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A Histologic and Physiologic Investigation of the Sensory Receptors in the Periodontal Ligament of the Cat

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A Histologic and Physiologic Investigation of the Sensory Receptors in the Periodontal Ligament of the Cat

by

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AUTOBIOGRAPHY

Joseph E. Kizior was born in Chicago, Illinois, on June 7, 1938. He graduated from St. Rita High School, Chicago, Illinois, in June, 1956. He took his predental studies at the College of Arts and Sciences of Loyola University in Chicago, Illinois. In June of 1962 he received his Doctor of Dental Surgery degree from Loyola University Dental School in Chicago, Illinois. Following graduation he served as a commissioned officer in the United States Army Dental Corps. He was accepted for graduate school in the Department of Orthodontics at Loyola University School of Dentistry in June, 1964.

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CHAPTER I

INTRODUCTION AND STATEMENT OF THE PROBLEM

Introductory Remarks

Irritability or excitability is described as the power of a living animal to respond in some way to a stimulus. Sensory receptors have been developed for the appreciation of the various stimuli. Most receptors show specificity, being especially adapted to one modality and relatively insensitive to all others.

Several types of sensory receptors have been reported in the periodontal ligament. These vary in their location, configuration, and relationship to the other cellular elements in the ligament. Also, these receptors have exhibited sensitivity to a variety of stimuli. Nevertheless, precise information associating individual receptors with specific stimuli is still lacking.

Statement of the Problem

This study is designed to obtain additional information about the receptors in the periodontal ligament. To accomplish this, the innervation of the periodontal ligament of the cat will be investigated.

This will be done by way of a histologic investigation and electro-physiologic experimentation. The information gained from these procedures will then be correlated.

CHAPTER II

REVIEW OF THE LITERATURE

The innervation of the periodontal ligament has been the object of considerable histologic and physiologic investigation. The earliest study was reported by Dependorf in 1913. He stated that in the human periodontal ligament there are networks of coarse and fine neurofibrils which terminate as fine pointed processes in the cementoblastic region.

Kadanoff (1928) demonstrated that the neurofibrils in the periodontal ligament extended from the apical region to the gingival margin, and were reinforced by fibrils entering the ligament through foramina in the alveolar plate. The fibrils ended in terminal plexuses, and some of them had small knob-like swellings at their ends. Kadanoff's findings were in variance with those of Dependorf in that he did not observe any fibers entering the cementum.

Lewinsky and Stewart (1936) found two sources of fibers in the periodontal ligament of the cat, apical and alveolar. The apical fibers ran vertically from the apex to the gingival margin being located in the more peripheral part of the ligament. They were reinforced at intervals by the alveolar fibers. These entered the ligament from the alveolar plate and divided into two parts, each extending either apically or gingivally. The nerves, regardless of their source, were made up of coarse and fine fibers. The coarse fibers stained dark brown, while the finer fibers stained black. The fibers ended differently in that the coarse fibers remained in the outer part of the ligament terminating in spindle-shaped end-organs. The finer fibers turned inward to the deeper part of the ligament where they broke up into fine arborizations without terminal endings.

In a study on the innervation of the human periodontal ligament, Lewinsky and Stewart (1936) showed that the general arrangement of the nerve fibers corresponded to that of the cat.

Van der Sprenkel (1936) reported different kinds of endings in the periodontal ligament of young mice. He found end-organs lying on bundles of collagenous fibrils and surrounded by a periterminal reticulum in the peripheral part of the membrane. These resembled the end-organs reported by Lewinsky and Stewart (1936) except that the terminations they observed were not associated with any particular cell. Free nerve endings were seen in the central part of the ligament. They extended into the layers of cementoblasts. These findings were not wholly in accord with those of the other investigators. It may be that the innervation of the periodontal ligament of the mouse is different from that of the primates.

Bradlaw (1936) studied the periodontal ligament of the monkey. His findings were similar to those of Lewinsky and Stewart (1936). In addition, he found terminal neural coils located in close proximity to the cementum.

Dockrill (1954) reported that some of the nerves in the periodontal ligament were encircling the tooth, while others appeared as if they were leaving the ligament at an angle and were about to wind around it. The winding nerves had knob-like swellings containing neurofibrils. These swellings immediately preceded free nerve endings. Dockrill suggests that the knob-like endings described by others may have been artifacts created by cutting the nerves during sectioning. In addition to the free nerve endings, he also observed looping coils.

Because of their argyrophylic property, the collagenous fibers in the periodontal ligament complicate the identification of nervous tissue. Bernick (1957) described a method in which the tissues are treated with a proteolytic enzyme that will remove the collagenous elements and suppress their staining. He maintained that this enzymatic hydrolysis is essential, since only 10 per cent of the sections are of value after staining with silver.

Sections treated in this manner revealed two types of nerve endings. Medullated fibers terminated as elongated spindle-like structures throughout the stroma. Nonmedullated fibers united to form a fine arborization from which delicate fibrils arose and terminated as free endings among the stroma cells, cementoblasts, and cementum. The presence of fibrils in the cementum is believed to be the result of their being "trapped" as secondary cementum is laid down.

Rapp, Kirstine, and Avery (1959) studying human teech found large neural trunks in the center of the periodontal ligament. They also reported highly organized neural terminations throughout the ligament. These appeared as large, ovoid structures consisting of interweaving medullated and nonmedullated fibrils. Connective tissue fibers were found around the periphery of these structures. They also observed free neural endings throughout the ligament. No neurofibrils were observed penetrating the cementum; instead, as they approached the cementum, they formed loops and passed back toward the center of the ligament.

Most of the studies on the innervation of the periodontal ligament agree that the neural source is derived from the apical region and the alveolar bone proper opposite the root surface. However, there are differences as to the extent of the branching and the mode of termination of the nerves. The consistent appearance of neural terminations in the various studies suggests some type of function.

The histologic observations gave rise to research using the tools of the physiologist. Pfaffmann (1939) was the first to make use of electro-physiological methods in the study of dental nerves. Workers before him speculated about the physiologic properties of dental nerves on the basis of clinical observation or histologic sections. The earliest of these was Bell (1835) who showed clinically that the dentine itself was acutely sensitive to pain. Peaslee (1857) described various types of pressures that can be detected and localized by the teeth. Black (1887) stated that the sense of touch resided wholly in the periodontal membrane, while the pulp gave rise to pain whatever the stimulus. Stewart (1927) was able to show that after the removal of the dental pulp there was no alteration in the ability of the tooth to detect tactile stimuli. Pulpal nerves, according to Stewart, were not involved in touch and proprioception. He presumed that this sensation is mediated along the large myelinated nerves in the periodontal ligament.

Gerard (1923) stated that pain is conducted by unmyelinated and small myelinated fibers, whereas touch is conducted by large myelinated fibers. Windle (1926) applied this general knowledge of peripheral nerve function to his study of pulpal nerves. He showed that the dental pulp is supplied by small nerve fibers. Therefore, he concluded that these fibers conduct pain. To support his findings, he further demonstrated that the cells in the Gasserian ganglion which reacted to the removal of the dental pulp were the cells for small or medium pain fibers.

Brashear (1936) attempted to demonstrate the selective nature of the distribution of sensations in the teeth on the basis of nerve fiber diameters. He studied the fiber components of pulpal and periodontal nerves in the cat and in man. He found that the pulpal fibers varied from two microns to nine microns in diameter, and that the periodontal fibers ranged in all sizes from two to fourteen microns. Citing the evidence presented in the electro-physiologic studies of Heinbecker, Bishop, and O'Leary (1933), he concluded that the smaller fibers in the pulp mediate pain sensations while nerve fibers in the membrane mediate tactile and other sensations including pain. These observations were verified when he examined sections of the Gasserian ganglion taken from animals whose dental pulps were extirpated. He found that cells which exhibited chromatolysis were of the type that gave rise to small fibers. Cells associated with larger fibers remained unaffected.

After reviewing the work of Windle (1926) and Brashear (1936), Lewinsky and Stewart (1936) suggested that the two types of nerve fibers in the periodontal ligament transmit different impulses. They associated the thick fibers and their specialized end-organs with tactile sensations and the finer fibers with pain.

Van der Sprenkel (1935) attributed the following function to the periodontal ligament end-rings. The end-rings lay flatly on the collagenous bundles which normally are in a resting state. When pressure is applied the bundles became taunt, and the end-rings change their form. He considered this change as a sufficient stimulus to activate the nerves associated with the respective end-rings.

Pfaffmann (1939) recorded discharges from dental nerves during a series of experiments. In so doing, he determined that the threshold value of the canine tooth in an adult cat was from two to three grams. This was done with a series of calibrated bristles.

In another experiment, he applied pressure to the intact tooth with a glass rod, and obtained a pattern of discharges which showed a high voltage spike of about 250 to 600 microvolts at the moment of contact. This was followed by a steady asynchronous discharge of lower voltage showing a gradual diminution in the potential magnitude as the pressure was maintained. The discharges stopped immediately after the pressure was removed. The endings that were responsive to this pressure were shown to be located in the periodontal ligament, since there was neither a diminution in response to pressure nor an increase in the pressure threshold after removal of the pulp.

Pfaffmann also recorded discharges from single fiber preparations. Some endings gave only a few impulses whereas others discharged for five minutes or longer. This was taken as evidence of the varying adaptation time of the different endings. With greater pressures the frequency of response was higher and the adaptation time longer. The initial frequencies were shown to vary with the different rates of force application. Directional sensitivity was also demonstrated by the individual fibers. Pressures against only one surface of the tooth were effective for a particular fiber. This suggests that only one type of deformation of the ending is necessary in initiating a discharge.

The conduction velocity of the fibers was estimated from the form of the diphasic impulses when the inter-electrode distance was known. The rates for the faster conducting fibers were found to be 24 to 60 meters per second. Erlanger and Gasser (1937) have shown that these rates are characteristic for large fibers. Histologic studies demonstrated that these fibers and their associated end-organs are located in the periodontal ligament. This further supports the fact that the tactile or proprioceptive receptors are in the periodontal ligament.

The study of impulses due to noxious stimuli was complicated because they were masked by discharges from pressure endings. The teeth were treated with agents that are related to pain, such as hot or cold water. This resulted in impulses of a lower voltage and a slower conduction rate than those initiated by pressure.

Ness (1954) using rabbit teeth employed two force systems--a weight-and-pully system in which forces of different magnitudes were applied in the same direction, and a system using traction in which forces of the same magnitude were applied in different directions. He inserted a platinum wire electrode to record discharges from the entire nerve trunk as well as single fiber preparations. The following observations were made from his experiments: (1) the threshold value for the rabbit incisor tooth was found to be one to two grams; (2) the threshold value for the cat canine tooth did not vary much from this; (3) when pressure was applied to the intact tooth, the discharges recorded from the entire nerve trunk were similar to those obtained by Pfaffman (1939). In addition to these, Ness also found discharges of a small amplitude being recorded in the absence of overt stimulation. Ness divided the nerve discharges into three distinct patterns, and the association of a specific spike size and adaptation time with each pattern has been taken as evidence for three distinct types of receptors. These he referred to as slow-adapting, fast-adapting, and spontaneously discharging. When the measurements were collected, the different types of discharges presented the following distribution:

	Mean	S.D.
29 slow-adapting units	0.92 mV.	0.63
5 fast-adapting units	2.65 mV.	1.91
5 spontaneously discharging units	0.17 mV.	0.04

The most numerous were the slow-adapting receptors which also discharged the longest in the presence of a sufficient stimulus. Responses from fast-adapting receptors consisted of one or two large spikes at the "on" or at the "off" of the stimulus. Gray (1959) defined this type of ending as a phasic receptor and stated that the response of phasic receptors is dependent on the time course of the stimulus application. Spontaneously discharging receptors maintained a steady frequency of discharge with only some variation after a long period of time. The location of these different receptors was assumed to be the periodontal ligament.

Ness also reported that all three types of receptors showed directional sensitivity. When the stimulus was applied in the most sensitive direction of a receptor, the response frequency was greater, the threshold value less, and the discharge longer. Citing the evidence presented by Boyd and Roberts (1953) that their "slowly adapting" responses originated from stretch receptors whose directionality was a property of their individual orientation, Ness reasoned that the directionality of the periodontal receptors could be a property of the orientation of the individual receptor. Clark (1947) concurred with this reasoning by suggesting that the different alignments of receptors in the skin may be the basis of perception of mechanical stimuli. Each receptor would then have an axis whose direction would determine the direction of stimulation to which the receptor would be most sensitive. Ness further speculated that the axis of such a receptor need not be that of the nerve terminals themselves, but that of connective tissue fibers. If this is so, then the discharge frequency would be a property of the extensibility of the connective tissue.

Loewenstein and Rathkamp (1955) evaluated the pressoreceptive modality of human teeth. Using calibrated esthesiometers, they determined that the threshold values ranged from 0.948 to 4.533 grams with an average of 2.535 grams, thus classifying the teeth as highly sensitive structures. In other experiments an attempt was made to locate the pressoreceptors. Threshold values of pulpless teeth were compared with normal teeth, and in all cases the values were higher for pulpless teeth. A similar study comparing the thresholds of denervated teeth and the same teeth covered with a metallic cap showed no difference at all. These results were interpreted to mean that the dental pressoreceptors are located intradentally as well as in the periodontium.

Kruger and Michel (1962) determined the portion of the sensory trigeminal complex that was innervated by the buccal cavity. They accomplished this by using a steel microelectrode that was advanced in a series of penetrations along the dorsal surface of the sensory trigeminal complex. At each penetration mechanical stimuli were applied to various structures in the buccal cavity until some activity was encountered. The teeth were stimulated individually and the properties of the resulting neural discharges were reported. Fast-adapting discharges were elicited by gentle touching. Slow-adapting neurons responded to a sustained stimulus by initially displaying a rapid acceleration of impulse discharges and then maintaining a slower rate until the stimulus was removed. The present findings concerning the tactile and directional sensitivity of the teeth concur with the earlier works of Pfaffmann (1939) and Ness

(1954). On one occasion Kruger and Michel recorded the following distribution of trigeminal neurons excited by tooth stimulation:

	Fast Adapting	Slow Adapting	Total
Touch	29	13	34
Pressure	9	23	32
Tapping	4	. . .	4

There was no evidence of any neurons specifically excited by noxious stimulation of the teeth. The authors do not disprove their presence but suggest several possible interpretations as to why they have missed identifying them. The simplest lies in the difficulty encountered in applying a noxious stimulus to the pulp and then identifying the appropriate pain neuron. Another clue is provided by the anatomical arrangement of the trigeminal fibers. Only a few of the incoming fibers of the trigeminal nerve do not bifurcate. Upon these few fibers falls the burden of pain impulse transmission, unless the tactile fibers of the descending branch also conduct pain. It has been suggested that they conduct pain after excessive activation. This reasoning is speculative, and was offered as an explanation of the difficulty encountered in isolating pain neurons.

Jerge (1963) studied the neurons in the trigeminal mesencephalic nucleus of the cat. He classified them on the basis of their peripheral fields and physiological characteristics into three types: (1) those innervating the muscle spindles of the masseter, temporal, and medial pterygoid muscles; (2) those innervating dental pressure receptors of a single tooth, and (3) those innervating pressure receptors of two or more adjacent teeth.

He observed thirteen units in the trigeminal mesencephalic nucleus which were responsive only to pressure applied to an individual tooth. In studying these units he concurred with the earlier reports of Ness (1954) and Pfaffmann (1939) that the receptors which activated the units were located in the periodontal ligament of the tooth. The average threshold value for these units was found to be 1.8 grams. This value varied with the direction of the stimulus, the minimum being recorded when the pressure was applied in the most sensitive direction of the tooth. A unit could be discharged by pressure in any direction up to 90° from the most sensitive direction. Throughout the remaining 180° of the tooth circumference, the unit was non-excitable. In addition Jerge was able to show that the tooth itself has no directional preference. The arrangements of the receptors will respond to pressure regardless of the direction from which it is applied. Some of these units appeared to be fast-adapting, while others were slow-adapting. Four of the units were of the former type and responded with a short burst of one to two spikes to a weak stimulus and with ten to twenty spikes to a strong stimulus. The remaining units were slow-adapting.

Jerge (1963) also studied the latency of the units that were related to the pressure receptors of the teeth. These showed a variation in latency which was attributed to the properties of the receptors themselves. The minimum latency was recorded when the direction of the stimulus coincided with the axis of maximum sensitivity for the receptors. This direction was characterized by a maximum in the frequency of responses and a minimum in the stimulation threshold.

The second type of dental pressoreceptor units differed from the first in that they innervated from two to six teeth. Each of the teeth in such a group had an axis of preferred stimulation along which the frequency of discharge was high, the threshold low, and the latency period short. All of these units were found to be quick-adapting. The threshold values for the units that innervated several teeth were found to be between two to six grams. The values increased from tooth to tooth as the stimulus was applied to the posterior teeth. Jerge concludes by stating that the findings reported on the units agree with the histological evidence establishing the first-order nature of the mesencephalic nucleus cells.

Moyers (1965) reported a study in which periodontal pressoreceptor thresholds were determined by means of an electronic device permitting dynamic loading of human teeth. The mean threshold recorded was 0.692 grams. Moyers questions the reliability of using only tactile receptors as guides in determining thresholds. Many factors such as head position, emotional make-up, environment, and physical fitness of the individual can influence the perception of pressure. Moyers cited a study in which six out of fifteen subjects reported thermal sensations with force application. He advocates the multimodality concept in receptors and suggests that more physio-psychological investigations are needed to solve this problem.

CHAPTER III

METHODS AND MATERIALS

Introduction

Ten healthy, adult cats were used in this study. The animals were used first in the physiologic experiments, and were later sacrificed to obtain the jaws for the histologic appraisal of sensory receptors in the periodontal ligament.

Two sets of data were collected, one dealing with the physiologic responses of the periodontal receptors to certain magnitudes of force application. The second deals with the histologic assessment of the sensory receptors in the periodontal ligament.

The methods and materials utilized during the physiologic experiments of this research were also employed in the research titled, "A Correlation of the Functions and Diameters of the Sensory Fibers in the Mandibular Nerve of the Cat," M.S. thesis, Loyola University, 1966, by John William Cuozzo.

Physiologic Experimentation

I. Preparatory procedures.

The following procedures preceded each experiment:

A. Preparation of the Animals for Surgery

All the animals were received from the Research Animal Department at the Stritch School of Medicine of Loyola University, Chicago, Illinois. They were under the care of this department until the day of the experiment. The weights of the animals ranged from 2.8 to 4.5 kilograms.

One and four-tenths grams per kilogram body weight of Urethan (Merck) in isotonic saline solution was injected intraperitoneally for anesthesia. This dosage was found to be effective in establishing a long lasting anesthetic plane suitable for surgery.

When the desired level of anesthesia was attained, the fur was shaved from the region of the lower border of the mandible. The exposed skin was scrubbed with an antiseptic soap, dried, and the surgery was started.

B. Surgical Procedure

The inferior alveolar nerve was exposed before it entered the mandibular canal. Dry skill specimens showed the mandibular foramen to be from four to seven millimeters above the lower border of the mandible (Figures 1 and 2). This area was reached by making an incision which extends from behind the mandibular angle to

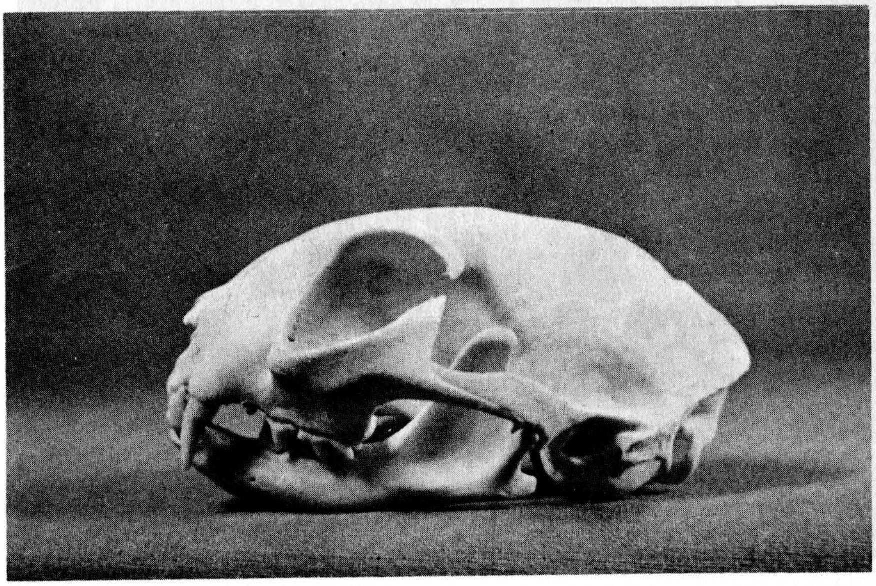


FIGURE 1

LATERAL VIEW OF THE CAT SKULL

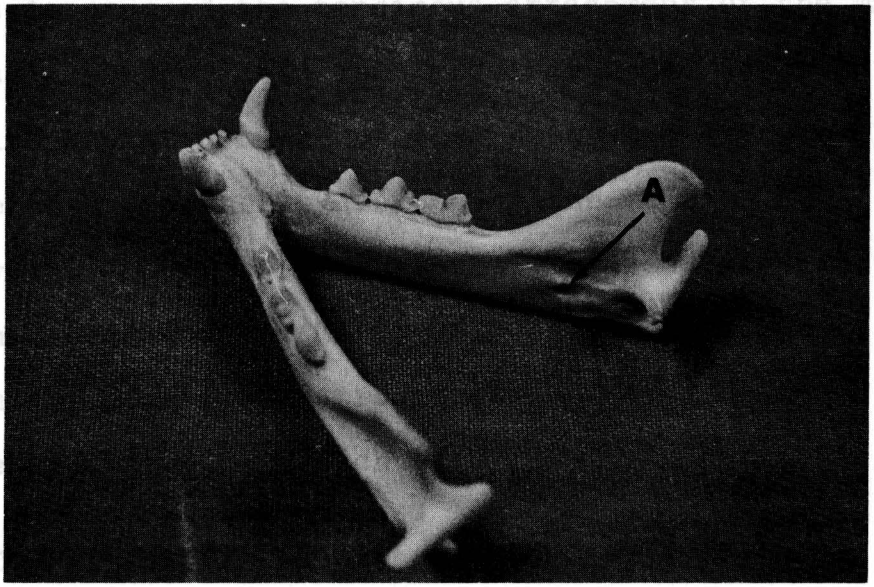


FIGURE 2

MEDIAL VIEW OF THE CAT MANDIBLE

A--Mandibular foramen

the symphysis along the medial side of the inferior border of the mandible. Retraction of the skin and underlying fascia exposed the tendinous attachment of the masseter muscle (Figure 3). This attachment was severed together with the attachment of the internal pterygoid muscle beneath it; thereby, exposing the mandibular artery, vein, and nerve just as they entered the mandibular canal (Figure 4). The thin connective tissue sheath surrounding these structures was removed, and they were separated from each other.

C. Attachment of Electrodes

A pair of modified sleeve electrodes were placed around the inferior alveolar nerve to record the resulting impulses. Each electrode was made from a round, pure silver .010 inch wire. The electrodes were assembled into a single unit in a manner which facilitated their placement around the nerve and insured their contact with it. The free ends of the electrodes were connected directly to a shielded cable. The jack from this cable led directly into the input terminal of a preamplifier.

After the electrodes were placed around the nerve, the incision was sutured loosely, and the area was kept moist with Tyrode's solution. This solution is an electrolyte composed of 0.90% NaCl; 0.02% KCl; 0.02% $CaCl_2$;

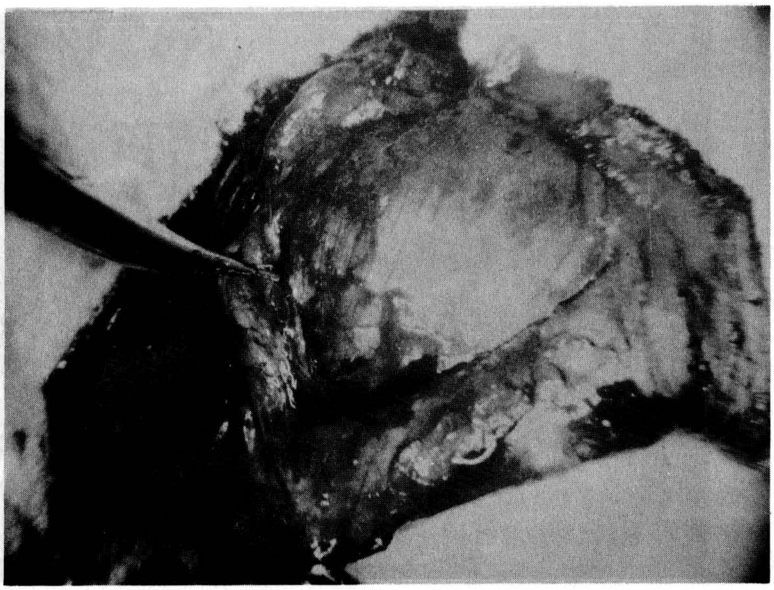


FIGURE 3

EXPOSURE OF THE MASSETER MUSCLE

Exposure of the masseter muscle following the retraction of the skin and overlying fascia.

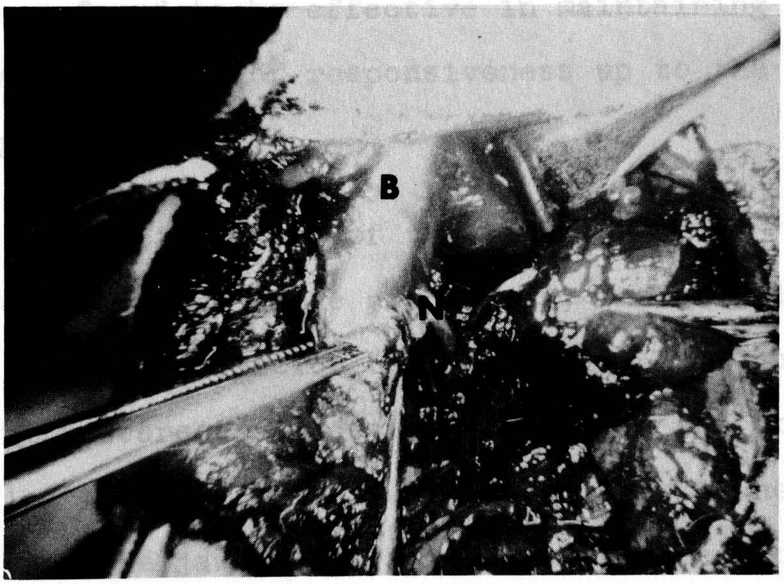


FIGURE 4

THE INFERIOR ALVEOLAR NERVE EXPOSED

B--Lower border of the mandible

N--The inferior alveolar nerve overlying the probe

0.01% $MgCl_2$; 0.10% glucose; 0.10% $NaHCO_3$; and 0.05% NaH_2PO_4 in water. It was prepared prior to each experiment and was found to be effective in maintaining the nerve at a peak level of responsiveness up to the time of actual experimentation.

D. Immobilization of the Jaws

Following the above procedures, the animal was placed in a supine position on a specially built platform having a stereotaxic device mounted at one end (Figure 5). This device was used to orient the head and to immobilize the jaws. The animal was secured to the platform with retention straps (Figure 6).

The main part of the platform is one-half inch thick, and its over-all measurements are twenty-eight inches in length and twelve inches in width. Its underside is slotted to hold the retention straps. One end of it is modified by being narrowed to a five inch square section on which the stereotaxic device is mounted.

The stereotaxic device is also made from the poly-vinyl chloride material. It consists of five vertical posts which are attached to the platform at different locations. One post is placed at the superior end of the section; the other four are placed in pairs along its edges. Each post is one-half inch thick and measures

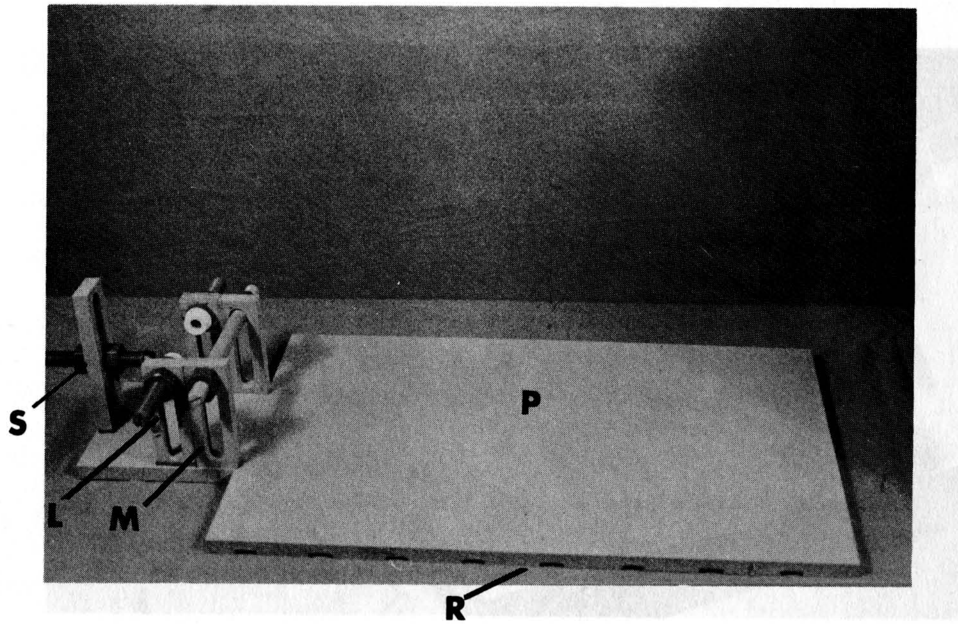


FIGURE 5

THE VINYL PLATFORM AND THE STEREOTAXIC MOUNT

S--Superior post

L--Lateral posts supporting the lateral skull bar

M--Lateral posts supporting the mandibular stabilizing bar

P--Platform

R--Slot for retention straps

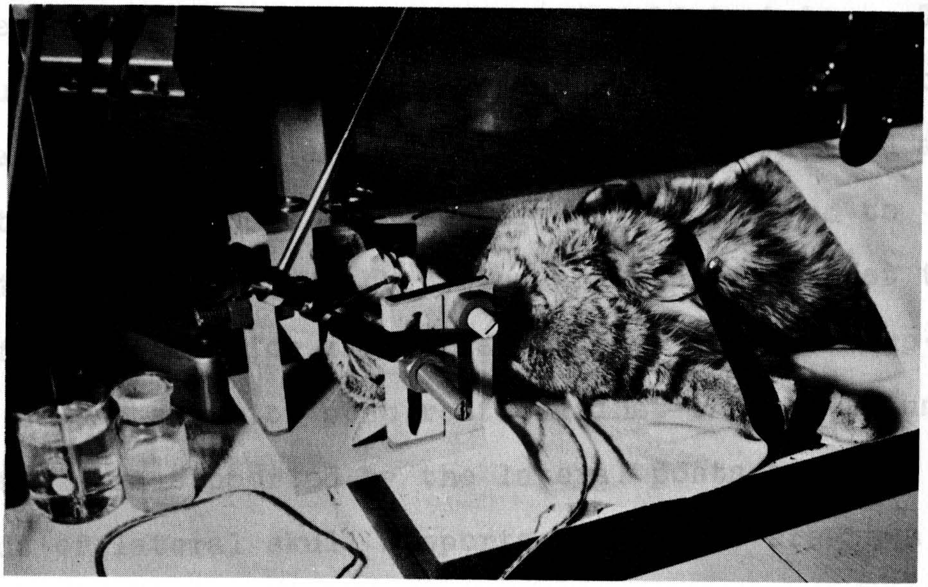


FIGURE 6

THE ANIMAL POSITIONED IN THE STEREOTAXIC DEVICE three and one-half inches in height and one and a half inches in width, excepting the superior post which measures five inches in height. All the posts are slotted to allow for the movement of a cylindrical one-half inch bar. The bars supported by the superior post and the two anterior posts along the edges are similar. They are threaded at the outside end, and a hard rubber tip is attached to their inside end. The rubber tips are rested against the head of the animal for maximum stability. The bar supported by the superior post rests against the calvarium; while the bars supported by the lateral posts serve as ear rods or lateral skull supports. The bars are then locked or set at the desired positions with the threading mechanisms at their outer ends.

The two posts nearest the main section of the platform support a single bar which rests firmly against the lower border of the mandible.

The jaws were kept open with an acrylic-resin bite block. It was fabricated of auto-polymerizing resin and inserted on the side of the mouth opposite the area of surgery.

Following the preparatory procedures described above, the animal was transferred to an electrically shielded cage for the physiologic portion of the experiment (Figure 7). The shielding of this case was by means of a double layer of copper screening that was stretched over its wooden framework. Nonconducting brass screws and corner brackets were used to hold the framework together. The cage measures sixty inches in length, twenty-four inches in depth and forty inches in height. There are two doors on the front side of the cage, each covered with a double layer of screening material. During the experiment the doors are closed to provide an added measure of shielding. The cage was grounded by cables to the plumbing in the laboratory.

II. Force Producing Instrument and Recording System

A. Force Producing Instrument

A specially designed force producing instrument (Figure 8) was used during the experiments to apply forces to the teeth. It was built by the P. A. Sturtevant Co., Addison, Illinois.

Essentially the instrument consists of two major components--the torque wrench with its adapter, and the fixture on which this is mounted. These components were arranged so that the magnitude and direction of force could be changed or set as desired. A description of each component will show how this is accomplished.

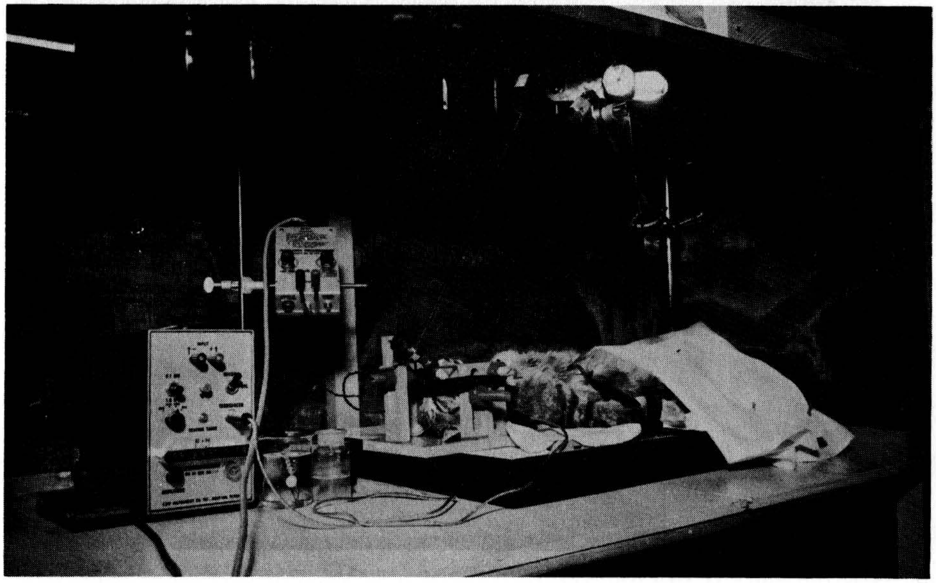


FIGURE 7

THE ANIMAL PLACED IN THE SHIELDED CAGE

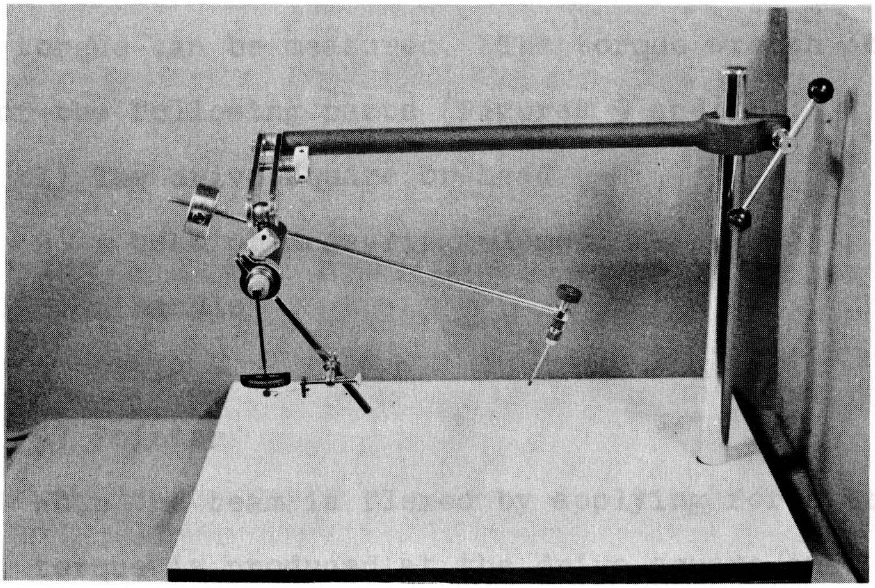


FIGURE 8

FORCE PRODUCING INSTRUMENT

(Parts labelled in Figures 13 and 14.)

A torque wrench is a measuring tool similar to a micrometer, vernier caliper, and other measuring devices. It is set or calibrated so that the resistance to turning or torque can be measured. The torque wrench consists of the following parts (Figures 9 and 10):

1) The drive square or head
2) A beam or measuring element
3) A handle
4) Scale
5) Pointer

When the beam is flexed by applying force at the handle, torque is produced at the drive square end. The amount of this torque can be calculated by using the formula: T = F x D, which is an algebraic expression of the torque law. All torque wrenches are designed to follow the principles of torque law which is derived from the fundamental law of the lever. This law states that the force times the distance equals the moment or torque about a point. In calculating the amount of torque produced about the drive square, the lever length (represented by D), is multiplied by the amount of force applied at the handle (represented by F). Lever length refers to the distance from the center of the drive square to the point on the handle where the pulling force is concen-

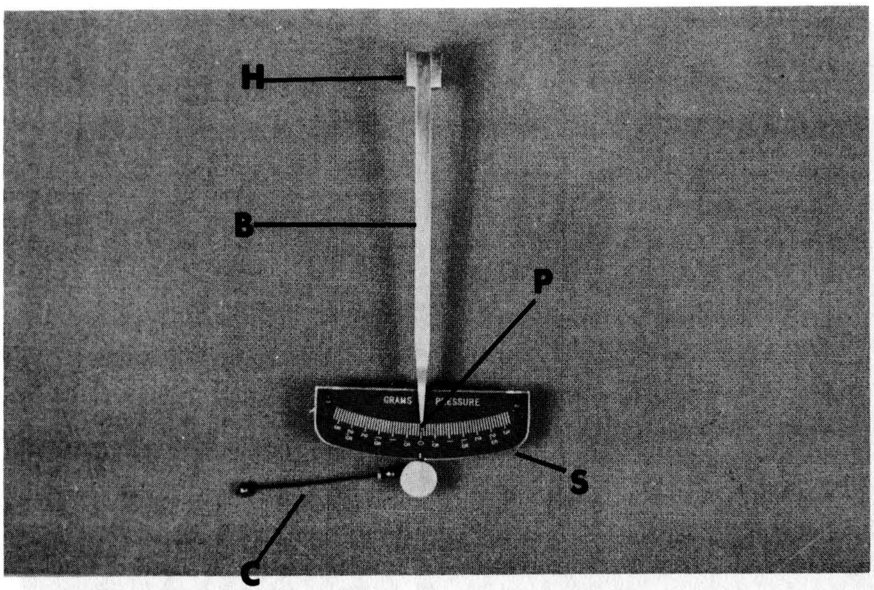


FIGURE 9

A TORQUE WRENCH WITH THE PARTS LABELLED

B--Beam

H--Head

S--Scale

P--Pointer

C--Cord

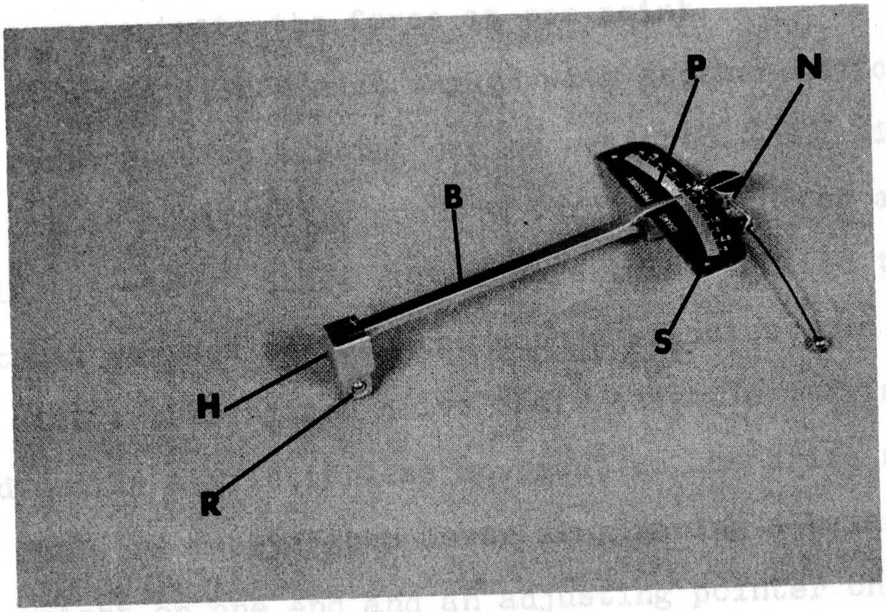


FIGURE 10

SIDE VIEW OF A TORQUE WRENCH WITH THE PARTS LABELLED

H--Head

R--Drive square

B--Beam

P--Pointer

S--Scale

N--Handle with cord attached

trated. This distance is always measured at 90° to the direction of the force. Torque wrench handles are designed to concentrate the force at one point.

A torque wrench must engage with another device so that resistance to turning can be measured. Engaging devices can be modified to adapt a torque wrench for a particular service. Since forces were to be applied to the teeth at various angles, the torque wrenches were coupled with a bearing and drive shaft assemble. This produced nearly a frictionless movement as the drive square rotated 360°. A twelve inch lever arm, having a balancing counter-weight on one end and an adjusting pointer on the other, was attached to the rotating drive square. The relationship of the pointer to the long axis of the tooth determined the angle at which the force was directed. The rotating lever arm was balanced to maintain any set pointer-to-tooth relationship. To insure that the applied force described a 90° angle with the lever as prescribed in the torque law, the following modifications were made:

1) A circular disc having an engraved line cross its center was attached to the handle beneath the scale (Figure 11).
2) To concentrate the pulling force to one point

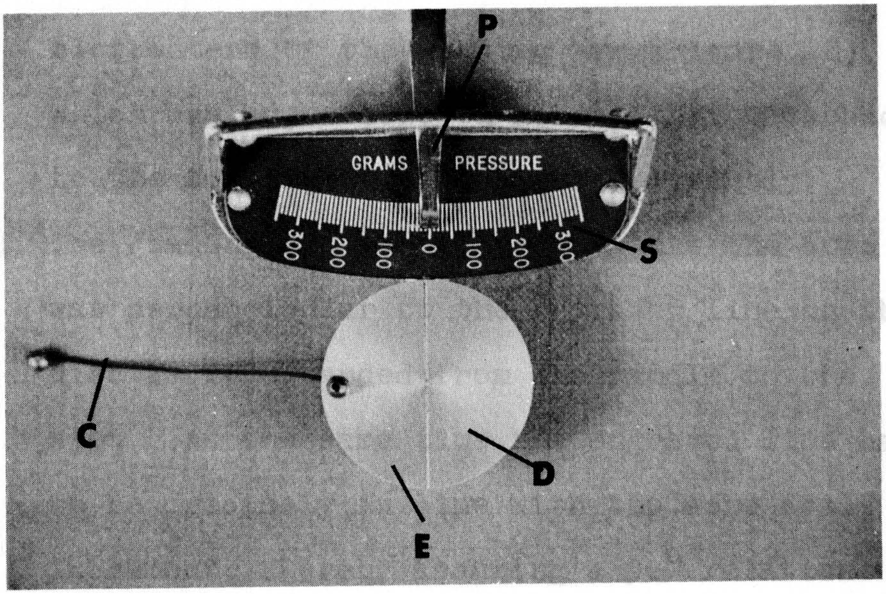


FIGURE 11

A TORQUE WRENCH SCALE AND ASSOCIATED PARTS

S--Scale

P--Pointer

D--Circular disc

E--Engraved line on circular disc

C--Cord

on the handle, a cord was attached to it behind the disc.

3) The cord extended from the handle to the slotted end of the loading arm (Figure 12), which was adjustable on a beam that attached to the bearing and drive shaft assembly.

4) The loading arm was placed so that the cord was perpendicular to the engraved line on the disc as it extended from the handle to the slot. At the same time the engraved line had to be precisely in line with the zero scale increment; thereby assuring a 90° relation of the force to the lever arm.

5) The amount of applied force was controlled by moving the slotted end of the loading arm toward or away from the handle by means of a threaded screw.

All torque wrenches were calibrated so that the maximum allowable inaccuracy would not exceed 2 per cent of the full scale reading. Forces ranging from 0.2-300 grams were applied to the teeth. To maintain the utmost degree of accuracy, it was necessary to use several torque wrenches. In so doing, the inherent accuracy of the torque wrenches was exploited, since the working range was

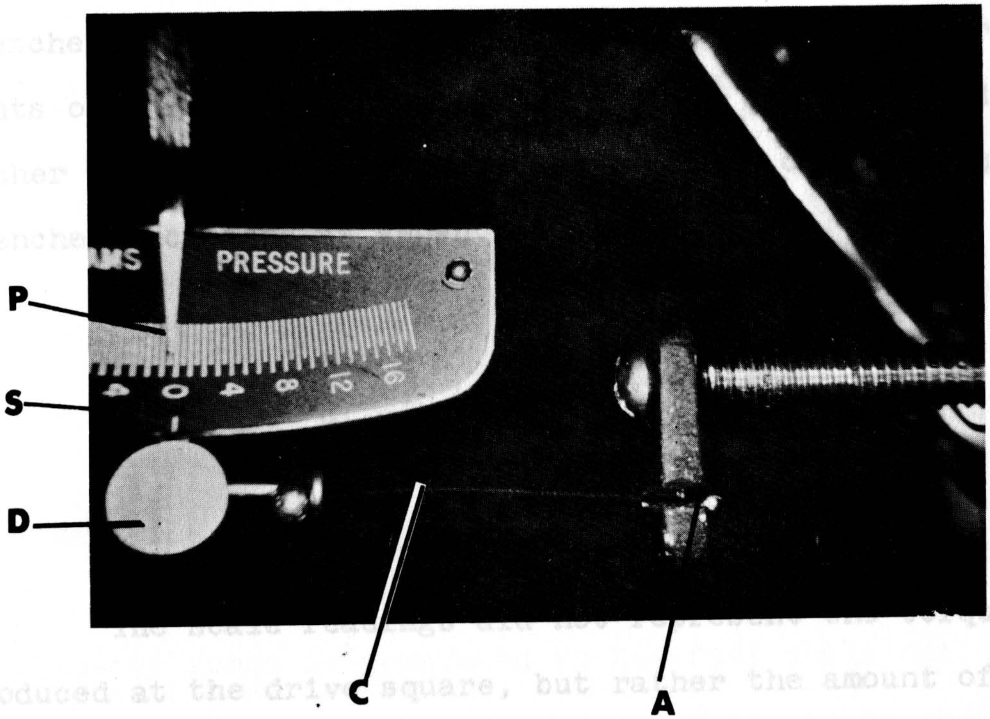


FIGURE 12

THE CORD EXTENDING FROM THE TORQUE WRENCH HANDLE TO THE SLOTTED END OF THE LOADING ARM

S--Scale

P--Pointer

D--Circular disc

C--Cord

A--Slotted end of the loading arm

limited to the mid-two quarters of the scale or between 20 to 80 per cent of deflection. The use of five torque wrenches also allowed for a better spacing of the increments on the scale so that true readings were obtained rather than estimates. The scale readings on the torque wrenches increases as shown below:

No. 1	0-3 gms.
No. 2	0-6 gms.
No. 3	0-16 gms.
No. 4	0-60 gms.
No. 5	0-300 gms.

The scale readings did not represent the torque produced at the drive square, but rather the amount of force applied directly to the teeth. This conversion to a direct force reading is explained by referring to the Torque Law, which as written previously, is $T = F \times D$.

In solving for F, the equation is rewritten as, $F = T/D$. In the instrument the torque force was produced at the drive square end and transmitted through the bearing and drive shaft assembly. The resulting torque force at the end of the assembly generated a new "compressive" force. This force was directed to the tooth by the pointer end of the lever arm. It varied directly with the length of the lever arm. For example: If a 40 in.

gram torque wrench is deflected to the full scale range, then at a distance of one inch from the axis of the drive shaft 40 grams of compressive force is generated. Algebraically, this can be written as:

$$T = F \times D$$

$$40 \text{ in. gm.} = F \times 1 \text{ in.}$$

$$F = \frac{40 \not{\text{in}}. \text{ gm.}}{1 \not{\text{in}}.}$$

$$F = 40 \text{ gm.}$$

If the point of force application is increased to a distance of twelve inches from the axis of the drive shaft, as was done in the instrument, only 3.33 grams of compressive force is generated at the full scale deflection of a 40 in. gram torque wrench. This can be shown algebraically as:

$$T = F \times D$$

$$40 \text{ in. gm.} = F \times 12 \text{ in.}$$

$$F = \frac{40 \not{\text{in}}. \text{ gm.}}{12 \not{\text{in}}.}$$

$$F = 3.33 \text{ gm.}$$

The increment lines on the scales of the torque wrenches were engraved so that the applied force could be read directly in units of grams when a twelve inch lever arm is used. This lever arm was kept throughout all the experiments.

The torque wrench together with the bearing extension and the loading and balancing arms comprises the torque wrench assembly (Figure 13). This assembly was suspended from a fixture (Figure 14) which also contributed to the versatility of the instrument by having readily adjustable parts. The fixture consists of a rigid base, a vertical post, and two movable arms. The base measures eighteen inches in width and twenty-eight inches in length. A twenty-two inch vertical post extends from the base. A freely movable horizontal extension arm, capable of being rotated 360° and raised or lowered as desired, is attached to the post. A vertical assembly holding arm is attached to the horizontal arm at its other end. The vertical arm rotated 360° in a direction perpendicular to the horizontal arm. The torque wrench assembly is suspended from this vertical arm by means of a ball and socket arrangement.

B. Recording System

The impulses initiated as a result of the applied forces to the teeth were recorded by a precision system. The system extended from the electrodes surrounding the nerve to the recording physiograph. The electrodes were connected directly to a shielded cable, whose jack led

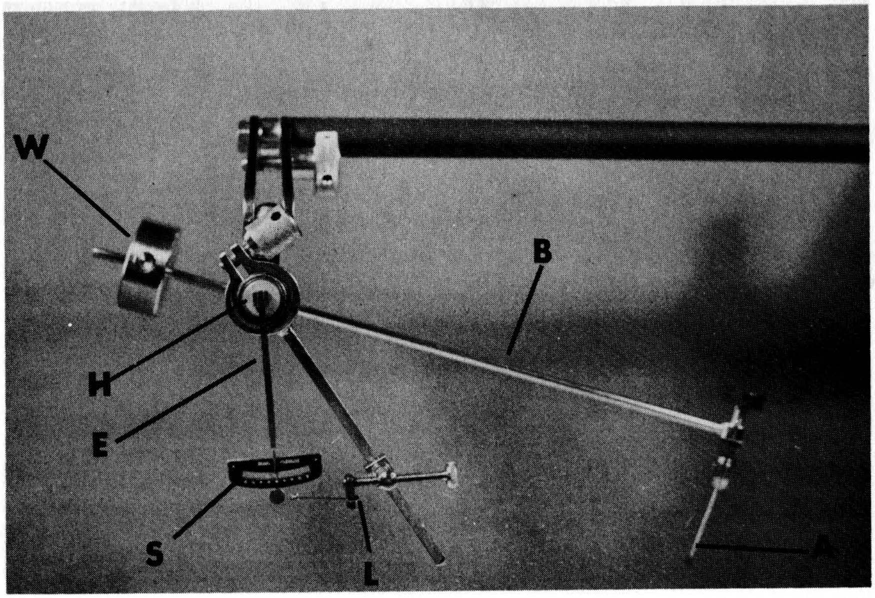


FIGURE 13

THE TORQUE WRENCH ASSEMBLY

B--Balancing arm

A--Adjustable pointer on the balancing arm

W--Counter-weight on balancing arm

H--Head of torque wrench

E--Beam

S--Scale

L--Loading arm

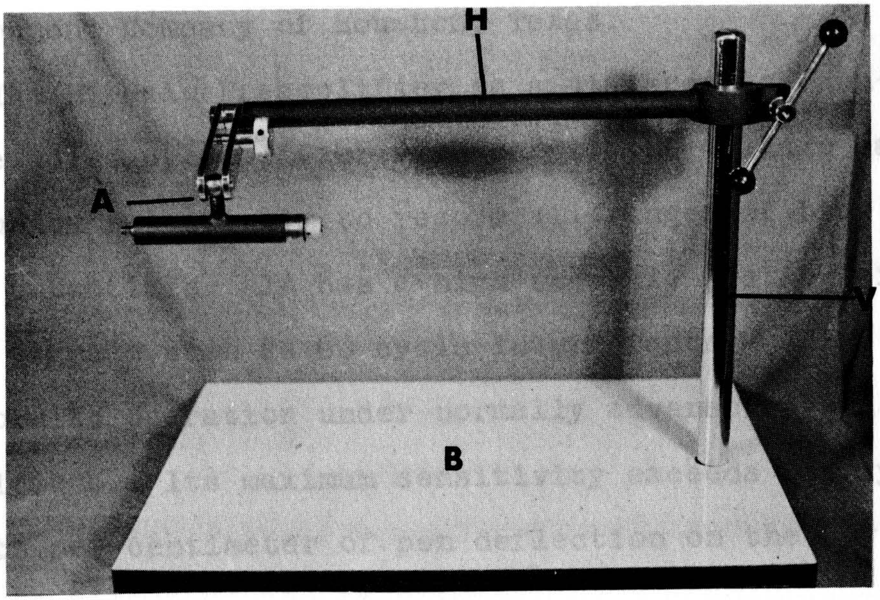


FIGURE 14

FIXTURE OF THE FORCE PRODUCING INSTRUMENT

B--Base of the fixture

V--Vertical post

H--Horizontal rotating arm

A--Vertical assembly holding arm

into the transducer input terminal of the Hi-Gain Preamplifier, Model No. 93-300-70, manufactured by the E & M Instrument Company of Houston, Texas.

The Hi-Gain Preamplifier is a differential condenser, coupled preamplifier with sufficient voltage gain and frequency selectivity to record all ranges of bioelectric potentials. It has a high rejection ratio for in-phase signals such as 60 cycle interference. This allows for its operation under normally adverse recording conditions. Its maximum sensitivity exceeds thirty microvolts per centimeter of pen deflection on the physiograph.

The preamplifier led into one of the four channel amplifiers of the physiograph, which was kept next to the shielded cage. The physiograph was manufactured by the E & M Instrument Co. It can be operated as a single channel recording device, or in conjunction with a large selection of accessories to provide simultaneous multichannel recordings of a variety of physiologic phenomenon.

A typical physiograph channel consists of a transducer, an amplifier, and a pen recorder.

The transducer converts almost any known physiological event into proportional electrical signals suitable for processing and recording by the physiograph system. The electrical signals are connected to the input of the physiograph amplifier. The output of this amplifier is connected to the pen recorder of the physiograph.

The pen recorder consists of a pen motor, an electrically driven stylus, an inking system, and a moving paper chart which is driven at a predetermined speed. The electrical impulses from the amplifier are received by the pen motor. This motor drives the recording pen. The excursions of the pen across the moving chart are proportional to the physiological activity, and provide a permanent record of the experiment.

The main frame assembly of the physiograph is a basic four channel housing designed for the installation of recording channels and accessory plug-in modules. It also provides power distribution, control, and monitoring circuits. Six compartments are provided for plug-in modules--four for amplifiers or accessories, two for the paper control unit, and an additional accessory plug-in module.

The paper control unit governs the recording chart speed in any one of twelve fixed gradations from 0.01 to 5 cm./sec. This unit also records time signals every 1, 5, 30, or 60 seconds. The pen recording the time signals is also an event marker. It deflects upward when activated by the panel mounted event marker push button switch, the stimulator accessory plug-in module, or by an external marker jack. All time and mark events were recorded on the fourth channel.

An impedance Pneumograph Model No. 93-800-70 was used for quantitative measurement of the animals respiratory rate. It was also built by the E & M Instrument Company. A pair of needle electrodes was placed in the sides of the animals' thorax to monitor all its respiratory movements. A small alternating current of approximately two microamperes is passed through the electrodes. The voltage across these electrodes is directly proportional to the subject impedance. Minute voltaic variations resulting from impedance changes are amplified and detected as either a direct coupled or a condenser coupled signal for recording on the physiograph. The third channel was used for the respiratory recordings.

Undesirable respiratory movements of the supra and infra hyoid muscles often caused deflections of the pen in the nerve recording channel. The use of the Impedance Pneumograph made it possible to correlate these movements with any interferences observed in the nerve recording channel.

III. Force Application

The mandibular canine tooth was tapped with forces of varying magnitude and direction. As the forces were applied to the canine tooth, permanent recordings were made of the resulting nerve trunk potentials. The sequence of stimulations for each experiment was as follows:

A. Single light taps were delivered intermittently to the canine tooth with a glass rod from three directions:

1) incisal
2) labial
3) lingual

B. One to eight light taps were delivered to the canine tooth with a glass rod in rapid succession. The taps were from three directions:

1) incisal
2) labial
3) lingual

Each tap or series of taps was followed by a rest period of one to three minutes.

C. Sustained forces were delivered to the canine tooth by means of the versatile force producing instrument. These forces were directed to specific surfaces of the tooth at measured angles for a period of time.

1) Single threshold forces from 0-3 grams were applied in gradations of 0.2 grams from these directions:

    a) incisal--along the long axis of the tooth

    b) labial

    c) lingual

    The time of each force application was 0-20 seconds.

2) Single forces ranging from 0.2 grams to 16 grams were applied to the canine tooth in gradations of two grams from these directions:

    a) incisal--along the long axis of the tooth

    b) labial

    c) lingual

    The time of each force application was 0-20 seconds.

3) Single forces ranging from 0.2 grams to 60 grams were applied to the canine tooth in gradations of 10 grams from the following directions:

a) incisal--along the long axis of the tooth

b) labial

c) lingual

The time of each force application was 0-20 seconds.

4) Single forces ranging from 0.2 grams to 300 grams were applied to the canine tooth in gradations of 40 grams from the following directions:

a) incisal--along the long axis of the tooth

b) labial

c) lingual

The time of each force application was from 0-20 seconds.

The procedures as outlined above were followed routinely for each experimental animal. In addition, other procedures were carried out to obtain certain required data. These were as follows:

1) Clipping off the crown of the canine tooth and recording the resulting acivity.

2) Applying sustained single forces to the canine tooth which ranged from 1 to 20

pounds (Avoirdupois weight). These forces were applied along the long axis of the tooth at intervals of 30 to 60 seconds. The time of force applications was from 0 to 60 seconds.

Histologic Investigation

All the animals were sacrificed after the physiologic experiment was completed. The soft tissues were dissected from the jaws. The jaws were then removed from the head (Figures 15 and 16) of the animal and placed in the fixing solution.

The fixative was a 10 per cent formalin solution. It penetrates the tissue rapidly and does not deteriorate. The jaws were kept in this fixative for four days during which time the volume of the fixative was maintained at twenty times the volume of the tissues. The specimens were then decalcified in a 10 per cent solution of nitric acid. The bone was assessed roentgenographically to ascertain when the decalcification was complete. Following decalcification the specimens were washed thoroughly in tap water and dehydrated in ascending concentrations of alcohol (50-70-95-100 per cent). Since paraffin was the embedding medium, the absolute alcohol was replaced by xylol prior to embedding. All of the specimens were serially sectioned with a rotary microtome. From each

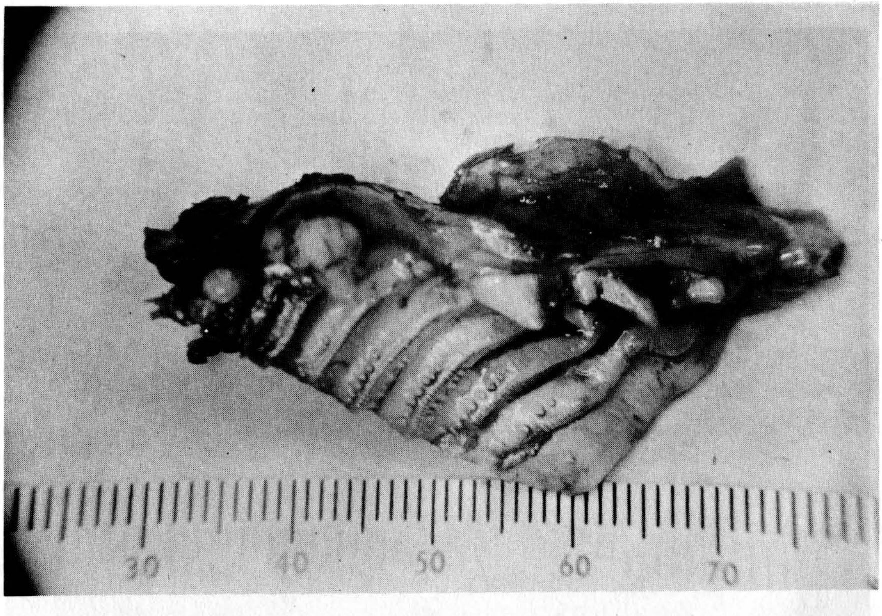


FIGURE 15

THE MAXILLA REMOVED FOR HISTOLOGIC STUDY

FIGURE 16

THE MANDIBLE REMOVED AND SECTIONED FOR HISTOLOGIC STUDY specimen one-half of the tissues were sectioned to a thickness of 20 microns; the other half to a thickness of 40 microns. Over 1,200 sections were obtained from the specimens. These were stained as follows:

1) Powers Modification of Romanes' Method of Impregnation with Silver Nitrate. With this method the neurofibrils appear to be a deep brown to black in contrast to the lighter staining of the surrounding non-nervous tissues.

2) The Hematoxylin eosin stain.

All the sections were examined under various magnifications using the Zeiss Standard Photomicroscope.

This instrument is equipped with an inclined binocular tube having two eyepieces of a 10x magnification. Four objectives were attached to a firmly mounted quadruple revolving nosepiece. They had the following magnifications: 4x; 10x; 40x; and 100x. Photomicrographs of the sections were obtained under the various magnifications by using Kodak Kodacolor high-speed thirty-five millimeter film.

CHAPTER IV

FINDINGS

Histologic Findings

The innervation of the periodontal ligament of the cat was studied histologically. Two sources of innervation were identified. One group of fibers entered the ligament from the apical portion of the alveolus. This group comprised the apical fibers. Other fibers entered the ligament through foramina in the alveolar bone. These are referred to as alveolar fibers.

The apical fibers were grouped into large neural bundles located centrally in the periodontal ligament (Figure 17). These fibers extend toward the gingiva parallel to the long axis of the tooth. The alveolar fibers diverged as they entered the ligament, some coursing gingivally and other apically (Figure 18).

The fibers were observed to vary in their size. The majority of the apical fibers were larger than the alveolar fibers. The apical fibers demonstrated little branching. They remained in the center of the ligament closely associated with the blood vessels. The alveolar fibers broke up into fine ramifications in all directions.

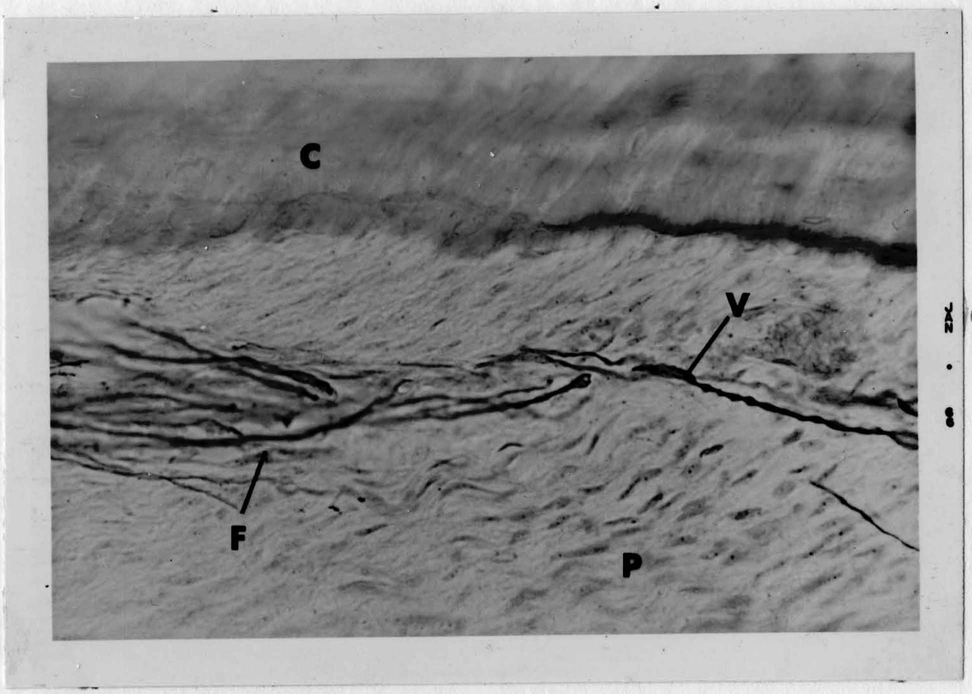


FIGURE 17

LARGE APICAL FIBERS CENTRALLY LOCATED IN THE PERIODONTAL LIGAMENT, AND CLOSELY ASSOCIATED WITH A BLOOD VESSEL

C--Cementum

P--Periodontal ligament

F--Apical fiber

V--Varicosity observed on an apical fiber

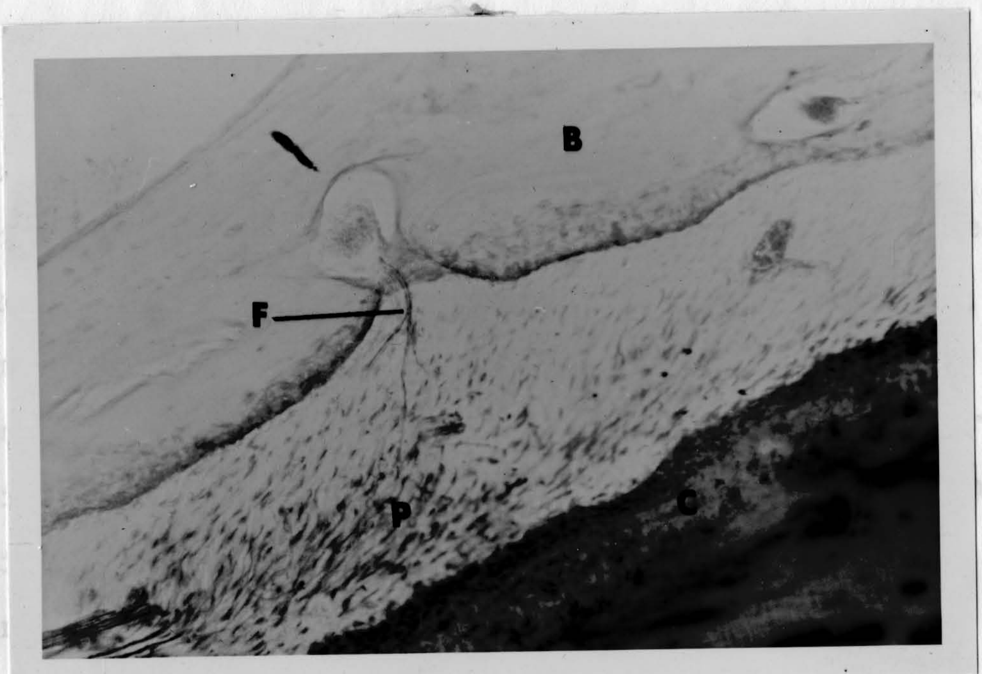


FIGURE 18

ALVEOLAR FIBERS AS THEY ENTER THE PERIODONTAL LIGAMENT AND DIVERGE APICALLY AND GINGIVALLY

B--Alveolar bone

F--Alveolar fiber

P--Periodontal ligament

C--Cementum

with a tendency toward the more medial part of the ligament.

Special emphasis was placed on the terminal portions of the fibers. Two types of nerve endings were identified in the periodontal ligament--highly organized terminations (Figures 19-21), and free nerve endings (Figure 22). The former appear as large ovoid structures consisting of myelinated and non-myelinated interweaving fibers. They were surrounded by a delicate connective tissue capsule. These highly organized nerve endings were observed in the apical one-third of the ligament. The larger size fibers were found to be associated with these nerve endings. In certain sections (Figure 23) the fibers can be seen as they enter the nerve ending.

The terminal portions of the small fibers comprised the free nerve endings. These were located throughout the ligament.

All of the fibers had a medium brown to dark brown appearance. The larger fibers presented numerous varicosities along their course. These were not found on the smaller fibers.

Physiologic Findings

The presence of two distinct types of nerve

KEY TO THE LABELS IN FIGURES 19-22

B--Alveolar bone

P--Periodontal ligament

C--Cementum

R--Receptor (encapsulated)

F--Free nerve ending

A--Apical fiber

V--Alveolar fiber

O--Blood vessel

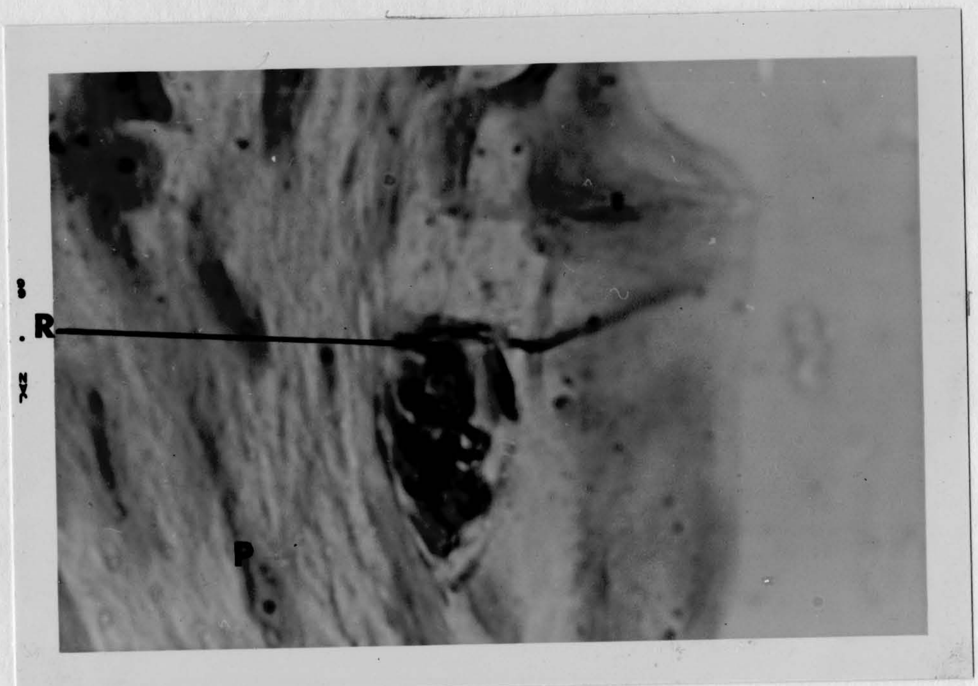
Magnification x100

Magnification x1000

FIGURE 19

PERIODONTAL RECEPTOR (Key to labels p. 60)

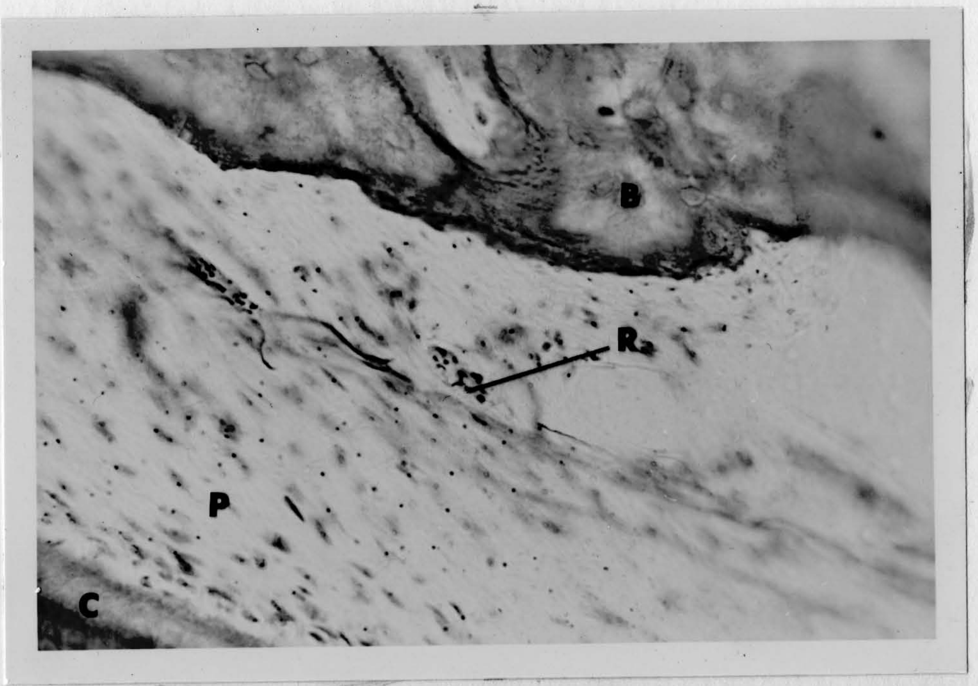
Magnification x100



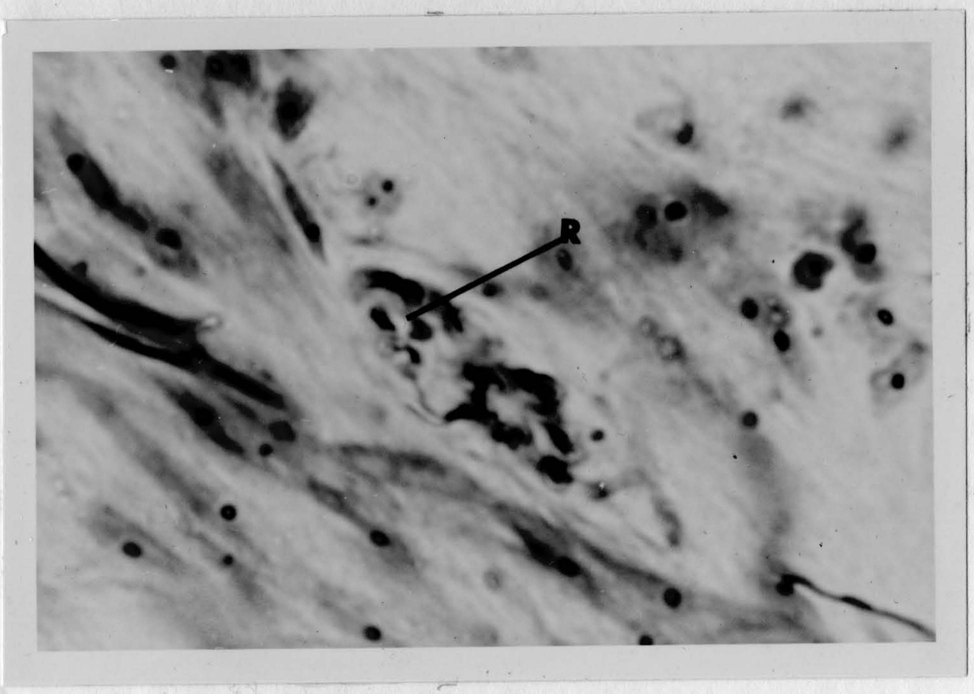
Magnification x1000

FIGURE 20

PERIODONTAL RECEPTOR (Key to labels p. 60)



Magnification x100



Magnification x1000

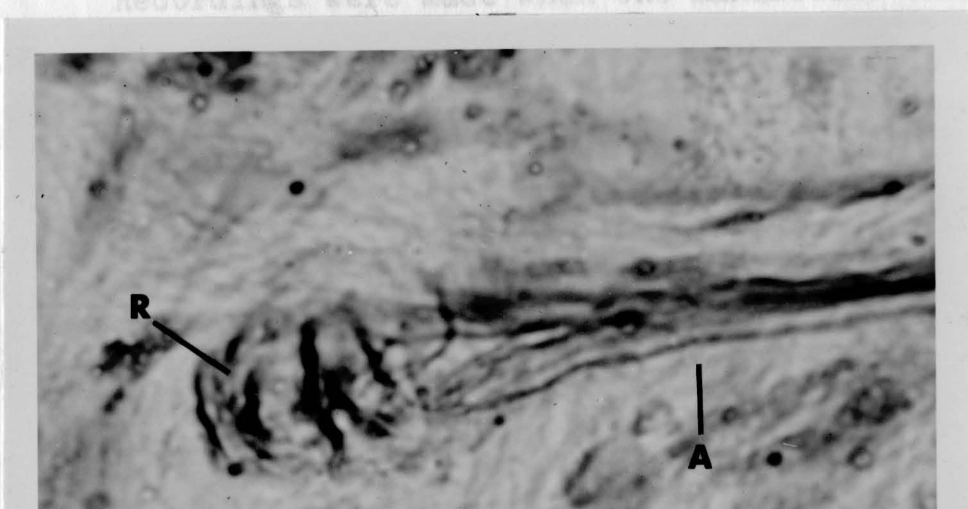
FIGURE 21

PERIODONTAL RECEPTOR (Key to labels p. 60)

FIGURE 22

FREE NERVE ENDINGS IN THE PERIODONTAL LIGAMENT (Key to labels p. 60)

Magnification x100



Magnification x1000

FIGURE 23

PERIODONTAL RECEPTOR AND ITS ASSOCIATED FIBERS. Fibers can be seen entering receptor (Key to labels p. 60) endings with a different distribution suggests that each type has a different function. Characteristic discharges are presumed to be initiated by each type of nerve ending. To substantiate this, the activity along the inferior alveolar nerve was recorded under different conditions.

Recordings were made when no overt stimuli were applied to the teeth (Figure 24). They showed spontaneous activity which was characterized by successive compound action potentials. These potentials did not demonstrate any definite pattern.

Recordings were made when the mandibular canine tooth was tapped with an insulated glass rod. A single diphasic compound action potential was recorded after each tap. This potential overshadowed the recorded background activity (Figure 25). The height of the recorded compound action potential varied with the velocity of the tap and the direction from which it was applied. It was increased as the velocity of the tap was increased. Taps of relatively equal velocity were delivered to the incisal, labial, and lingual surfaces of the mandibular canine tooth. The highest potentials were recorded following the incisal taps. When these taps were applied at intervals of 0.2 to 0.4 seconds, there was no observable change in the recorded action potentials (Figure 26).

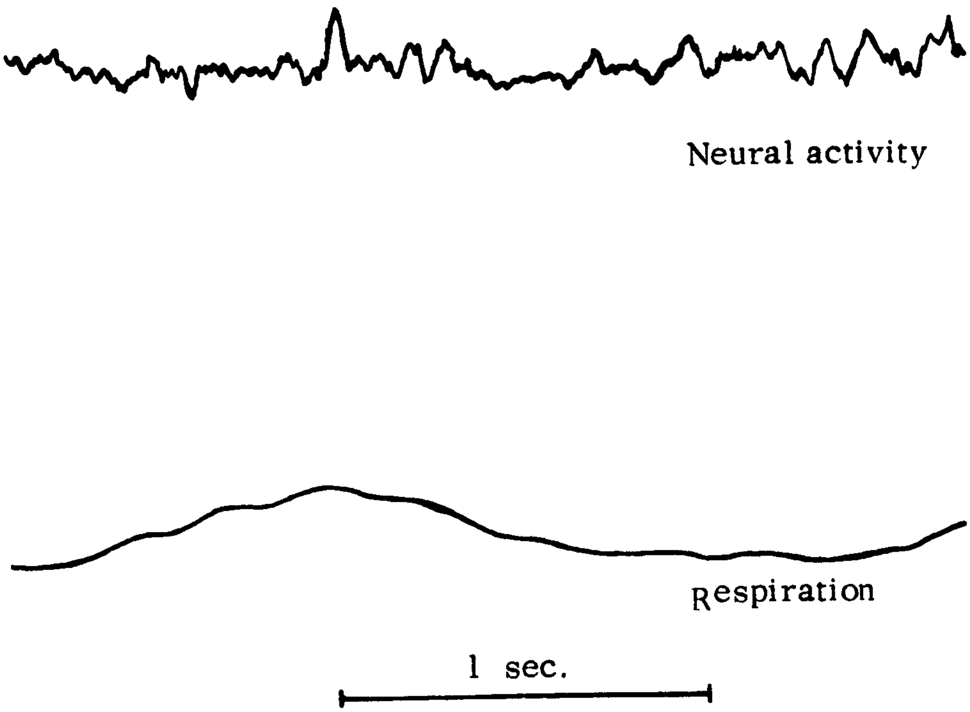


FIGURE 24

RECORDING FROM THE MANDIBULAR NERVE OF THE CAT SHOWING SPONTANEOUS ACTIVITY

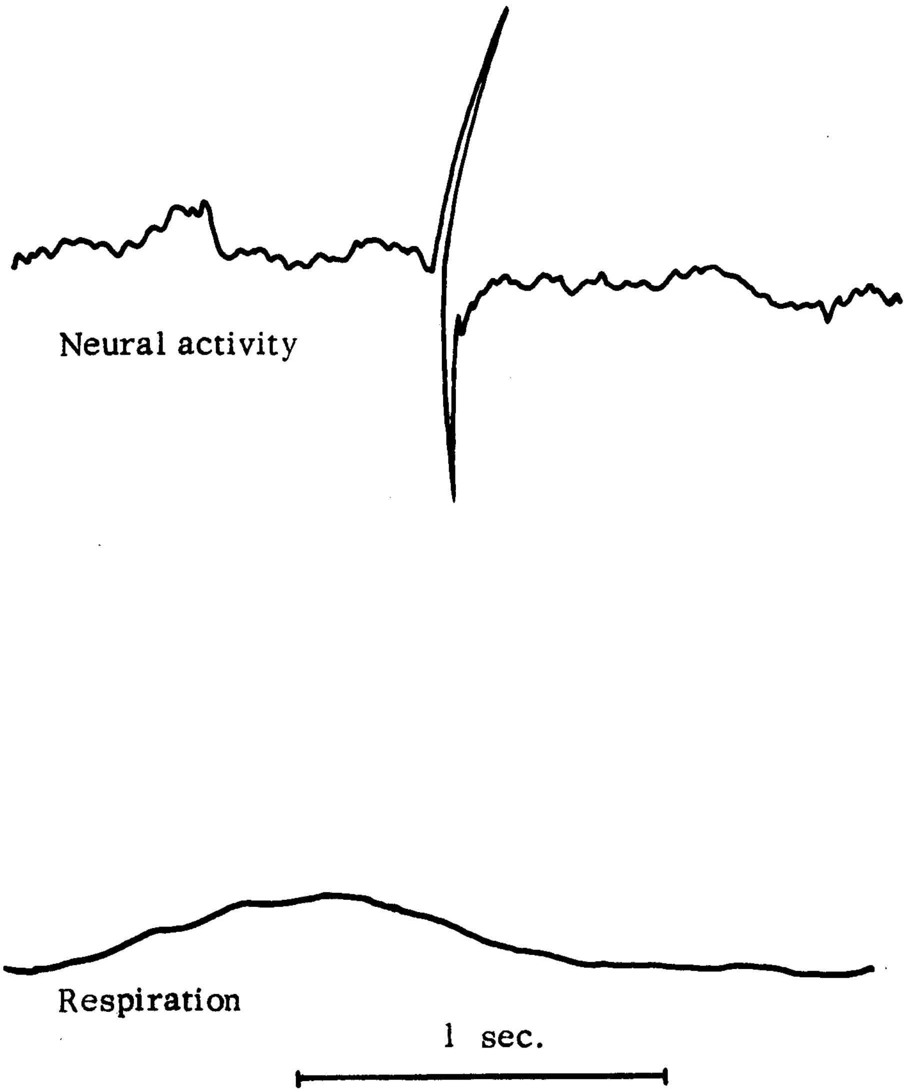


FIGURE 25

RECORDING OBTAINED FROM THE MANDIBULAR NERVE OF THE CAT FOLLOWING AN INCISAL TAP

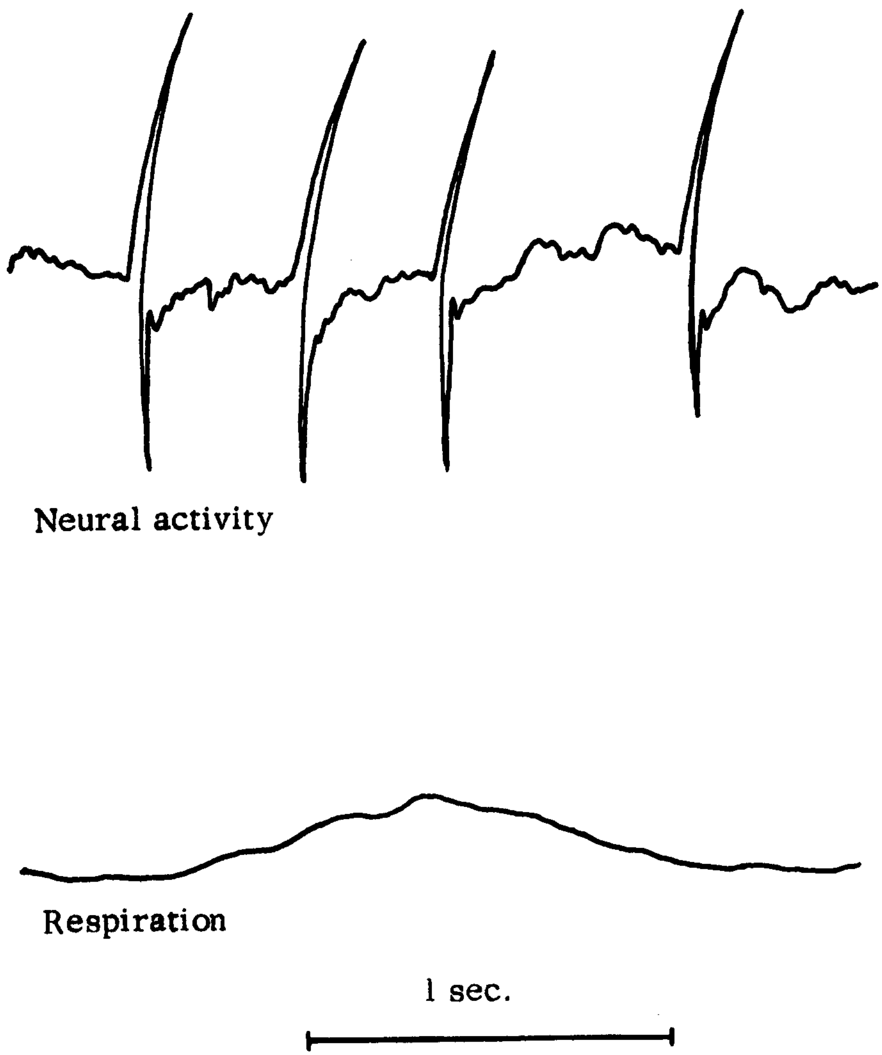


FIGURE 26

RECORDING OBTAINED FROM THE MANDIBULAR NERVE OF THE CAT FOLLOWING A SERIES OF INCISAL TAPS

Recordings were also obtained when sustained forces of known magnitude were applied to the tooth. The resulting patterns of activity were similar, each being characterized by initial high spikes followed by a steady asynchronous discharge of decreasing spike height until there was a return to the normal background level. This type of activity is referred to as adaptation. The duration of this activity varied with the different forces applied and is termed adaptation time. The shortest adaptation time measured was 0.14 seconds, while the longest recorded adaptation time was 2.3 seconds. These adaptation times are expressed as the percentage of the maximum time obtained for each animal.

Increases in the adaptation time were observed as the magnitude of the applied forces increased (Figures 27 and 28). When the light forces ranging from 4 to 20 grams were applied to the tooth, the adaptation time increased markedly per unit increment in the force (Figure 29). Figure 30 shows that 45 per cent of the maximum adaptation time recorded had occurred when the force application reached twenty grams. Thereafter the adaptation time continued to increase as forces ranging from 20 to 550 grams were applied, but the increase per unit force increment was notably less. Only a slight

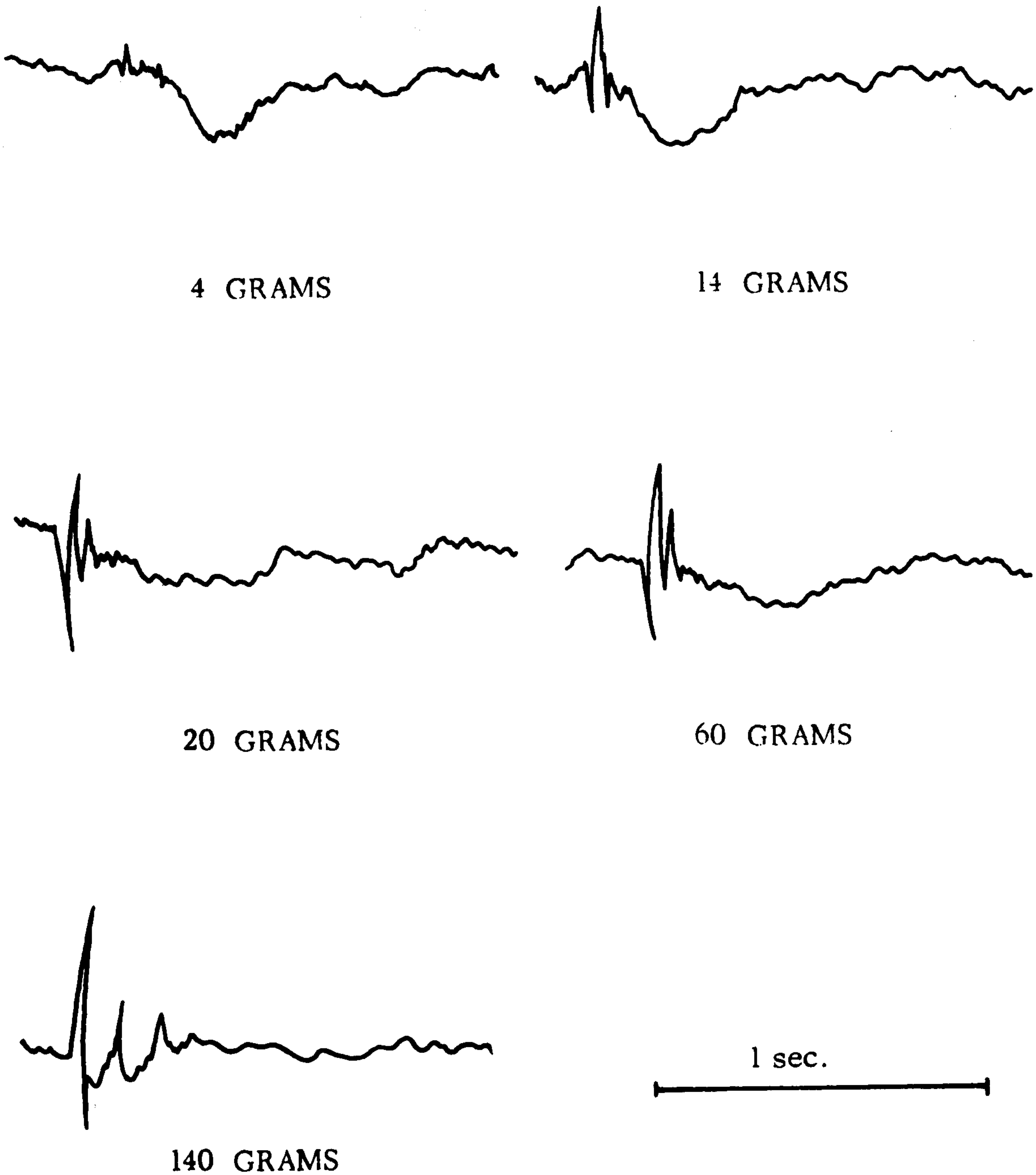


FIGURE 27

RECORDINGS FROM THE MANDIBULAR NERVE OF THE CAT SHOWING THE ADAPTATION TIME FOLLOWING DIFFERENT FORCE APPLICATION (CAT NO. 1001)

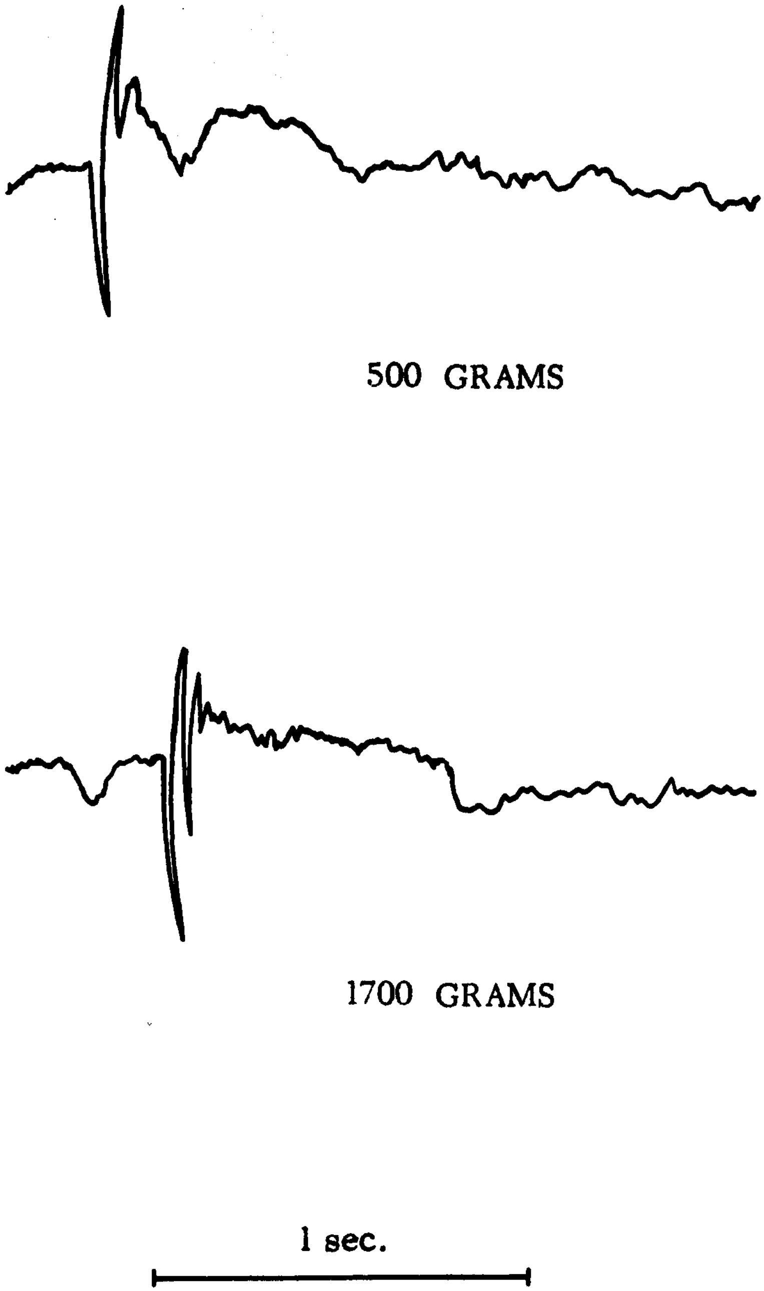


FIGURE 28

RECORDINGS FROM THE MANDIBULAR NERVE OF THE CAT SHOWING THE ADAPTATION TIME FOLLOWING DIFFERENT FORCE APPLICATION (CAT NO. 1001)

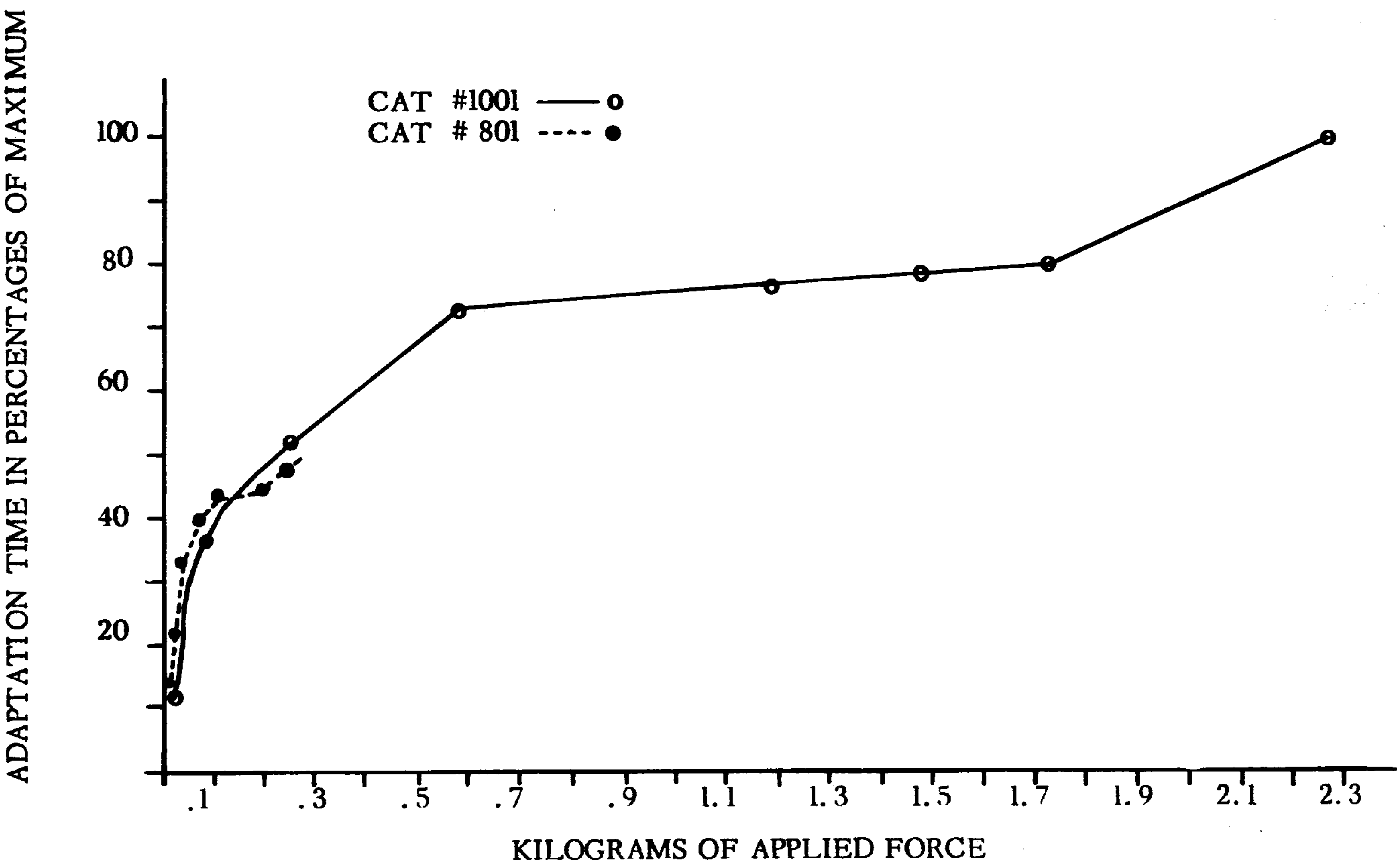

CAT #1001
CAT # 801
100
80
60
40
20
ADAPTATION TIME IN PERCENTAGES OF MAXIMUM
.1
.3
.5
.7
.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
KILOGRAMS OF APPLIED FORCE

FIGURE 29

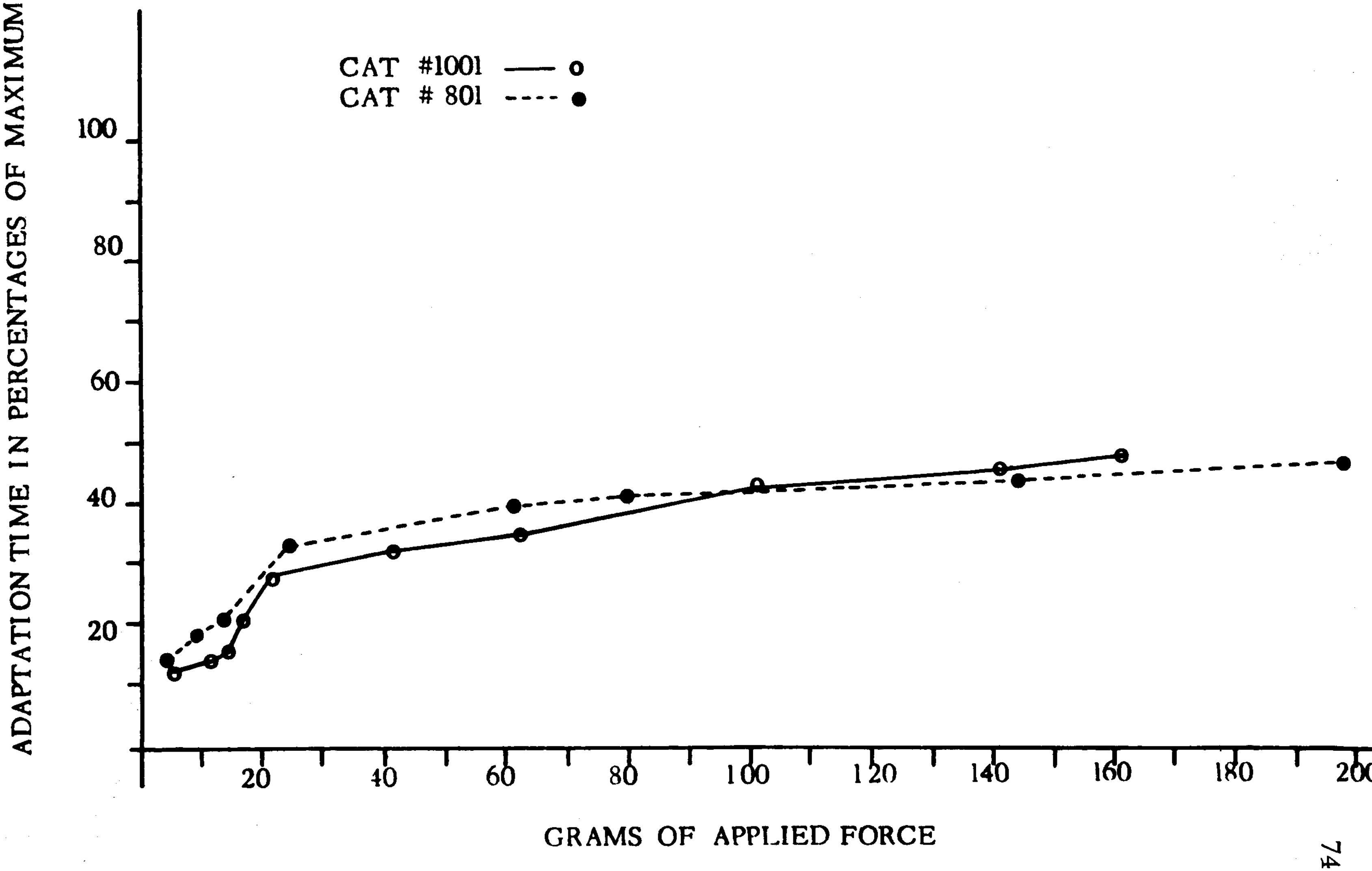

CAT #1001
CAT # 801
100
80
60
40
20
ADAPTATION TIME IN PERCENTAGES OF MAXIMUM
20
40
60
80
100
120
140
160
180
200
GRAMS OF APPLIED FORCE

FIGURE 30

increase in the adaptation time was observed as forces of a heavier magnitude (550 to 1,700 grams) were applied.

A marked increase in the adaptation time was observed again as forces above 1,700 grams were applied to the tooth. Of particular interest was the concurrent change in the respiratory recording with still heavier forces (Figure 31). A much greater inspiratory effort was recorded at this time. This was interpreted as an indication that pain was being experienced by the animal.

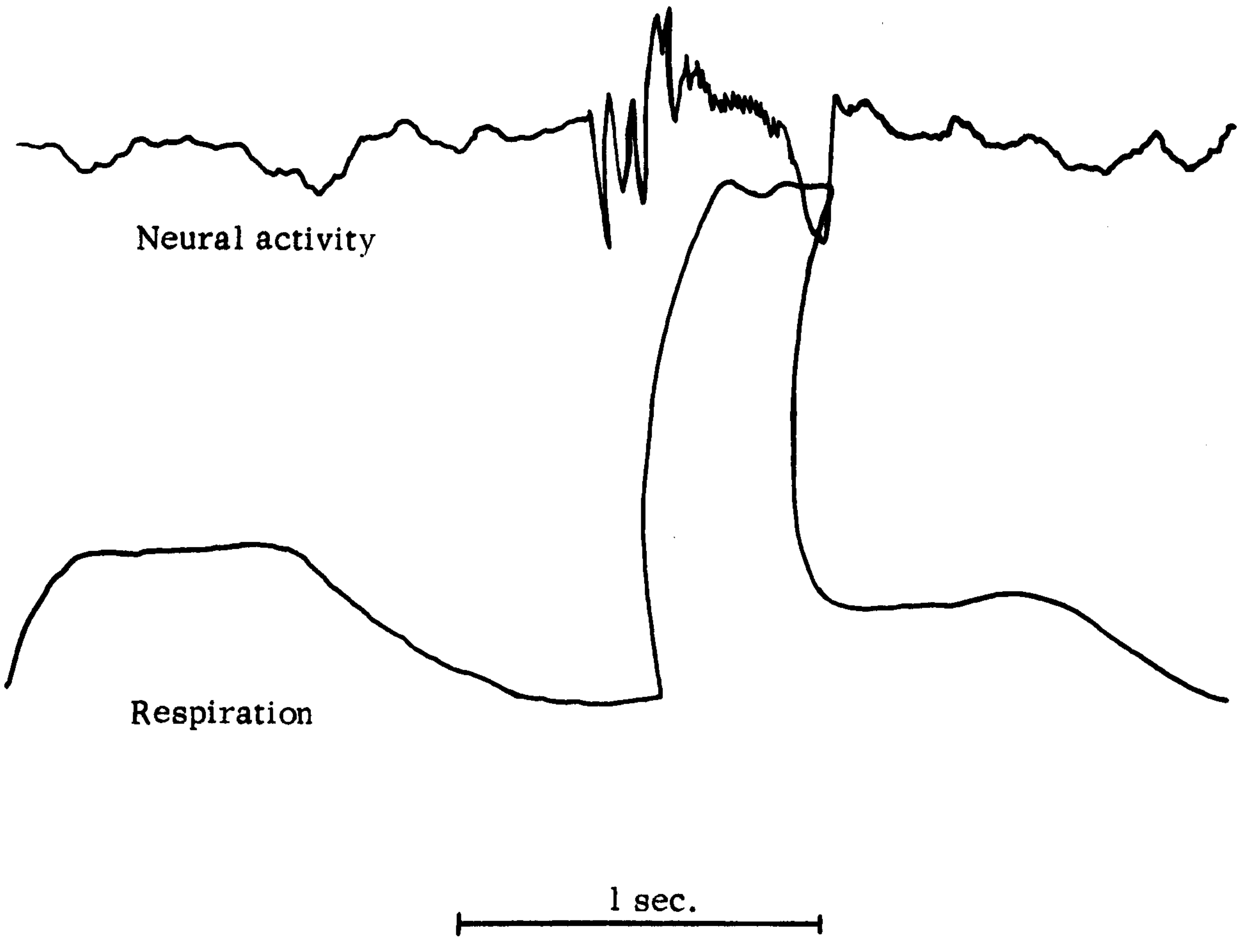


FIGURE 31

RECORDING FROM THE MANDIBULAR NERVE OF THE CAT FOLLOWING THE APPLICATION OF A 9,000 GRAM FORCE (CAT NO. 1001) Note the increase in respiratory amplitude.

CHAPTER V

DISCUSSION

The sensory receptors of the periodontal ligament were studied in this research. Two avenues of approach were used to gain information regarding these receptors; one was histologic, while the other was physiologic. The histologic aspect of this study served as a means of positively identifying these receptors and establishing their location in the periodontal ligament. The data obtained from the physiologic experiments contributed toward a better understanding of their function. The findings obtained from these two methods will be correlated in this discussion, so that a more meaningful relationship between the structure and the function of these receptors will be had.

Two types of receptors were identified in the periodontal ligament of the cat; one was the ovoid, encapsulated sensory end-organ, while the other was the free-nerve ending. The former appeared to be highly organized, consisting of both myelinated and non-myelinated interweaving fibers. The free-nerve endings were comprised of the terminal portions of the smaller fibers.

There are conflicting reports in the literature concerning the types of receptors in the periodontal ligament. Two types of receptors have been identified in the periodontal ligament by Avery (1959), Bernick (1957), and Lewinsky and Stewart (1936). This is in agreement with the findings in this research. Van der Sprenkel (1935) and Orban (1944) reported three types of receptors. A possible explanation for these differences in the types of sensory receptors in the periodontal ligament was offered by Dockrill (1954). He suggests that the swellings resulting when a nerve fiber is cut may be erroneously identified as knob-like endings. Varicosities observed along the course of the large fibers also can be incorrectly identified as receptors. To prevent this, thick sections of twenty and forty microns were prepared for this study. By studying the different depths of focus, the outline of the receptors can be followed and often even the continuity of the nerve fiber with the end-organ can be observed. This is shown in Figure 23. Finally, the apparent difficulty encountered in the staining of nervous elements can lead to varying interpretations of these structures. The Powers modification of Romanes technique for staining of nerve fibers in paraffin sections was employed in the preparation of the slides for this study. The consistent results obtained with this technique assured a more positive identification of the receptors.

A significant observation was made regarding the location of the receptors in the periodontal ligament. Free-nerve endings were found freely distributed throughout the ligament. The ovoid, encapsulated receptors were observed in the apical one-third of the ligament. The significance of this observation from a functional point of view will be discussed later.

In an attempt to learn more about the function of the two types of periodontal receptors, records of electrical potentials were obtained from the inferior alveolar nerve trunk when various stimuli were applied to the mandibular canine tooth. The interpretation of these records is prefaced by a discussion of some related factors. The inferior alveolar nerve is comprised of many sensory fibers. Some of them are associated with periodontal receptors. Others are associated with receptors located in the skin of the chin, gingiva, mucous membrane, dental pulp, and other related structures. Various stimuli such as thermal changes, tongue movements, and salivation can activate these receptors. This results in a series of impulses being propagated along the alveolar nerve. Discharges carried by the visceral efferent fibers supplying the blood vessels in the area also contribute to this activity. Collectively this activity is termed "spontaneous" or "background" activity. It must be distinguished from activity when pressure is applied to the tooth. To facilitate this, the recording electrodes were placed around the inferior alveolar nerve just ahead of the lingual nerve. This limited the receptive field somewhat, since impulses carried by the lingual nerve were not recorded. Nevertheless, spontaneous activity initiated by the remaining receptors was still observed. A typical illustration of this background activity is seen in Figure 24.

Spontaneous discharges were also observed by Ness (1954) when no stresses were applied to either the ipsilateral incisor or to the mandibular symphysis. These discharges were obtained from a few randomly dissected fibers of the incisor nerve. Ness attributed these discharges to one type of periodontal receptor. This conclusion appears to be rather speculative since there is no positive evidence that the discharges were carried by fibers associated with periodontal receptors. Adding to the speculation of this conclusion, is the fact that these discharges could be modified by applying pressure to either the symphysis, ipsilateral incisor, or the inter-incisal papilla. On the basis of this it is more logical to assume that a number of receptors responding to a variety of non-specific stimuli contributed to the spontaneous discharge, rather than a specific type of periodontal receptor.

Changes in potential were recorded from the inferior alveolar nerve when different forces were applied to the tooth. Different compound action potential were induced as these forces were applied. To give this discussion continuity, the potentials obtained when the mandibular canine was tapped will be considered first. This will be followed by a discussion of potentials obtained when sustained forces were applied to the tooth.

A single diphasic compound action potential was recorded when the tooth was tapped. This action potential masked or overshadowed the background activity. This indicated that the tapping of the tooth activated a new group of receptors which were not discharging during the background activity. The evoked potentials resulting from the tapping of the tooth can be explained as follows. The moment the glass rod contacted the tooth, the threshold level of a certain number of pressure sensitive receptors in the periodontal ligament was reached. They responded by discharging at the same time in phase. These discharges were propagated along the inferior alveolar nerve. When they reached the electrode, they were recorded as a diphasic compound action potential which was greater than that of the background activity. The background activity returned after this potential was illicited.

Tapping the tooth at intervals of .2 and .4 seconds resulted in a series of diphasic compound action potentials which varied directly with the increase in the intensity of the taps. From this it is evident that the sensory receptors are capable of signalling differences in stimuli. The basis for the relationship between the properties of the stimulus and the frequency of discharge by the receptor can be explained by referring to the properties of the individual receptors.

Sensory end-organs are excitable tissues. It is widely held that the cause of impulse initiation in receptors is the result of a change in their electrical potential. This change is confined to the receptor and is graded according to certain characteristics of the stimulus. Before the receptor can initiate an action potential, the stimulus must reach an intensity sufficient to lower its membrane potential to a certain critical level. When this occurs, an action potential is initiated and propagated along the associated afferent fiber. Various investigators have recorded these potential changes in mechanically activated receptors. The ovoid receptors identified in the periodontal ligament resemble other mechanically activated receptors. They are surrounded by a connective tissue sheath and show interweaving fibers. It may therefore be reasoned that these receptors are also activated when they are deformed by forces applied to a tooth. Gray and Sato (1953) have reported that changes in the receptor potential increase with the degree of deformation of the receptor. This increase continues to a certain point beyond the threshold level, and then tapers off to a constant value. Katz (1950) has also shown that the frequency of discharge by such a receptor is linearly related to the magnitude of the receptor potential change. Consequently, a greater deformation of the receptor will result in an increased frequency of discharge along the afferent fiber. These observations correlate well with the findings obtained in this study.

When the tooth was tapped, some receptors in the periodontal ligament were activated to the degree of initiating a discharge. That is, their receptor potential changed to the point where threshold levels were reached. This level is not the same for all receptors. Landgren (1952) has shown, for example, that pressure sensitive units in the carotid sinus do not initiate discharges at the same time. They exhibit differential sensitivity. The individual receptors initiated discharges at different levels of pressure. Each receptor was specific for a certain pressure or stimulus value. All the receptors responded to the same energy (pressure). However, they did so at different threshold levels. Periodontal receptors also exhibit differential sensitivity. If this were not so then all the receptors would respond to every applied stimulus regardless of its intensity. This is not true, since different action potentials were recorded as the strength of the stimulus varied.

Since periodontal receptors exhibit differential rates of sensitivity, it can be reasoned that the lighter taps will initiate threshold level potentials in a few receptors, while heavier taps will increase the number of receptors reaching threshold level. This was observed during the experiments when taps were delivered to the canine tooth from the incisal direction. When the taps were varied in intensity from light to heavy, then correspondingly lower and higher action potentials were recorded. This observation is important from a morphological point of view. It supports the fact that although these ovoid, encapsulated receptors showed structural similarity, they differed in their sensitivity to the same type of stimulus; namely pressure.

Action potentials of different magnitudes were observed when the tooth was tapped with forces of similar magnitudes from different directions. This indicates that the differential sensitivity is not only dependent on the strength of the stumulus, but also on the direction from which it was delivered. During the experiments it was observed that the evoked potentials following incisal taps were of a greater magnitude than those from the labial or lingual direction. This demonstrates the directional sensitivity of the periodontal receptors. This variable sensitivity to changes in the direction of the stimulus can be explained on the basis of receptor location. The ovoid, encapsulated receptors were observed around the tooth in the apical one-third of the ligament. Consequently forces directed along the long axis of the tooth activate more receptors to threshold level than those from the labial or lingual direction.

Jerge (1963) gives further credance to this reasoning by reporting that the majority of the receptors respond to some degree when pressure is applied to the tooth regardless of the direction from which it is applied to the tooth regardless of the direction from which it is applied. Because of their location, however, the threshold level is not attained in all receptors. He further reports that some periodontal receptors discharge only when the pressure is applied up to approximately 90^{o} from the direction of maximum threshold. This is evidence that directional sensitivity is directly related to the location of the receptor, rather than to any particular receptor property. Based on this reasoning, one may expect that if the same receptor could be repositioned in the ligament, its particular directionality would be changed.

When sustained forces of known magnitudes were applied to the mandibular canine tooth, a series of compound action potentials was recorded following each application. The pattern of these action potentials was quite similar, each being characterized by initial high compound action potentials followed by a steady asynchronous discharge of progressively decreasing action potential amplitudes. This progressive decrease in the electrical activity is termed as adaptation, and its duration is expressed as adaptation time.

Different adaptation times were obtained when sustained forces of a known value were applied to the tooth. The pressure sensitive receptors in the periodontal ligament experienced a change in their potential the moment that the force was applied. When this change reached the threshold level, a volley of discharges was initiated. This volley is represented by the first compound action potential. The succeeding potentials varied in size, all being smaller than the first. This can be interpreted as follows. The moment the sustained force was applied, different magnitudes of potential change occurred in the various receptors. In some, the potential changes were well above threshold level. In others they were equal to or slightly above this level. In these the amount of discharge was less as was the period of time required for them to adapt. In receptors whose potential change was well above threshold level, the amount of discharge was greater and the adaptation time longer. Consequently, these receptors were not discharging in phase; but asychronously since some of the receptors are becoming adapted, while others are still adapting and may be discharging at a high rate. Eventually all the receptors adapt and the activity is reduced to an almost constant level. This is observed in the potentials returning to the base activity.

The data illustrates that there has been a progressive increase in the adaptation time as the magnitude of the sustained forces increased. However, this increase did not occur proportionately (Figure 29). This figure shows that there is a marked increase in the adaptation time as the light forces ranging from 4 to 20 grams were applied. Further demonstration of this is seen in Figure 30, which shows that 45 per cent of the maximum adaptation time occurred by the time the force application reached 20 grams. Thereafter, the increase in adaptation time per unit force increment decreased as forces from 20 to 550 grams were applied. Only slight increases in the adaptation time were observed when forces ranging from 550 to 1,700 grams were delivered to the tooth. These differences in the adaptation time are due to the amount of displacement the individual receptors undergo. This varies with the strength of the stimulus and the location of the receptor in the ligament. Since all the sustained forces were delivered from the incisal direction, directional sensitivity did not modify these findings. The strength of the stimulus, however, did influence the observed adaptation times.

Similar increases in the adaptation time can be associated with a certain range of force application. When forces ranging from 4 to 20 grams were applied to the tooth, marked increases were observed in the adaptation time per force increment. This suggests that only a small increase in the applied force is required to activate additional receptors to threshold level, and to change further the potential of those already activated. Gray and Sato (1953) have reported that certain mechanoreceptors reach threshold level after a displacement of a few tenths of a micron. Their findings invite a more detailed speculation about periodontal receptors based on the observations made in this research. Since it takes so little deformation to activate mechanoreceptors, it could be suspected that slight increases in force application activate a sizeable number of receptors. After a certain force magnitude, very few receptors remain that have not reached threshold level. Thereafter, as the force magnitude continues to increase the receptors experience only greater deformation. This results in receptor potential changes of a greater magnitude, and consequently longer periods of adaptation. However, the increases in the adaptation time are not as pronounced. This speculation is substantiated in this research. The marked increases in the adaptation time observed when the lighter forces were applied demonstrates that more receptors were activated with each force increment.

The increase in adaptation time was reduced when forces ranging from 20 to 550 grams were applied. This can be explained by referring back to the histologic findings. Only two types of receptors were identified in the periodontal ligament. Since free-nerve endings are associated with pain, the ovoid, encapsulated receptors must be sensitive to these lighter force applications. However, only a small number of these receptors was identified in the periodontal ligament. By the time the force magnitudes reached this range, most, if not all, of the receptors were already activated to the threshold level. Therefore, the number of remaining receptors activated by forces in this range is minimal. Changes in the potential of these receptors probably accounts for the increase in the adaptation time observed with these forces.

As the applied forces were increased to magnitudes ranging from 550 grams to 1,700 grams, only slight increases in the adaptation time were noted. These are represented by the slow rising plateau seen in Figure 29. At these force ranges the threshold level was probably reached in all responding receptors at the instant the force was delivered. It can be speculated that the maximum receptor potential changes also occurred in the majority of the responding receptors when forces were applied in this range. The receptors are probably discharging at near peak level. As the forces are increased in this range, only slight increases were observed in the adaptation time. These are probably due to minor increases in the potential of those receptors where this was possible. The minimal increase in adaptation indicates that these were few in number.

An appreciable increase in the adaptation time was again noted when force magnitudes greater than 1,700 grams were applied. The plateau of relatively little increase in adaptation time has now ended. The recordings obtained with high force magnitudes present a different pattern of activity than those associated with the lighter forces. The initial compound action potentials maintain their height for a longer period of time before a decrease is observed, and the decreasing discharges continue for longer periods of time. This suggests that another type of receptor started to discharge, and its associated fiber activity was being recorded. Since there were only two types of receptors in the periodontal ligament, and one of these, the encapsulated nerve ending, has been identified in behavior with the lighter forces; the other type of receptor, the free-nerve ending, must be associated with this activity.

Gasser (1943) has not only classified fibers in sensory nerves, but he has also correlated their physiological and anatomical properties. He has associated small, unmyelinated fibers with sensations of pain. These types of fibers were observed throughout the periodontal ligament. Their terminal portions comprise the free-nerve endings seen in the histologic sections. Anatomists and histologists alike have identified free-nerve endings in the cornea of the eye. Lewis (1942) and others have shown that only the sensation of pain can be evoked from the cornea. On the basis of this, it is logical to assign the function of pain sensitivity to the periodontal free-nerve endings. Substantiating this is the fact that forces above 1,700 grams are considered painful dental stimuli. Therefore, it is not surprising that activity is initiated in these endings at this time. The discharges following the application of these forces were prolonged as compared to the non-painful stimuli. The adaptation time was observed to be considerably longer. These observations were confirmed by Adrian (1928) in his research dealing with pain.

Supporting evidence that free-nerve endings are activated by stimuli of higher magnitudes is shown in Figure 31. This figure represents the potentials obtained when a force of nine kilograms was applied to the tooth. This magnitude of force applied to a tooth will cause pain. As expected, a marked increase in the adaptation time was observed when this force was applied. Also, while this force was being applied to the tooth, there was a marked change in the recorded respiration. The inspiratory cycle was longer during this heavy force application. This was interpreted as an indication that pain is being experienced by the animal. Ranson and Billingsley (1916) have reported that one of the several reflexes associated with pain is altered breathing. P. Bard in the textbook, Medical Physiology, also mentioned the fact that an alteration in the respiratory activity is observed when painful experiences are encountered. This change in the respiratory record was taken as conclusive evidence that pain receptors were activated. It is further reasoned that some of these pain receptors were probably activated when forces of 1,700 grams were applied to the tooth. The observable increase in the adaptation time substantiates this, even though there was no apparent change in the respiratory activity at this force level. Painful stimuli of a greater intensity are necessary to produce an alteration of the respiratory cycle.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This research was designed to obtain information about the structure and function of the sensory receptors in the periodontal ligament of the cat. Two approaches were employed in obtaining this data; one was histologic, the other physiologic.

Histologic sections of the periodontal ligament were prepared in thicknesses of twenty and forty microns. The Powers modification of Romanes method for staining nerve fibers in paraffin sections was used. Consistent results were obtained with this method.

Two distinct types of receptors were identified in the histologic sections. One type appeared as ovoid, encapsulated structures, and they were observed in the apical one-third of the ligament. The other type were free-nerve endings. These were observed throughout the ligament.

A series of physiologic experiments involving the application of various forces to the mandibular canine tooth were conducted to ascertain the function of each type of receptor. Taps were delivered to the tooth with an insulated glass rod. Sustained forces were applied by means of a specially built force producing instrument. This instrument allowed the operator to control both the magnitude and direction of the applied forces.

The inferior alveolar nerve was exposed before it entered the manidbular canal. Pure silver wire electrodes were placed around this nerve. The neural activity initiated by the force applications was recorded on one of four channels of an appropriate paper recorder (the physiograph). The respiratory rate was recorded simultaneously over another channel by means of an impedance pneumograph.

Graded, sustained forces from 0 to 9,000 grams were applied to the tooth during the experiments. The activity recorded during the application of these forces was evaluated in terms of the observed adaptation times, since this property can be attributed to the receptors. The adaptation times were expressed as percentages of the maximum adaptation time recorded for any one animal. Marked increases in the adaptation time were observed as the force application was increased from 4 to 20 grams. Relatively small increases in the adaptation time occurred per increment of force application from 20 to 1,700 grams. When the magnitude of the applied force surpassed

1,700 grams, the adaptation time began to increase noticeably, and significant changes were observed in the recorded pattern of nerve activity.

Physiologists have established that free-nerve endings are associated with painful sensations. It is reasoned that the forces above 1,700 grams activated the free-nerve endings and their associated fibers. This is substantiated by the changes that occurred in the respiratory recording when the force was increased to nine kilograms. The marked inspiratory effort recorded at that time indicated that the animal was experiencing a painful sensation. Therefore, it is concluded that the pain threshold was reached with the application of 1,700 grams sustained force.

Since free-nerve endings are associated with the heavier forces, the activity recorded with the lighter forces must be initiated by the other type of receptors, the ovoid, encapsulated terminations. These appeared to be sensitive to one form of energy, pressure. The observed increases in the adaptation times indicate that the individual receptors probably have different threshold levels. This threshold level is also influenced by the location of the receptor in the ligament. This was demonstrated by the differences in the potential amplitudes when the direction of the stimulus was varied. Forces directed along the long axis of the tooth evoked the highest potentials, indicating that the greatest number of receptors were probably activated at this time. This can be explained structurally by the fact that the ovoid, encapsulated receptors were observed in the apical one-third of the ligament, and consequently would be subject to greater distortion by incisally directed forces than by forces from other directions.

On this basis it can be concluded that there are two types of functional receptors in the periodontal ligament: (1) the ovoid, encapsulated receptors having low thresholds for force application, and (2) the free-nerve endings having thresholds which are reached only when the applied force exceeds 1,700 grams. These are associated with painful sensations.

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APPROVAL SHEET

The thesis submitted by Dr. J. E. Kizior 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.

5/20/66
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