staining technique tended to negate this observation.

C. Eight Week Old Cat

The coronal and sagittal sutures in the eight week old cat, stained with hematoxylin-eosin, revealed a reduction in the bony projections, associated with a more regular pattern and a lower level of mitotic activity around the bone (See Figure 22). Again, a mitotic count was not performed but rather, this is a subjective observation. The region of previous pre-osteoblastic and osteoblastic layers was reduced, whereas, the fibrous layer of connective tissue running parallel to the bone was widened. The middle layer appeared the same size to the one week and four week old cats, however, the blood vessels were more widely dilated.

Periodic acid-Schiff sections of the eight week old cat, appeared similar to that seen with hematoxylin-eosin, i.e., a decrease in the cambial layer and an increase in the capsular layer.

Silver nitrate sections of the coronal and sagittal sutures revealed a decrease in the overall number of nerve fibers and endings in the middle layer of the suture. This decrease in number of nerve fibers was seen in both, nerves associated with blood vessels and nerves not associated with blood vessels.

II. Mid-Palatal Suture

A. One Week Old Cat

The mid-palatal suture stained with hematoxylin-eosin revealed a multi-laminar surface with a distinct separation of various zones or layers. The outer perimeter of the suture appeared to have old bone with new bone being laid down on top of this surface of old bone. Numerous mitotic figures were seen at the periphery of the new bone. As we progress across the suture to the opposite side of the bone, a fibrous layer is observed which has an abundance of collagenous fibrous tissue. This traversing fibrous tissue is parallel to the bone surface. The cells in the mitotic layer were seen to run at right angles to the bone surface. Approximating these fibrous layers was the center or middle layer of the suture where blood vessels were in great abundance, particularly in the posterior one-third of the suture (See Figure 23).

Adjacent to this central area was another fibrous layer, running parallel to the bone. A multi-cellular layer with mitotic figures, similar to that previously described on the other side of the central area was observed. There was a small amount of new bone seen atop old bone. Reversal lines separated the old and new bone tissue. Lucannae were seen in the old bone. In comparing the anterior, middle, and posterior area of the suture, the

posterior area of the suture appeared to demonstrate the greatest abundance

of blood vessels and nerve fibers.

The mid-palatal suture, stained with periodic Schiff in a one week old

kitten gave the observation of a distinct separation of various layers in

the suture. There are bony processes or spicules of bone present. Adjacent

to this is the cambial layer, with numerous osteoblasts, probably depositing

bone which is at right angles to the older bone. This layer has extensive

mitotic activity, and is much thicker in a one week old kitten. Adjacent to

this layer is a fibrous layer, which is this in a one week old cat and stains

a pinkish color. This fibrous layer is seen to run parallel to the bone.

The next layer is called the middle layer. Blood vessels and nervous tissue

are located in this middle layer. This layer also appears to run parallel to

the bone. A second fibrous layer, cambial layer, and bone is seen on the

other side of the middle layer as observed on the opposite side (See Figure 24)

The mid-palatal suture, stained with silver nitrate in a one week old

kitten, had numerous nerve fibers and endings located around blood vessels in

the middle layer of the suture (See Figure 25). These nerve fibers were not

prominent around the lateral areas of the suture. The greater majority of

these nerves appeared to locate in the posterior one-third of the palatal

suture. Other nerves were seen that were not associated with blood vessels.

Nerves did not appear to traverse through the surrounding bone as they did

in the periodontal ligament.

B. Two Week Old and Four Week Old Cats

Hematoxylin-eosin, periodic acid-Schiff and silver nitrate staining of the

mid-palatal suture revealed no significant difference from that previously

described under coronal and sagittal sutures.

C. Eight Week Old Cat

The mid-palatal suture of an eight week old cat, stained with hematoxylin-

eosin, were also quite different. The bony projections, or multi-laminar

surface, were greatly reduced and assumed a more regular pattern with a lower

level of mitotic activity around the bone. A mitotic count was not performed

but, rather, this is a subjective observation. The region of previous pre-

osteoblastic and osteoblastic layers was greatly reduced, whereas the fibrous

layer of connective tissue running paralled to bone was widened. The middle

layer appeared the same in size to one week and four week old cats, however,

the blood vessels were more widely dilated.

Periodic acid-Schiff sections of the eight week old cat in the mid-palatal

suture region appeared similar to that seen with hematoxylin-eosin, i.e., a

decrease in the cambial layer with a concomittant increase in the capsular

layer. The middle layer stained blue in color, the capsular layer was a pinkish

blue, and the cambial layer was a reddish purple. The significance of the

difference in color between these layers will be discussed in the discussion

section of this thesis.

Silver nitrate sections of the mid-palatal suture of the eight week old

cat appeared to demonstrate a decrease in the overall number of nerve fibers

and endings in the middle layer. This concurred with studied reported by

Pritchard, Scott, and Girgis (1956).

III. <u>Nerve Fiber Diameter</u>

Nerve fiber diameters were measured in the mid-palatal suture involving nerves associated with blood vessels on two different days. The mean and standard error of the mean were calculated and plotted on a histogram for each day (See Table 3). "t" tests were then performed and demonstrated no statistical significance at the .05 level, hence the measuring technique proved to be consistent. This was done on all cats from ages one to eight weeks and the results are shown in Table 4. Nerve fiber diameters were then plotted for all cats on one histogram (See Table 3).

Nerve fiber diameters were then measured in the mid-palatal suture of nerves <u>not</u> associated with blood vessels on two different days. The mean and standard error of the mean were calculated and plotted on a histogram for each day (See Table 5). "t" tests were then performed to demonstrate no statistical significance at the .05 level, hence, the measuring technique proved to be consistent. This was done on all cats from ages 1 to 8 weeks and results are shown in Table 6. Nerve fiber diameters were then plotted for all cats in this category, on one histogram (See Table 5).

"T" tests were then performed to determine if there was a statistical significance between nerve fiber diameters of nerves associated with blood vessels vs. nerves <u>not</u> associated with blood vessels and the results showed there was a statistical difference at the .01 level. (See Table 7)

COMPARISONS OF PERIODONTAL LIGAMENT TO SUTURES

When comparing the periodontal ligament to the sutures, it appears that there is a greater number of nerve fibers in the periodontal ligament than in the sutures. However, this was an observation and was not verified statistically.

"T" tests were performed comparing nerve fiber diameters of the periodontal ligament vs. the nerve fibers in the sutures associated with blood vessels (See Table 7). The results showed there was a statistical difference at the .01 level. It should be pointed out here that the means of measuring the nerves at the apex of the periodontal ligament was to find a nerve bundle and measure the distinguishable fibers across this bundle once. This was done only once to prevent recounting of the same fibers.

In the sutures, due to a decrease in the number of nerve fibers, all nerves that could be seen were measured.

DISCUSSION

Histology

Sutures

The connective tissue which were observed in the mid-palatal and coronal
sutures have a definite layered arrangement. Pritchard, Scott, and Girgis
(1956) presented a detailed account of five sutural layers. Weimann et al
(1955) described the sutural ligament as consisting of three layers. The
author's findings concur with those of Pritchard et al (1956) which demonstra-
ted some slight variation in the histology of a suture. This variation may be
due to developmental changes. There is a decrease in the cambial layer and an
increase in the capsular layer with an increase in age. The middle layer of
the suture remained relatively constant between one and eight weeks of age,
but the blood vessels and nervous tissue within this middle layer did not
remain constant with age. The fibroblasts appeared to reduce in size with age.
The osteoblasts decreased in number with age, whereas, the number of osteo-
clasts appeared to increase with age.

The blood vessels and nervous tissue appeared to be located only in the
middle layer of the sutures. The greatest number of nerve fibers in the mid-
palatal and coronal sutures were associated with blood vessels. Nerves associ-
ated with blood vessels, according to Orban (1957) and Ralston (1960), are
unmyelinated sympathetic nerves which are involved in reflex regulation of
vasodilation. If this is so, the possibility of a sympathectomy may be consi-

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dered for a future study.

Nerve fibers not associated with blood vessels were also observed to be coursing throughout the mid-palatal and coronal sutures. These are believed to be associated with both pain and tactile response.

Latham et al (1966), found that the cranial and facial sutures in man were very active growth areas during the first four years of life. At four years this activity had diminished to the point where the sutures appeared histolo-gically to be quiescent. They concluded that after age three, the sutures were sites of union between two bones with local remodeling occuring as required by growth of adjacent structures. Baer (1954) supports this finding. Since this investigation was performed with cats, the sutures appeared histologically to be quiescent earlier in age.

The articular surfaces of mature sutures present a convoluted interlocking pattern which results from differential growth along the sutural border (Yen, 1963). However, in the older eight week old cat, these convoluted interlocking patterns are reduced.

Periodontal Ligament

Most studied on the periodontal ligament have emphasized the morphology of connective tissue fiber bundles since these have the most obvious functional significance. Orban (1957) reported that fibers of the alveolar group appear to provide the main support for the tooth against displacing forces. This author observed the apical group of connective tissue fibers projecting from the region of the apex of the tooth, longitudinally upwards toward the gingivae. The middle group of connective tissue fibers in the periodontal ligament appears

to traverse in more of a oblique direction, at right angles to the long axis of the tooth. The fibers at the upper one third of the periodontal ligament are also traversing on an oblique angle but appear to attach at the alveolar crest in the eight week old cat. In the one week and four week old kittens, the alveolar crest consisted of bone spicules, hence, no bony attachment was seen.

Furstmann et al (1965) points out that these principle periodontal fibers are not fully developed or attached to the alveolar bone surface until eruption of the tooth is completed and into occlusion. The investigation generally confirmed this, however, the general directional pattern of these periodontal fibers does appear to be set.

The nerve supply to the periodontal ligament follows closely the vascular pathways. Lewinsky et al (1936), Bernick (1957), etc., described the nerve supply to the periodontal membrane arising from two sources: the dental nerve penetrates the alveolar bone at the apex of the tooth and gives off branches to the surrounding ligament space; the interalveolar and interradicular nerves give off branches which pass laterally into the periodontal ligament through the cribiform plate of the alveolar bone, join the branches from the dental nerve, and course occlusally together. These findings were confirmed here for the cat. The dental nerve fiber bundles of the periodontal ligament tended to be located in the center of the periodontal ligament as they progressed superiorly. The interalveolar nerves appeared to be localized around the periphery of the alveolar bone it penetrated. The apical area of the periodontal ligament demonstrated the greatest concentration of nerve bundles.

Simpson (1964) in studying extracted pre-molar teeth, gave a very similar description of the nerve supply in the periodontal ligament adding, that definite end organs were seldom seen, with fine simple endings being the rule. The author's findings appeared to agree with this.

Bernick (1957) describes the nerves as ending in an aborization. This investigation appeared to demonstrate an aborization-like termination of nerve endings. This is difficult to demonstrate using the Ungewilter's silver nitrate method due to the limitations of sections thickness.

Van der Sprenkel (1936), Kadanoff (1929), Rapp et al (1957) and Bernick (1959) claimed that nerve fibers extended through the cementoblast layer. This finding was not observed by the author in this investigation. The nerve fibers in the periodontal ligament that coursed in the direction of the cementoblast layer of the tooth were occasionally observed to curve back from the tooth.

The nerve endings in the periodontal ligament appeared as "free" nerve endings and nerve endings with knob-like swellings at their terminus. In this investigation, no connective tissue capsules around nerve fiber ending or Pacinian corpuscles were observed in the periodontal ligament, in agreement with Lewinsky et al (1936), Bernick (1957) and Simpson (1964). Kizior (1968) and Avery (1959) reported Meissner-like nerve endings in the periodontal ligament of cats and humans. The author agrees with this term, "Meissner-like" because the outer connective tissue capsular covering was not observed. Cauna (1959) reported Meissner corpuscles to grow in size with age, therefore, young animals demonstrated smaller Meissner-like endings. This investigation did not concur, however, the length of the study did not warrant this type of an investigation.

Techniques

Feindel et al (1947), Gardner (1956), Miller (1964) and Boyd (1958) have all demonstrated material well stained with the methylene blue technique. These sections with methylene blue provides excellent contrast between nerve and background tissue, the nerve staining blue-black while the surrounding tissues remained colorless or light blue. The concentration of methylene blue in the staining solution influences this contrast. The author was unsuccessful with the methylene blue technique in this investigation. The concentration of methylene blue used initially was .02% as advocated by Boyd (1958). When this did not appear satisfactory, .05% methylene blue concentration, as advocated by Gardner (1956) and Miller (1964), was used. This also proved unsatisfactory.

The metallic salts are reported to be deposited on the connective tissue as well as on nerve fibers. The author, using the Ungewilter's urea silver nitrate technique, found this to be incorrect. The connective tissue fibers were stained a colorless to a colorless to a pale yellow material. The nerve fibers were seen to be a brownish-black with excellent results.

Sections of ten, twelve, fifteen, and twenty microns were obtained for silver impregnation with optimum results seen with the thicker fifteen and twenty micron sections. Restaining of these sections two and three times was also beneficial. Over-stained sections (four times) demonstrated some vascular walls and endothelial cells.

It is difficult to photograph the spray-type nerve endings because they ramify to a depth beyond the depth of focus of the microscope. This problem could be avoided if the methylene blue sections were satisfactory. Efforts

to compensate for this were performed by the "rolling-camera" technique, i.e.
taking an exposure of the nerve fiber and moving to the next serial section
to pick up the nerve fiber, where it left off in the previous section, and re-
photographing. Other efforts to solve this problem in the future might include
double exposures made at two planes of focus on the same ending. Another would
be to photograph the endings on motion picture film while the microscope is
being focused through the entire depth of the ending.

Hematoxylin-Eosin sections were also gathered for a general orientation
of the periodontal ligament and cranio-facial sutures collected.

Periodic acid-Schiff staining was performed to show the mucopolysaccha-
rides and their relative PAS positive for younger connective tissue (pinkish
color), or PAS negative for older connective tissue (blueish-purple color).
The direction of the connective tissue fibers was also observed in the perio-
dontal ligament, the mid-palatal and coronal sutures.

Nerve Fibers and Nerve Endings

The mean diameter of the nerve fibers and nerve endings were also observed
at 450 times magnification with the light microscope. The results demonstra-
ted a significant statistical difference of these nerve fiber diameters at the
.01 level in the periodontal ligament when compared to the nerve fibers in the
sutures. Also, "t" tests were done to compare nerve fibers associated with
blood vessels versus nerve fibers not associated with blood vessels. The
results again showed a significant statistical difference at the .01 level
(See Table 7). These results would tend to suggest that the nerve fibers with
a mean fiber diameter of four microns in the periodontal ligament are A-delta
fibers for proprioceptive response. Those nerve fibers with a mean fiber

diameter of one micron in the sutures are C fibers for pain and tactile response. Those nerve fibers associated with blood vessels are for vasomotor control.

Kizior (1968) stated that free nerve endings in the periodontal ligament elicit pain. The author would tend to question this concept of correlating free nerve endings with pain because the mid-palatal and coronal sutures have appeared to demonstrate free nerve endings in this investigation. Clinically, in orthodontics, we perform a splitting of the mid-palatal suture in certain patients and these patients have not been observed to complain with pain. Yet, these free nerve endings are observed to be present.

The presence of many nerve bundles and nerve fibers coursing in proximity to blood vessels confirming the observations of Gardner (1956), and Stilwell (1956).

Ham (1930) described the free nerve endings as a fine beaded terminal arising from a naked nerve fiber. Cauna (1959) found free nerve endings in the connective tissue terminating as free fading filaments. The free nerve endings observed in this study terminated as either very fine enlargements or simply faded out of optical resolution.

The posterior area of the suture appeared to demonstrate the greatest abundance of blood vessels and nerve vessels. Sicher (1965; personal communication) suggests that the mid-palatal suture is not an active growth area but rather an "adaptive" (adjusting) growth site. During active growth periods, the mandible is continually enlarging in width from bone apposition on its lateral aspects on the molar region. Sicher feels the mid-palatal suture must "adapt or adjust" for the maxilla, in this posterior region, to accomodate for this mandibular widening. Thus, creating a more harmonious growth between

maxilla and mandible.

SUMMARY AND CONCLUSIONS

The presence of nerve fibers and nerve endings in the periodontal ligament of the mandibular lateral incisor and mandibular cuspid teeth was demonstrated. Also, the mid-palatal, sagittal, coronal and maxillo-zygomatic sutures were studied histologically in six kittens. Two kittens were intravitally perfused with a .02% or .05% methylene blue solution. Four kittens were used for study by metallic impregnation with the Ungewilter's urea silver nitrate technique. Specimens were stained with hematoxylin-eosin and periodic acid-Schiff techniques and studied histologically. Sagittal, frontal, and horizontal planes of section were used. The horizontal planes of section proved to be the most beneficial in measuring nerve fiber diameters.

There is a striking morphological similarity between the periodontal ligament and the sutural ligaments. The histology of the periodontal ligament and the sutural ligaments were compared, in the cat to man, and were observed to be similar. Five distinct layers in the sutures were observed to differentiate histologically with age. Fibroblasts, osteoblasts, and osteoclasts were also seen to differentiate with age in both the periodontal ligament and the sutural ligaments.

The vascular network and nerve supply of the periodontal ligament and sutural ligaments were seen to vary with age. Nerve fibers and nerve endings appeared to decrease with age in both the periodontal ligament and sutural ligaments. The vascular network for the periodontal ligament and sutural liga-

ments showed a concomittant dilation with age which connotes relaxation of the walls. The majority of nerve fibers lie in close proximity to the blood vessels. Because of this close vascular relationship, sensory fibers and motor fibers are believed to run together.

Only very occasional free nerve endings were observed in the sutural liga-ments. This is slightly different from the periodontal ligament, but it is to be understood that the sutural ligaments are protected from the external environment and are not subjected to the masticatory type stresses found in the periodontal ligament.

Nerve bundles enter the periodontal ligament from two sources; (1) fibers arise from the dental nerve as it penetrates the apical cribiform plate and then gives off terminal twigs that innervate the stroma of ligament as it courses gingivally.(2) the second group of fibers, perforating branches of the inter-alveolar nerves, ascent through the bone and penetrate the cribiform plate at various levels to unite with the ascending dental nerve. The greatest fiber concentration appears in the apical region of the tooth. Nerve fibers in the sutural ligaments arise from periosteum and dura mater; they are sparse and generally course in approximation to blood vessels near the middle of the suture.

Free nerve endings were observed in both ligaments (but were more sparse in the sutural ligaments) and may arise from myelinated or unmyelinated fibers. After loss of their myelin sheath, the fiber may divide or singly take a tor-tuous course and end in small terminal expansions or fade out of optical resolution. They are present throughout the ligament, but are most numerous

in the apical region of the tooth. None of the fibers were observed to enter the cementum. Only occasional free nerve endings were found in the sutural ligaments with no particular suture having a higher ratio. It should be pointed out here that the posterior one-third of the palatal suture was where the majority of the nerve fibers were found. Free nerve endings are regarded to function in pain reception and possibly tactile. Those endings in close association with blood vessels are considered to be autonomic in function.

Small knob-like endings occasionally were present in the periodontal ligament with no specific area of concentration. These endings are club shaped and may be single extensions of small fibers, or occasionally, the fiber bifurcates and forms two endings. Their function is unknown, however, it has been regarded to function in deep touch and pressure sensation. Complex and encapsulated nerve endings were not observed in the periodontal ligament or the sutural ligaments.

Nerve fiber diameters were measured in the periodontal ligament at the apex for a control, and then the nerve fibers in the sutural ligaments were also measured. Nerve fibers in the sutures were divided into those associated with blood vessels and those not associated with blood vessels.

The conclusions reached in this investigation are:

1. Nerve fibers and nerve endings are located in the sutures studied by this investigator.

2. The number of nerve fibers appears to have some correlation with age in the periodontal ligament and sutural ligaments of young kittens, i.e. a decrease in number of nerves with an increase with age.

3. Innervation of the periodontal ligament arises from dental nerves at the apex and laterally from interalveolar nerves.

4. Nerve fibers are more numerous in the periodontal ligament than in the sutural ligament and they generally course along in close proximity to blood vessels.

5. The apical nerve fiber bundles in the periodontal ligament appear to have a tendency to be located in the center of the ligament as they progress superiorly.

6. Free nerve endings, nerve endings that end in a knob-like swelling and terminal aborizations were observed in the periodontal ligament.

7. No nerve fibers were found in the cementum as extensions of the innervation of the periodontal ligament.

8. There was a differential histological appearance from one age increment to the next in the periodontal ligament and sutural ligaments in kittens; even though this age increment was, on occasion, only one week apart.

9. In the present study, no encapsulated nerve endings were observed in the periodontal ligament or the sutural ligaments.

10. The mandibular cuspid appeared to reveal a greater number of nerve fibers present than the mandibular lateral incisor.

11. The mean diameter of nerve fibers at the apex of the periodontal ligament was found to be 4 microns.

12. The mean diameter of nerve fibers in the sutural ligaments was found to be 1 micron.

13. Blood vessels in the older cats had an enlarged appearance in both the periodontal ligament and sutural ligaments.

14. Blood vessels and nervous tissue was located in the middle layer of the sutures only.

15. In comparing the anterior, middle and posterior areas of the mid-palatal suture, the posterior area of the suture appeared to demonstrate the greatest abundance of blood vessels and nerve fibers.

16. The morphology of the periodontal ligament and the sutural ligaments in the cat is similar to man.

17. Ungewilter's urea silver nitrate staining technique appeared to be more consistent than the intravital method with methylene blue in the present study.

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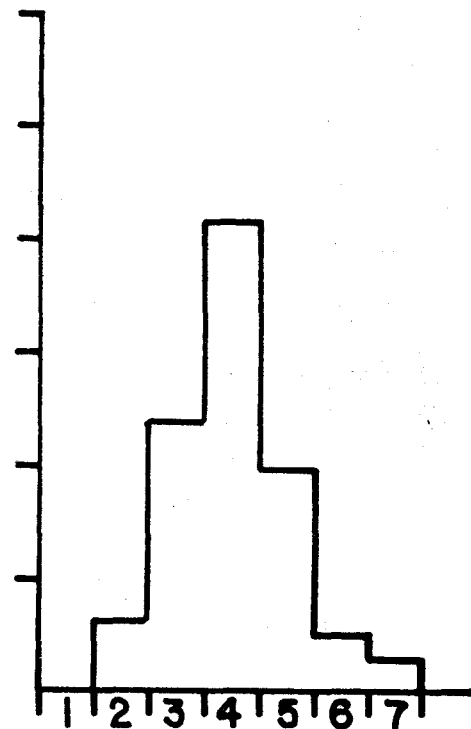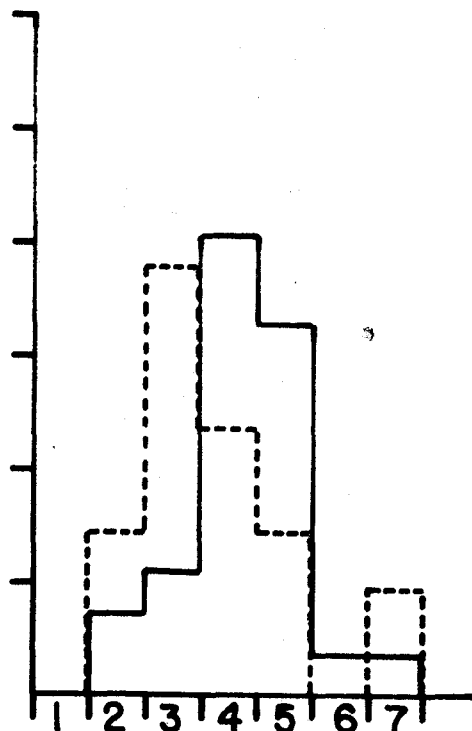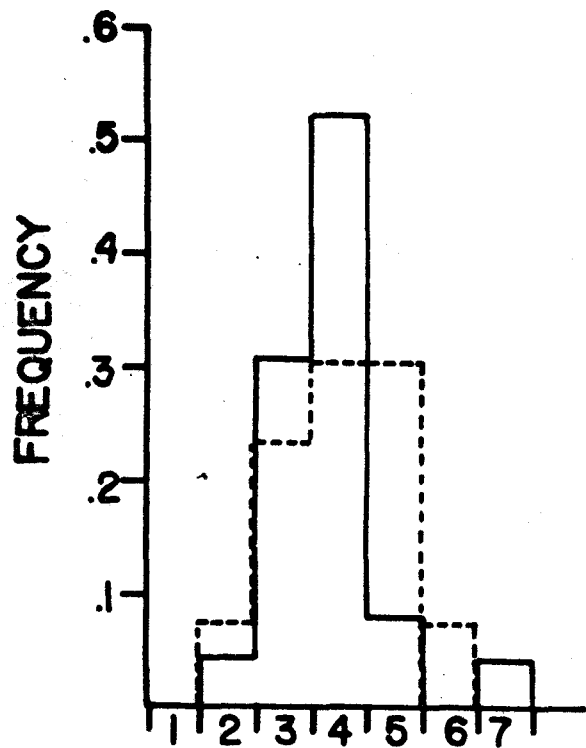
NERVE DIAMETER (MICRONS)

One Week Old Cat

_____ 1st measurement

---- 2nd measurement

Eight Week Old Cat

_____ 1st measurement

---- 2nd measurement

Composite of First
Measurements

TABLE 1.

50

One Week		Two Weeks		Four Weeks		Eight Weeks	
1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st. Meas.	2nd Meas.
$\bar{X} = 3.8$	$\bar{X} = 4$	$\bar{X} = 3.8$	$\bar{X} = 3.7$	$\bar{X} = 4.3$	$\bar{X} = 3.8$	$\bar{X} = 4.3$	$\bar{X} = 3.8$
$N = 23$	$N = 26$	$N = 25$	$N = 22$	$N = 21$	$N = 23$	$N = 27$	$N = 21$
$S\bar{X} = .2$	$S\bar{X} = .2$	$S\bar{X} = .2$	$S\bar{X} = .4$	$S\bar{X} = .2$	$S\bar{X} = .2$	$S\bar{X} = .2$	$S\bar{X} = .3$

$T = .84$	$T = .1$	$T = 1.4$	$T = 1.4$
No statistical significance	No statistical significance	No statistical significance	No statistical significance

Composite of First Measurements Taken

$$\bar{X} = 4.03$$
$$N = 96$$
$$S\bar{X} = .1$$

TABLE 2. Nerve Fiber Diameter Statistics in Periodontal Ligament

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NERVE DIAMETER (MICRONS)

One Week Old Cat   Eight Week Old Cat   Composite of First
                                                      Measurements

_____ 1st measure-   _____ 1st measurement
       ment
---- 2nd measure-   ---- 2nd measurement
       ment
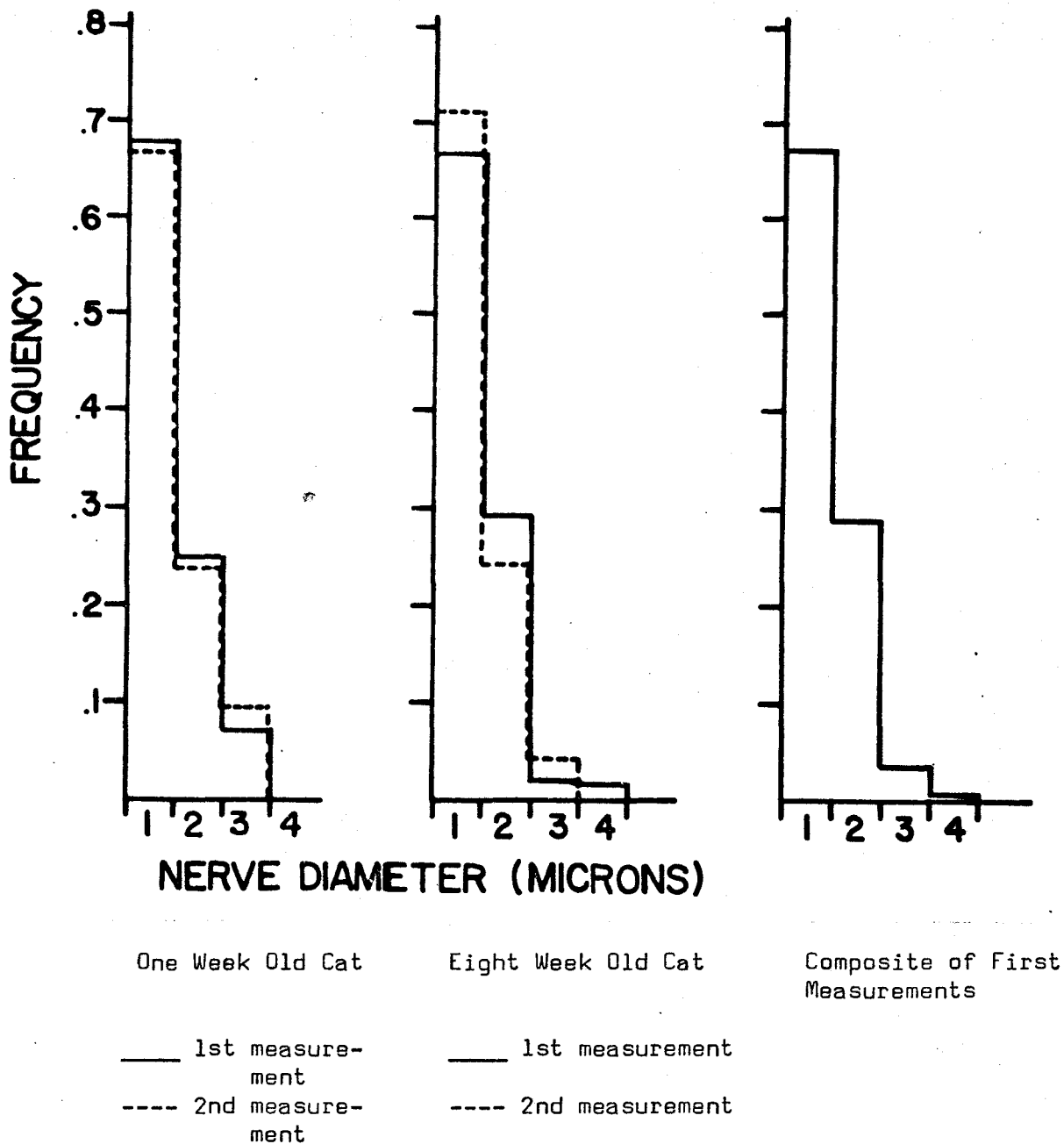
TABLE 3.

Sutures
Nerves Associated with Blood Vessels

One Week		Two Weeks		Four Weeks		Eight Weeks	
1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st Meas.	2nd Meas.
$\bar{X} = 1.4$	$\bar{X} = 1.3$	$\bar{X} = 1.4$	$\bar{X} = 1.3$	$\bar{X} = 1.3$	$\bar{X} = 1.3$	$\bar{X} = 1.4$	$\bar{X} = 1.4$
$N = 48$	$N = 45$	$N = 33$	$N = 39$	$N = 37$	$N = 30$	$N = 28$	$N = 21$
$S\bar{X} = .1$	$S\bar{X} = .1$	$S\bar{X} = .1$	$S\bar{X} = .1$	$S\bar{X} = .1$	$S\bar{X} = .8$	$S\bar{X} = .1$	$S\bar{X} = .3$
$T = .05$ No statistical significance		$T = .8$ No statistical significance		$T = 0$ No statistical significance		$T = 0$ No statistical significance	

Composite of First Measurements Taken

$$\bar{X} = 1.3$$
$$N = 146$$
$$S\bar{X} = .02$$

TABLE 4. Nerve Fiber Diameter Statistics in Sutures
Nerves Associated with Blood Vessels

TABLE 5.

Sutures
Nerves Not Associated with Blood Vessels

One Week		Two Weeks		Four Weeks		Eight Weeks	
1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st Meas.	2nd Meas.	1st Meas.	2nd Meas.
$\bar{X} = 1.1$	$\bar{X} = 1.1$	$\bar{X} = 1.1$	$\bar{X} = 1.1$	$\bar{X} = 1$	$\bar{X} = 1.1$	$\bar{X} = 1.1$	$\bar{X} = 1$
$N = 28$	$N = 29$	$N = 15$	$N = 19$	$N = 10$	$N = 14$	$N = 8$	$N = 10$
$S\bar{X} = .02$	$S\bar{X} = .01$	$S\bar{X} = .1$	$S\bar{X} = .05$	$S\bar{X} = 0$	$S\bar{X} = .1$	$S\bar{X} = .1$	$S\bar{X} = 0$

$T = 0$	$T = 0$	$T = 1.25$	$T = .27$
No statistical significance	No statistical significance	No statistical significance	No statistical significance

Composite of First Measurements Taken

$$\bar{\bar{X}} = 1.1$$
$$N = 61$$
$$S\bar{X} = .04$$

TABLE 6.   Nerve Fiber Diameter Statistics in Sutures
Nerves Not Associated with Blood Vessels

Nerves Associated with Blood Vessels vs. Nerves not Associated with Blood Vessels	Periodontal Ligament vs. Nerves Associated with Blood Vessels	Periodontal Ligament vs. Nerves Not Associated with Blood Vessels
T = 6.01 df = 206 = 2.61	T = -27.6 df = 241 = 2.6	T = 21.1 df = 166 = 2.6
Statistically Significant at the .01 level	Statistically Significant at the .01 level	Statistically Significant at the .01 level

TABLE 7. Nerve Fiber Diameters Comparing Nerves of Periodontal Ligament vs. Nerves of Sutural Ligaments

FIGURE 1

Sutures-Horizontal sections.
Figure one demonstrates the plane of section for the mid-palatal
suture. Note the horizontal plane of both the specimen and the
blade.

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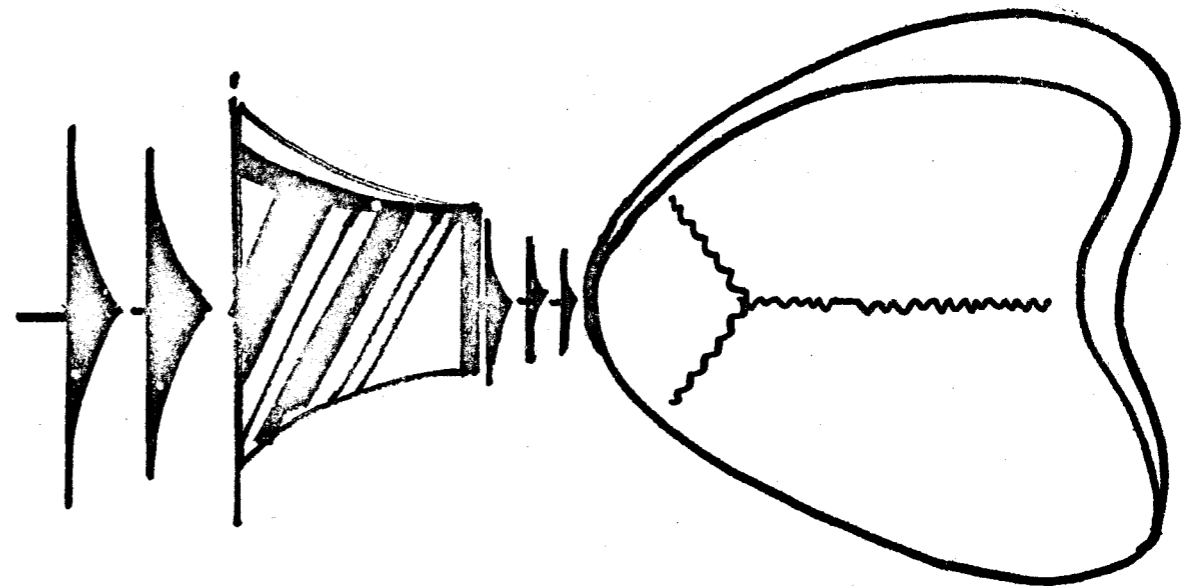
FIGURE 2

Periodontal ligaments - Longitudinal sections. Figure two demonstrates the longitudinal direction of the blade passing apically through the periodontal ligament.
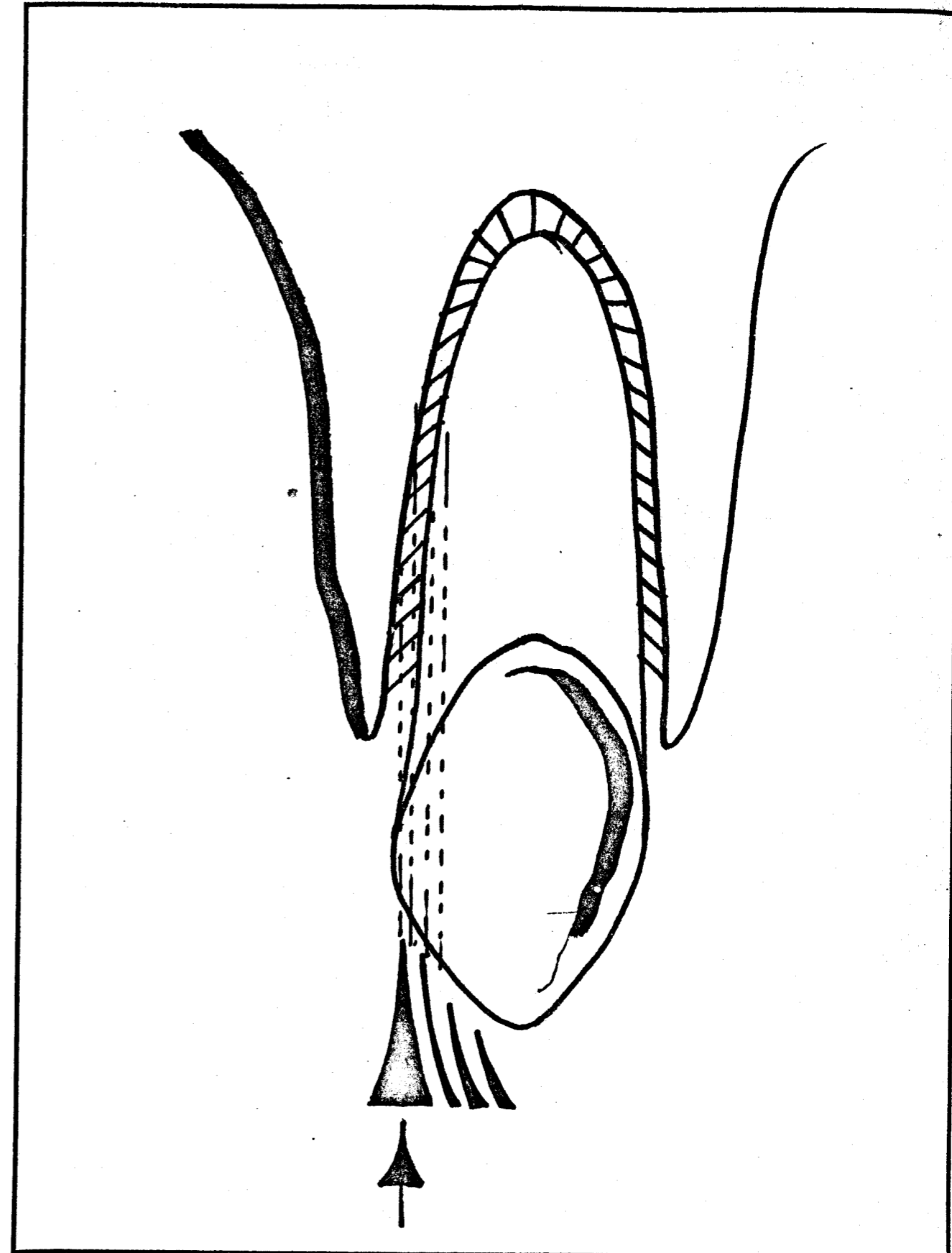
FIGURE 3

Methylene blue injected into carotid artery. This illustration
shows the methylene blue solution being injected into the carotid
artery in the first cat.

FIGURE 4

Methylene blue injected into the jugular vein. This illustration
shows the methylene blue solution being injected into the jugular
vein in the first cat.

FIGURE 5

Methylene blue injected into the heart. This illustration shows
the methylene blue solution being injected into the heart in
the second cat.

FIGURE 6

"Blueing" of the sclera of the eyes. This illustration shows the
sclera of the eyes turning blue and indicating the methylene blue
is circulating throughout the body.

FIGURE 7

Mid-palatal suture. This illustration shows the roof of the oral cavity after removal of the hard palate and mid-palatal suture.

FIGURE 8

Coronal and sagittal sutures. This illustration shows the exposed
cranium after removal of the coronal and sagittal sutures.
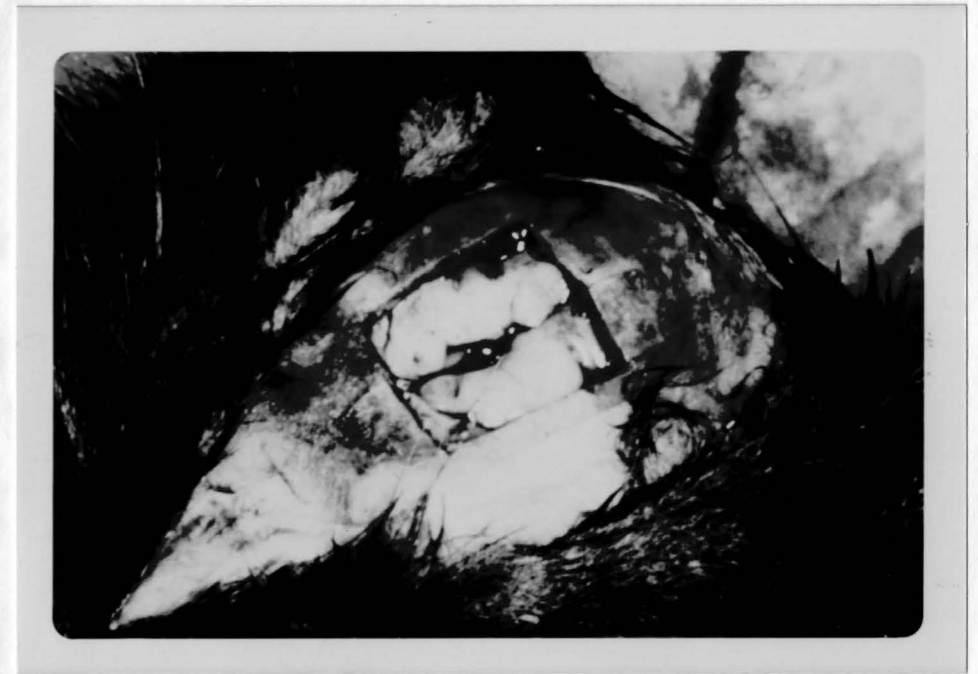
FIGURE 9

Specimens in oxygen chamber. This illustration shows the (from
left to right) cranial sutures, lateral incisor, canine, another
lateral incisor and palate specimens in the oxygen chamber before
fixed in the ammonium molybdate.

FIGURE 10

Oxygen chamber. This illustration shows the pressure tank and
attached oxygen tank used to oxygenate the specimens.
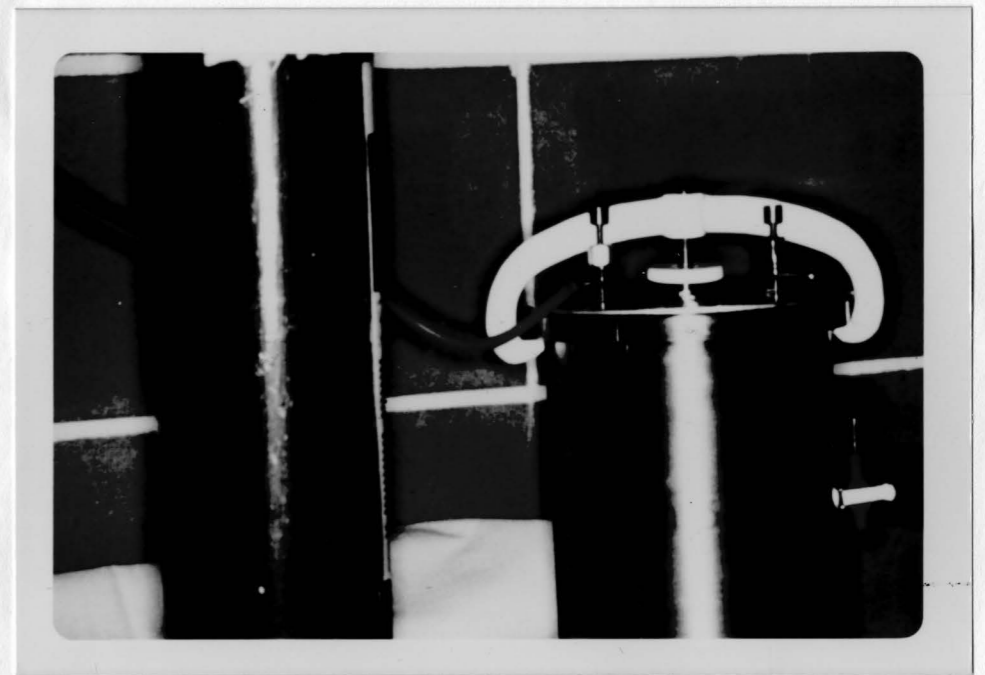
FIGURE 11

Direction of fibers in periodontal ligament with periodic acid-
Schiff. This illustration shows the fibers at the apex of the
tooth to be coursing in a longitudinal direction. However, those
near the middle of the tooth are more horizontal or run in a more
oblique direction.
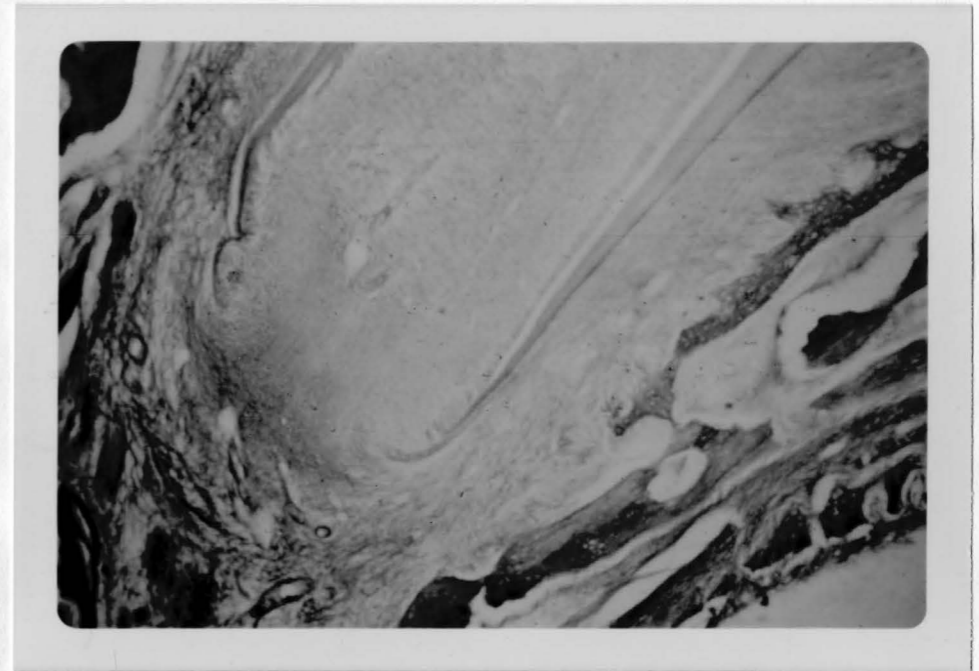
FIGURE 12

Ungewilder's urea silver nitrate staining of the periodontal liga-
ment. Note the numerous nerve fibers in the ligament which course
upward (towards the gingivae) in the central area of the ligament.

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FIGURE 13

Ungewilder's urea silver nitrate staining of the periodontal liga-
ment. This figure shows that some nerves terminate as free nerve
endings, whereas, others appear to end as knob-like swellings.
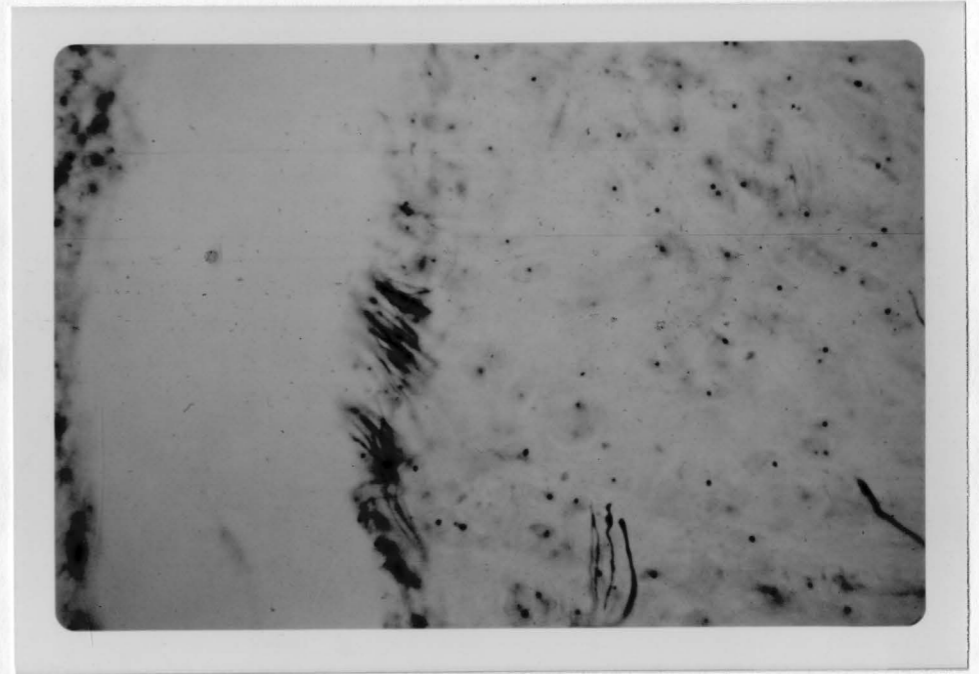
FIGURE 14

Hematoxylin-Eosin staining of 8 week old cat (periodontal ligament).
This figure shows less mitotic activity, more reversal lines,
fewer fibroblasts and osteoclasts with accompanying Howship's
lucannae.
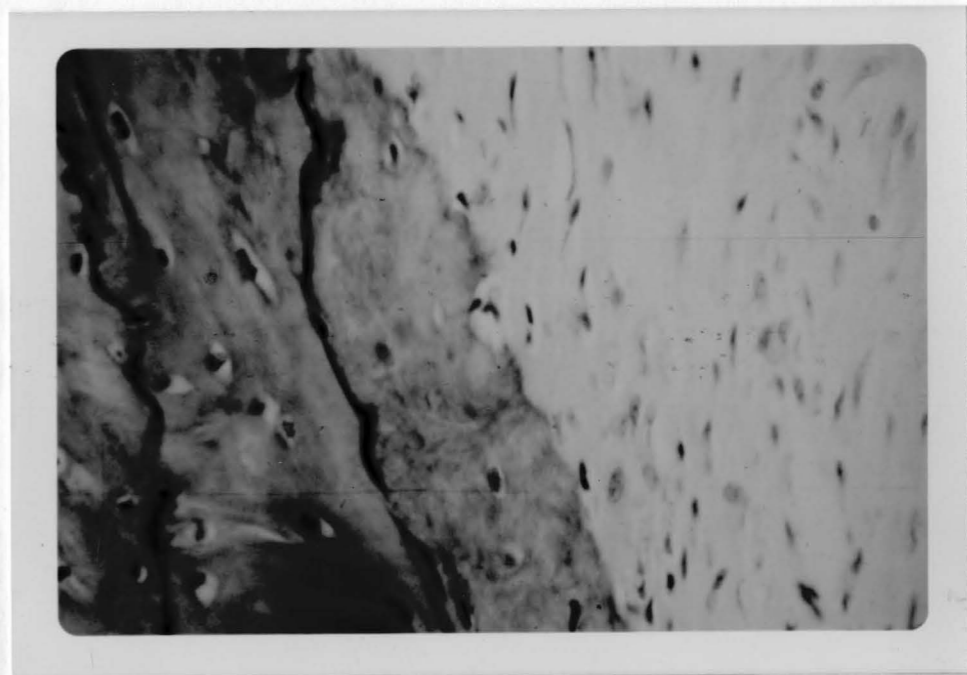
FIGURE 15

Silver nitrate staining nerve bundle at apex of periodontal ligament.
This illustration shows the nerve fibers, within a bundle, measured
to determine the mean diameters.

FIGURE 16

Coronal and sagittal sutures in one week old cat stained with
Hematoxylin-Eosin. This shows the multi-laminar surface of
the projecting cranial bones with clearly defined pre-osteo-
blastic and osteoblastic zones.

FIGURE 17

Middle layer of suture stained with Hematoxylin-Eosin. This
shows the middle layer of the suture where blood vessels can
be seen. Blood vessels and nerve fibers (with other stains)
were only seen in this middle layer of the sutures.
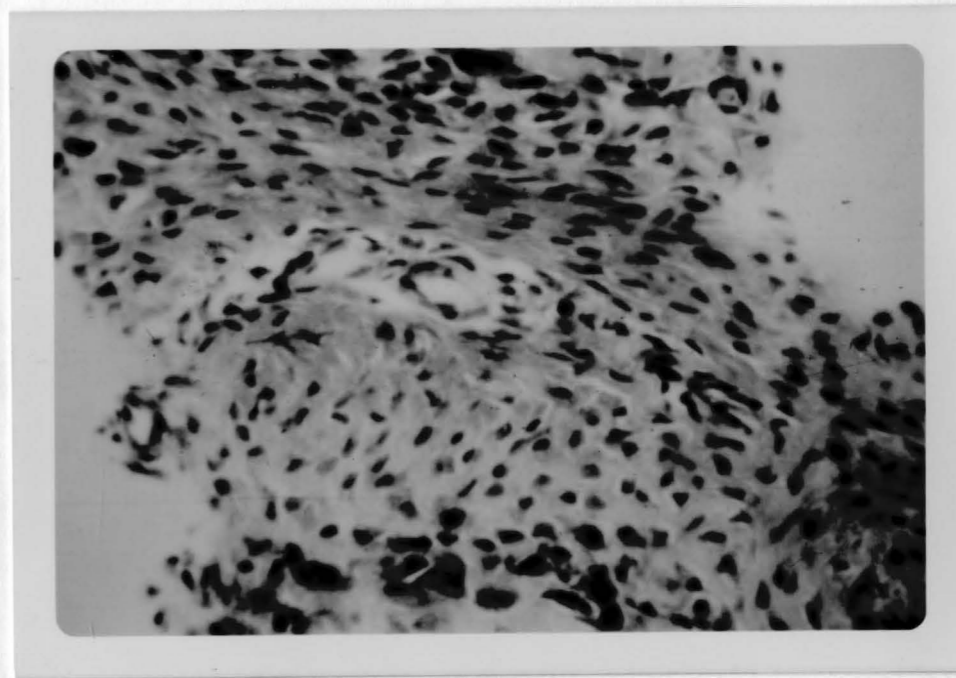
FIGURE 18

Coronal and sagittal sutures stained with periodic acid-Schiff
in one week old cat. This illustration shows a distinct sepa-
ration of various layers in the sutures. Note from top to
bottom the cambial layer (quite thick and staining pink).
The fibrous layer (thin in the young cat and staining pinkish-
blue). The middle layer (thick and contains blood vessels
and stains bluish-purple). The second fibrous layer, cambial
layer and bone.

FIGURE 19

Coronal and sagittal sutures stained with silver nitrate. This shows numerous nerve fibers and endings located around blood vessels in the middle layer of the suture only.
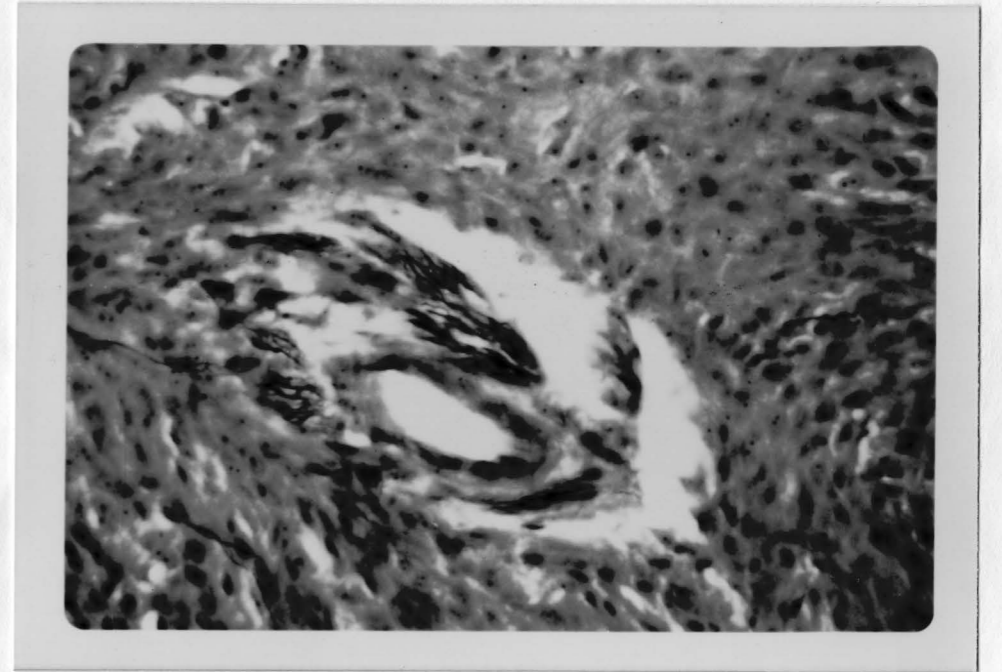
FIGURES 20 and 21

Coronal and sagittal sutures stained with silver nitrate. This
shows nerve fibers not associated with blood vessels in the
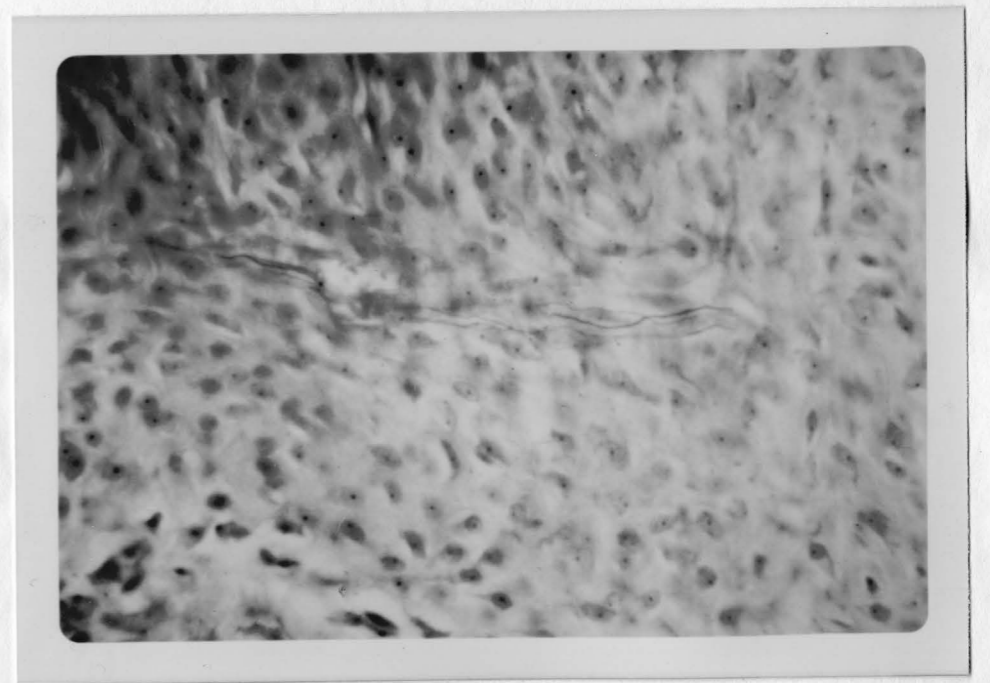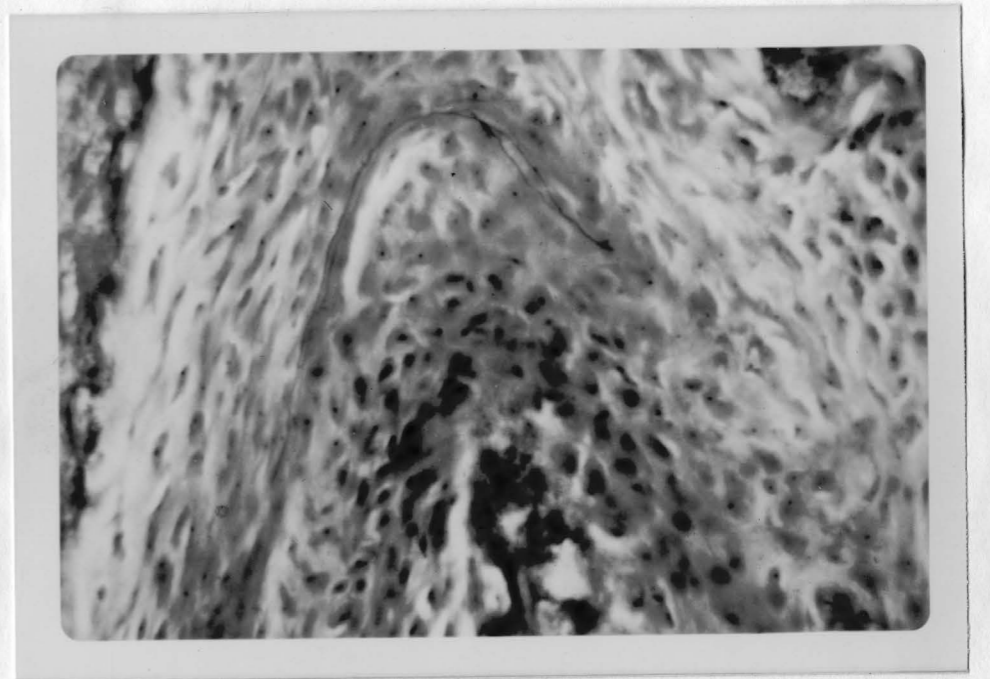middle layer of the suture.

FIGURE 22

Coronal and sagittal sutures stained with Hematoxylin-Eosin
in 8 week old cat. This illustration shows a reduction in the
bony projections, and a lower level of mitotic activity.

FIGURE 23

Mid-palatal suture stained with Hematoxylin-Eosin in one week
old cat. This shows a distinct separation of various zones
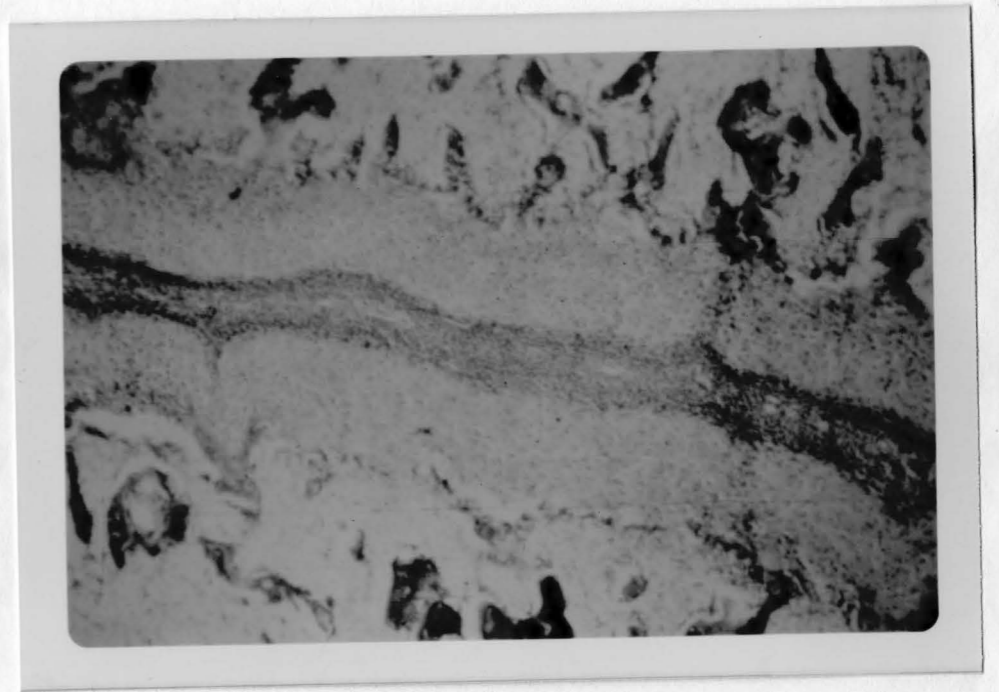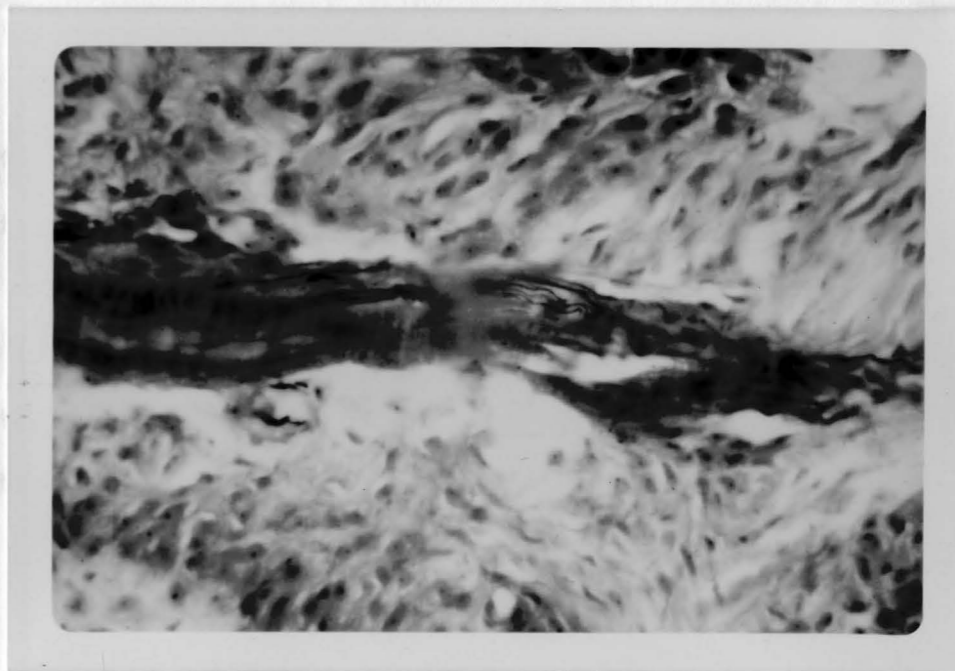or layers in the suture.

FIGURE 24

Mid-palatal suture stained with periodic acid-Schiff in one
week old cat. This illustration shows direction of fibers and
differentiation of various layers in the sutures.

FIGURE 25

Silver nitrate staining of mid-palatal suture. This illustration
shows the nerve fibers traveling along with the blood vessels in
the sutures.

APPROVAL SHEET

The thesis submitted by Dr. Roger Craig Nettune has been read and ap-
proved by three members of the faculty of the Graduate School.

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 require-
ment for the Degree of Master of Science.

DATE: July 1, 1972

Signature of Advisor