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A Study of the Apposition of Alveolar Bone Seen in Tooth Extrusion Using a Vital Staining Technique in Monkeys

John S. Theodorou
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A STUDY OF THE APPPOSITION OF ALVEOLAR BONE
SEEN IN TOOTH EXTRUSION USING A VITAL
STAINING TECHNIQUE IN MONKEYS

BY

JOHN S. THEODOROU

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

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BIOGRAPHY

John S. Theodorou was born in Oak Park, Illinois on May 8, 1927.

He was graduated from Oak Park - River Forest Township High School in Oak Park, Illinois in 1944. He entered Loyola University, Chicago, Illinois in June, 1944 as a pre-dental student.

He entered the United States Army during World War II in July, 1945 and served as a medical technician in Korea and the China-Burma-India Theatres.

Upon completion of active duty he returned to Loyola University to complete his pre-dental studies. He entered Loyola University School of Dentistry and was graduated with the degree of Doctor of Dental Surgery in June, 1951.

Dr. Theodorou practiced general dentistry in the Chicago area from August, 1951 to June, 1962.

In 1962 he was selected by the State Department, United States Information Agency, and participated as a foreign service specialist staff officer in the "Medicine - U.S.A." Cultural Exchange Exhibition to the U.S.S.R.

He began graduate studies in the Department of Oral Biology at

Loyola University, Chicago, Illinois in July, 1965.

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CHAPTER I

INTRODUCTION AND STATEMENT OF THE PROBLEM

A. Introductory Remarks:

The influence of forces on teeth and their supporting structures has been the object of numerous biophysical studies. Information still lacking is the relationship of alveolar bone apposition to varying force magnitudes. This study portends to explore this relationship when forces of varying magnitudes are applied to teeth for purposes of extruding them.

Apposition of alveolar bone during application of varying force magnitudes will be assessed by means of vital staining. Lead acetate vital staining has been selected because it is far more sensitive to changes in calcification tide than other types of vital stains. The influence of varying force magnitudes on calcification tide is the primary object of this research.

B. Statement of the Problem:

The purpose of this study is to observe the incremental rate of bone deposition as related to different force magnitudes when teeth are

being extruded. Lead acetate will be used as a vital stain.

CHAPTER II

REVIEW OF THE LITERATURE

PART A

Prior to the introduction of histologic, radiographic and experimental data, two conflicting theories were proposed to explain tissue changes coincident with tooth movements. Flourens (1847) advanced the theory that resorption of bone on the side of pressure and apposition on the side of tension occurred when teeth were moved in the alveolar process. Kingsley (1877) observed that tooth movement was possible because bone was elastic, compressible, and possessed the property of extensibility.

Black (1887) described the histologic findings in bone coincident with tooth movement in man from any cause such as loss of a neighboring tooth or continued pressure. He described apposition of bone by osteoblasts and resorption of bone by osteoclasts.

The classic work of Sandstedt (1904) marked the start of scientific research based on experimental data to account for tissue changes associated with orthodontic tooth movement. He observed osteoclastic activity, hemorrhage, thrombosis and undermining resorption of the

pressure side when forces of high magnitude were applied to the teeth. More uniform resorption of bone was observed when forces of lesser magnitude were used. Formation of new spicules of bone were seen on the tension side in the direction of the stretched periodontal fibers. These findings confirmed Flouren's theory that resorption of bone occurs on the pressure side and apposition on the tension side when pressure is applied to a tooth.

Talbot (1903) observed pathologic changes occurring in the alveolar process when orthodontic forces of high magnitude were applied to teeth.

Oppenheim (1909), using the incisor teeth of primates, concluded that new bone is formed on the tension side of the periodontal ligament. Bone is resorbed and space is created into which the tooth is allowed to move on the pressure side.

Oppenheim (1911) observed that orthodontic forces result in a complete transformation of the bony architecture by a rearrangement of bone spicules on both sides in a line perpendicular to the long axis of the teeth tipped labially. He cited the undesirable effects of using excessive forces in orthodontic tooth movement. Oppenheim made almost the same observation as Sandstedt, but came to different con-

clusions. While accepting the pressure theory, he explained the changes he found on the basis of Wolff's law of bone transformation (1892).

Johnson, Appleton and Rittershofer (1926) reinvestigated the problem of tooth movement using forces of known magnitudes. Their findings were similar to those of Sandstedt and other early investigators.

Schwarz (1932) acknowledged the scientific merit of Sandstedt's effort and in addition was the first to develop a correlation between force magnitudes and histologic changes. Schwarz postulated that in order to achieve biologic tooth movement, the forces should not be greater than capillary blood pressure, or 20-26 gms. per sq. cm.

He observed that there were four different degrees of biologic effect, depending upon the degrees of force used to move teeth. He defined them very simply as "weak," "medium," and "strong," and thusly gave to the clinical orthodontists an available scale for the absolute amount of the maximal force which could be applied to teeth.

He did not concur with Oppenheim on the concept of trabecular "rearrangement" occurring coincident with tooth movement.

Marshall (1931), studying the effects of excessive forces on the extrusion and intrusion of teeth, observed that the extruded central

incisor showed bone proliferation on the alveolar crests, whereas apical reabsorption occurred at the apical crest of the tooth which was intruded.

Orban and Gottlieb (1931) concurred with the findings of Johnson, Appleton and Rittershofer. They also found that teeth of animals will move under continued occlusal stress into new positions, and that the periodontal tissue and supporting bone are soon readjusted to accommodate the new requirements.

Orban accepted the view for the necessity of the use of light forces, mentioned first by Oppenheim (1911), by stating that, "every orthodontic moving of a tooth by means of appliances is an overstress in the biologic sense." This opinion was emphasized by Stuteville (1937) and Skillen (1940). The findings of Orban and Gottlieb concurred with Sandstedt's original conclusion, and with those of Schwarz.

Orban (1936), in a later study, disagrees with the findings and conclusion of Oppenheim (1911, 1933). Results obtained from animal experimentation, according to Orban, are not comparable to those found in man.

Stuteville (1938) dealt with injuries to the teeth and their supporting structures produced by orthodontic forces. He measured the force

exerted and the distance through which the teeth were moved. He observed histologically that traumatic forces caused a compression of the periodontal ligament between the surface of the tooth and the alveolar bone, which blocks the flow of the blood in the vessels in the pressure areas. If the pressure is maintained long enough, necrosis of the ischemic tissues results.

Moyers and Bauer (1950) discussed the periodontal response to the various types of tooth movements and some of the clinical implication of that process. Stasis in the periodontal vessels, which can be brought about with very little continuous force, is the first step in the series of pathologic changes making tooth movement possible.

During extrusion of teeth, so long as a very light force is applied, the essential periodontal changes will appear quickly and bone transformations will occur readily. Increasing the force often causes such a stretching of the fibers that periodontal blood flow is once more impeded.

Storey and Smith (1952), attempting to establish an optimum range of values for an applied force which will produce tooth movement without damage to the tissues, observed the following:

1. The surrounding tissues can support low values of forces up to a point without resorption of bone. By increasing the magnitudes of force, direct bone resorption occurs until an optimum point is reached.

2. Increasing the force beyond this optimum value will cause undermining resorption to take place.

No histologic evidence was presented to substantiate the tissue response in their experiments, however, they did include radiographic evidence of changes in the cribriform plate following orthodontic tooth movement.

Huettner and Whitman (1958), in their study of tooth extrusion, observed that the direction of the periodontal fibers was changed to follow the line of movement. No serious root resorption was seen, and new bone spicules were seen forming in the alveolar area formerly occupied by the apical end of the root. Resorption of the cementum and dentin was minimal and the vitality of the pulp tissues was maintained.

Jarabak and Fizzell (1963) emphasized the application of analytic mechanics and biophysics to orthodontics. They explained basically the how and why of orthodontic forces and tooth movement.

Their definition of tooth extrusion is as follows:

"... when a tooth is being extruded, it is being translated along its long axis. Extrusion is accomplished by applying an occlusal force to the bracket. The forces must be balanced in order to produce extrusion. Its root is held in the alveolus by periodontal ligaments that are being stretched. When a tensile force is applied for a time to the ligaments covering an area of the cribriform plate, there is an apposition of bone, which is the biologic means of attempting to reduce the unusual tension. This attempt would succeed except that the active element in the appliance continues to exert its gradually decreasing extruding force. The cribriform plate thickens, and it continues to support the tooth as long as the extruding force is not too great. When the active element reduces its force to the point where the tensile force in the fibers is no longer great enough to induce apposition of bone, the tooth ceases to move."

An understanding of the critical pressures and of the boundary conditions expressing their relations during tooth movement provides a helpful means of analyzing biologic responses to the orthodontic forces.

PART B

In order to make a biophysical assessment of the incremental rate of bone deposition as related to various magnitudes of forces, a vital staining technic was used.

The vital staining of bone was accidentally discovered and first reported on by Belchier (1736). He found that bone had a peculiar

affinity for madder. This marked the beginning of vital staining of bone.

Duhamel (1739) then recognized the significant importance of the fact that the sites of actively growing bone had a particular affinity for this dye. He used this principle to study growth and development of bone

Hunter (1771) utilized this procedure to study the growth of the mandible, and thereby advanced the theory of bone apposition and resorption as related to growth. Hunter's work was repeated by Brash (1924, 1934), who produced the same observations and conclusions. Hunter later used Alizarine, a derivative of madder, to further his studies on the growth of calcified structures.

Roentgenographically, Park (1930), found dense zones at the ends of long bones of children afflicted with lead poisoning. This became known as a constant diagnostic sign of lead poisoning.

Sodium flouride was used by Schour (1934) as a vital stain to study the incremental patterns of growth in dentin.

It was Gaffey (1931), who demonstrated roentgenographic evidence of "lead lines" both clinically and experimentally. He fed lead acetate to experimental animals and, with x-rays, was able to show definite bands of increased density in the tibia on the tenth day.

Okada and Mimura (1938, 1940, 1941, 1942), seeking to determine whether there is a differentiation in calcium tide, first used lead acetate as a vital stain of calcified tissues. It was administered intravenously to experimental animals and subsequently distinct brown-black lines could be observed histologically at the sites of calcification. As the lead acetate enters the blood stream, it deposits immediately as lead phosphate where calcification is occurring. At the time calcium phosphate collects, lead replaces calcium and precipitates as a lead phosphate. The lead does not move from the sites of deposition for a long time because of the lower solubility product of lead phosphate. This marked an important step in the study of bone growth since it was possible to mark the time lapse in growing bones of animals subjected to various experimental disciplines.

In mammals, intraperitoneal injection of alizarin red S will produce a sharp red line in the bone, dentin, and cementum calcifying at that time, and successive marks can be produced (Schour et al., 1941).

Weinmann and Sicher (1947) have asserted the extensive study of phosphorous as an experimental approach to the study of bone physiology and pathology. The sclerotic phosphorous bands, which are especially marked after the periodic intake of phosphorus, have been used in

determining the rate of apposition in the metaphysis and epiphysis. They also found that lead causes the development of osteosclerotic bands in the same growth zones.

Hiyama and Ichikawa (1952, 1953; Ichikawa and Hiyama, 1954) developed a technique for creating a time mark on the scales of fish by injection of lead acetate. This mark, in the form of a dark line, was used primarily as an aid in growth studies. Their method was to inject intermuscularly 0.1 ml. of solution of lead acetate varying from 1:199 to 1:999 for each 10 gms. of body weight. After "development" with various reagents, a lead line appeared in the scale. The process had little or no adverse influence and the lines lasted several months. This technique has been further developed and modified by Fry et al. (1960) who substituted lead disodium versenate for lead acetate and tested the results of many species.

Auskaps and Shaw (1955) found that a 1:99 aqueous solution of trypan blue, an acid dye of the azo group, injected intravenously in white rats and rhesus monkeys stained the mesenchymal tissues, bones, and dentin which were formed during the two week period that it remained in solution in the blood. This staining could be used to mark times and measure rate of growth in the same way as for alizarin and the tetracyclines. The

advantage of this trypan blue technique is that the mark is not lost by decalcification as is alizarin.

Lead acetate was fed to experimental animals by Vincent (1957), at the rate of 50 gm. /kg. weight in their food. Studying the rate of formation and of maturation of new osteons, he found in the histologic sections a dark brown zone in the long bones; this also appeared in microradiographs.

Milch, Ralb and Tobie (1957, 1958) found that thirty minutes after tetracyclines were injected into animals, all tissues would fluoresce. After six hours the fluorescence localized in areas of new bone formation and was retained there for over ten weeks. They state that this localization may be due to binding of either the unaltered drug or a metabolic derivative of it to calcium and/or bone matrix. Harris (1960) used tetracyclines for studying rates of bone growth. Frost, Villaneuva, and Roth (1960) administered achromycin to humans and laboratory animals in doses of 1 gm. per 75 pounds of body weight per day for three days. They later detected the drug in the bone areas which had been formed during the period of drug administration.

Okada and Asoda (1958) investigated the deposition properties of several other salts of heavy metals in bone. In their findings they observed that lead phosphate deposited most heavily, and had the lowest

solubility product of all the salts of heavy metals used. They found also that the form of lead in the blood varies with time. Initially it is in a labile form and can be readily deposited, but as time passes it changes into a more soluble form that does not become deposited in the hard tissues.

Kirpichnikov (1961) held carp fry in a solution of calcium 45 chloride, with an activity of two microcuries per liter, for one to two hours. The isotope accumulated mainly in the bones and had no effect on growth or survival.

Linhart (1963) found that bismuth has a high toxicity when given to animals intravenously but low toxicity when given orally. It forms lines in the long bones as it is laid down.

PART C

Dyes and Stains:

The distinction between technical dyes and biological stains is not clear. According to Gurr (1960): "Apart from a few natural colouring matters, microscopic (biological) stains consist of synthetic organic dyes that have, in some cases, been prepared by purification, standardization and perhaps modification of technical dyes, and in other cases prepared

directly from primary and intermediate products."

The subject of dye chemistry and nomenclature is a confused one. Conn (1961) presented a good discussion of the chemistry of dyes and the difficulties involved in classifying them. The work of the Biological Stain Commission has resulted in definite standards for over sixty of the most common dyes used in the biological laboratory, and has included in its publications (e.g. Conn, 1961) lists of preferred names of dyes.

There are a number of conflicting theories regarding the actual process which takes place when a dye acts on animal tissue. Singer (1952) reviewed the current theories and pointed out various factors which influence the process. Vital staining, or the coloring of living tissue without harming it, is a specialized area about which even less is known, Conn and Cunningham (1932). In most cases, vital staining is believed to be a phagocytic process rather than a chemical reaction Linhart (1963) but Enghusen and Enghusen (1961) disagree with this theory.

Baker (1958) stated that the special characteristics of vital dyes are penetration, specificity, and harmlessness. Dye solutions should be mixed and filtered just before use, as they may become toxic on standing Costello (1964).

Cocquio (1929) and Ludford (1931) pointed out hidden adverse effects of vital stains on eels and mice respectively.

CHAPTER III

MATERIALS AND METHODS

A. Animal Selection:

Six Rhesus macaque monkeys were used in this experiment. *Macaca mulatta* is the officially approved designation. Female monkeys were selected since they are more docile.

The close structural and physiologic similarity of the Rhesus macaque oral tissues to those of man make these monkeys useful for this research. The teeth of Rhesus macaque show certain similarities to the teeth of man, not only in gross and microscopic anatomical features, but also in sequence of development and in number. It is interesting to note that by the time the animal is six months old the eruption and calcification of the deciduous set of teeth usually is completed. The permanent dentition of the Rhesus macaque becomes completed during the eighth year, or only one year after the average age at which the first permanent teeth erupt in man. It is not unusual to find marked variation in the positions of the permanent teeth in the jaws of the Rhesus macaque. It is for these reasons that the Rhesus macaque affords unique opportunities for this research pertinent to

human function.

Preliminary experimental design required that the teeth of the monkeys selected have a full complement of permanent teeth with the exception of the third molars.

The present description of the macaques are offered and prepared with the needs of experimental design in mind rather than the demands of descriptive anatomy and have been technically described as follows: see Figure 1A, and 1B. The shape and general proportions of the dry skull of an adult Rhesus macaque monkey are adequately indicated by the illustration. The skeleton of the face has been profoundly modified by the masticatory apparatus, and that of the cranium to a lesser degree.

The canine teeth are long, and press against the anterior premolars of the lower jaw, the position of which is modified or distorted by the pressure, thus enabling these animals to crush and open hard-shelled fruits and nuts. Their anterior and median lower molars are four-cusped while the posteriors are markedly larger, and have cusps and a posterior talon. The inclined planes of the posterior teeth are very steep.

The inclination of the teeth from the medial line is exactly opposite from that observed in man. The crowns of all posterior maxillary teeth show an inclination toward the tongue. All mandibular posterior teeth

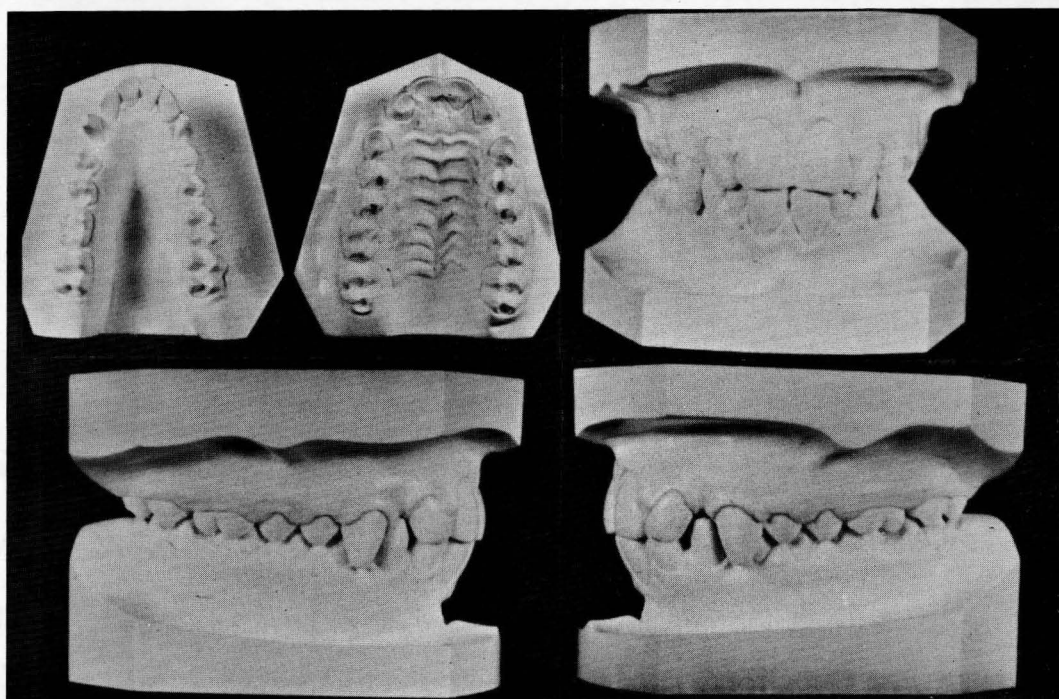


FIGURE 1

- A. FRONTAL AND LATERAL VIEWS OF A DRY SKULL OF A RHESUS MONKEY
- B. DIAGNOSTIC MODELS OF A RHESUS MONKEY

if inclined at all, have an axial direction toward the cheek.

The age estimation of the six Rhesus macaques in this experiment was based on known dates of birth observation on the dentition. The average ages of the monkeys were approximately four to six years, corresponding to the human age of twelve to eighteen years. The animals were purchased from Shamrock Farms, Middletown, New York.

The range of weights of the six monkeys in this study at the beginning of the experiment was 3,000 gms. to 4,700 gms.; the average weight being 3,820 gms.

The six experimental animals, for purpose of identification, were numbered at random from I-VI; number VI serving as the reference animal.

B. Animal Housing and Care:

The six experimental animals were kept in the primate colony established at the Franklin Boulevard Memorial Hospital Animal Research Center, Chicago, Illinois. This research center is operated under the auspices of the Department of Stomatology which is associated with Loyola University, School of Dentistry, Chicago, Illinois. This facility is also available to the Department of Orthodontics.

The monkey quarters consisted of six individual retreats constructed of meshed wire mounted to a heavy steel frame. The service doors are

made of panels which slide vertically and can be locked in position. Through this door the animal may be serviced from a divided feeding tray which is locked in place by flanges. The inside of the cage is accessible to the attendant by this door and by the removable metal bottom trays, the latter of which are removed for weekly cleaning.

Few animals respond so quickly to their diet as the monkey. Consequently, the most important single item in the care of a monkey is that of an adequate diet. The overwhelming nature of many cases of monkey disease has had its origin in subacute malnutrition. A proprietary guaranteed-analysis feed was provided to ensure the desired ration. This consisted of a daily ration of Rockland Laboratory Primate Diet in biscuit form. A second meal consisting of fresh food was given daily which consisted of one-half an orange and one banana each daily. The orange was injected with 5 cc. daily of Vi-Daylin multiple vitamins.

The above diet was altered, after placement and activation of the orthodontic appliances, by softening the biscuits with water into a mash consistency and providing an extra rich diet of bananas and vitamin preparations.

Temperature of the laboratory was thermostatically controlled and

maintained at a level simulating that of their native habitat.

A preventive medicine program was followed by medication of all animals soon after arrival as a prophylaxis against the enteric and pneumonic infections that appear so frequently in monkey colonies. This consisted of a continuous weekly administration of penicillin G during the entire course of the experiment. The animals were observed on a regular basis.

C. Animal Handling:

Due to the stress from transportation and other environmental changes, a two week colonization period was needed until the animals became better adjusted to their new quarters and in order to condition and treat them with antibiotics if necessary.

The first problem encountered when dealing with the monkey was restraint. The monkey was controlled by means of a "squeeze cage" Figure 2. This device is a smaller auxiliary cage which was attached by means of flanges to the larger home case. When the "squeeze cage" is in position the service door is opened and the former is separated by a drop door which is also opened. When both doors of the trap cage as well as the service door are open at the same time, the animal may

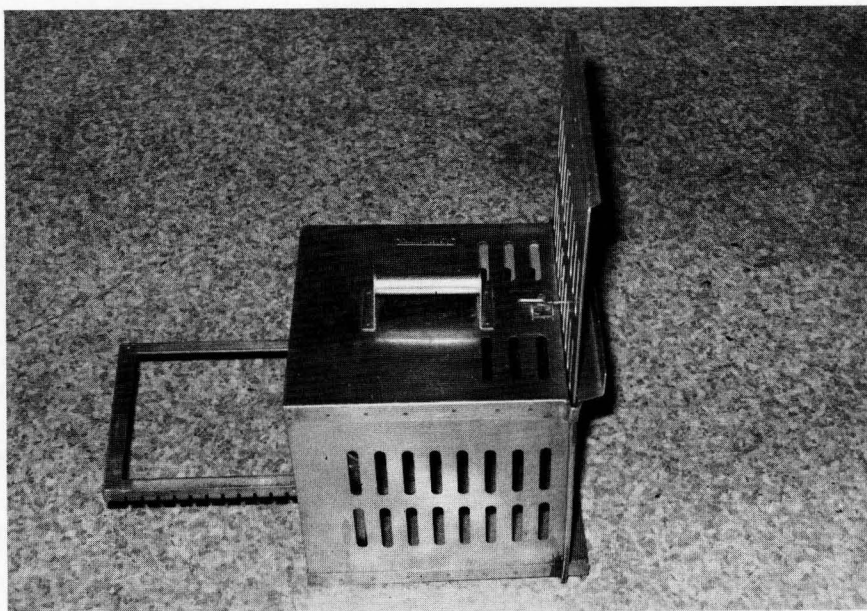


FIGURE 2
MONKEY SQUEEZE CAGE

pass freely between the compartments, which they learn to do readily. By closing the appropriate trap doors upon the animal as it enters the "squeeze cage," it may be imprisoned in the "squeeze cage."

The monkey is then forced against a sliding door and the legs are made available for parenteral injections. To handle the animals double leather gloves were worn to protect the handler from bites and scratches. This method has the advantage of exciting the animal less than by any other means, yet allows for faster handling and less trauma.

Monkeys may have chronic, subclinical infections with any number of potentially pathogenic organisms. Many infections in monkeys are communicable both to other monkeys and to man, as well as potentially fatal to the sick animal. Aseptic operative procedures, therefore, were practiced utilizing sterile surgical gloves, face mask, cap, gowns, and instruments. The monkeys were individually transferred from the cages to the animal operating room where all experimental procedures were performed.

D. General Anesthesia:

Manipulation of the appliances was done under general anesthesia obtained by intravenous injection of sodium nembutal (2 per cent) at the

rate of 25 mgs. per 1 Kg. of body weight.

Preparatory to general anesthesia the monkey was accurately weighed in the squeeze cage. The weight of the cage was then subtracted from the total weight, and from the ensuing figure the correct dosage of nembutal was determined, prepared, and recorded Table I.

The monkey was forced against a sliding door and the legs were made available for parental injection of the anesthetic solution. The monkey was grasped firmly by one leg, which was then scrubbed with soap and water, shaved, and cleansed with alcohol in preparation for the intravenous injection. Parenteral administration is usually preferred to intramuscular as the intravenous route requires one-half the dose and becomes effective immediately. The preferred site for injection was the saphenous vein. A 21 gauge, 1-1/2 inch needle and syringe were used with the foregoing method.

While under anesthesia, the monkey was immediately transferred from the squeeze cage to the operating table and placed on its back with its legs outstretched. Gauze strips were used to tie each appendage securely to the operating table. A suture was placed through the anterior portion of the tongue which was then pulled out to one side in

TABLE I

ANIMAL WEIGHTS AND LEAD ACETATE INJECTIONS

	Monkey I	Monkey II	Monkey III	Monkey IV	Monkey V	Monkey VI
Date	7-13-66	7-13-66	7-15-66	7-15-66	7-15-66	7-15-66
Body						
Wt. Kg.	4.59	3.40	3.45	3.80	4.68	3.00
Lead Ac.						
c. c.	4.6	3.4	3.4	3.8	4.7	3.0
Date	7-20-66	7-20-66	7-22-66	7-22-66	7-22-66	7-20-66
Body						
Wt. Kg.	4.50	3.30	3.42	3.70	4.74	3.29
Lead Ac.						
c. c.	4.5	3.3	3.4	3.7	4.7	3.3
Date	7-27-66	7-27-66	7-29-66	7-29-67	7-29-66	7-27-66
Body						
Wt. Kg.	4.53	3.25	3.40	3.75	4.60	3.60
Lead Ac.						
c. c.	4.5	3.3	3.4	3.7	4.6	3.6
Date	8-2-66	8-2-66	8-5-66	8-5-66	8-5-66	8-2-66
Body						
Wt. Kg.	4.54	3.20	3.53	3.82	4.40	3.63
Lead Ac.						
c. c.	4.5	3.2	3.5	3.8	4.4	3.6
Date	8-10-66	8-10-66	8-12-66	8-12-66	8-12-66	8-10-66
Body						
Wt. Kg.	4.30	3.30	3.55	3.87	4.10	3.61
Lead Ac.						
c. c.	4.3	3.3	3.6	3.9	4.1	3.6

order to prevent any obstruction of the airway. Butyn sulfate and metaphen ophthalmic ointment was placed in each eye as a precautionary measure in order to prevent post-operative infection and drying. During anesthesia, each animal received a prophylactic I.M. injection of 300,000 units of penicillin G. The heart and respiration rates were checked regularly during operative procedures.

The preceding steps were carefully followed for each monkey during all experimental procedures.

E. Selection of Teeth for Extrusive Tooth Movement:

The left maxillary second premolar was selected to study tooth extrusion for the following reasons:

1. The crown of the maxillary second premolar is more ideally shaped morphologically for orthodontic band placement than the first premolar.

2. The maxillary incisors are in the premaxillary bones and with the canines are embedded in more dense cancellous bone; the premolar teeth are embedded in spongy bone.

3. The canine, with its long tapering root, and the first and second molars would act very satisfactorily as anchor units in moving the second

premolar.

The experiment was confined to the maxillary arch, since three separate experimental procedures were to be conducted simultaneously. Manipulation and control of the appliances, thereby, would be simpler.

F. Preliminary Preparation of Experimental Animal:

The following initial steps ensued after the monkey was anesthetized by the method previously described:

1. A dental prophylaxis was done on each monkey in order to have a clean field of operation. Calculus and stains were removed.
2. Specially designed preformed acrylic trays and a heavy rubber base material were used to take impressions of the teeth and the surrounding tissues. Vel-Mix stone models were prepared from the impressions and from these working models, the appliances were then fabricated.
3. The monkey was returned to its cage and laid on its side with the head facing downward after completion of the above steps. Proper positioning of the animal is important in order to prevent obstruction of the airway by oral secretions.
4. When the respiration and heart rates appeared uniform the tongue was pulled out to one side and the suture was removed.

The maxillary right first premolar was removed earlier to facilitate a separate experimental procedure.

G. Design of Force System and Selection of Force Magnitudes:

To accomplish the stated goal of this experiment it was necessary to devise a force system that would produce a controlled pure extrusion of the second premolar tooth. The extrusive force should be applied to the crown of the tooth on the long centerline. It was impossible to accomplish this with a single force, therefore an appliance was designed that would develop a pair of extrusive forces. These two forces were to be applied to the buccal and lingual surfaces of the premolar tooth.

This required a design of appropriate active elements and adequate structure to resist the reciprocal intrusively directed forces and moments. The ultimate design considered, from the geometric point of view, was a sturdy rectangular frame attached to a canine and the molar teeth and containing the premolar tooth that was to be extruded. The two active elements that delivered the extrusive forces were attached to the long sides of the steel rectangular frame.

The magnitude of an optimal extrusive force for the teeth of a monkey is not known. Consequently, reference was made to Jeffry and

Kostiwa (1965) who assumed that sixty per cent of the force value for the movement of a human tooth could be used for a monkey tooth.

Their work, subsequently indicated that perhaps forty-five to fifty per cent of the force for a human tooth would have been a better estimate. A range of empirical values from 180 to 200 gms. has been suggested by various investigators for the extrusion of a human premolar tooth.

Reducing these suggested figures, it would appear that estimates for the monkey might be about eighty to ninety gms. On this basis five separate magnitudes of force, bracketing these estimates, were chosen for use in this experiment. These were 25 gms., 50 gms., 75 gms., 100 gms., and 150 gms. These forces were arbitrarily assigned to the experimental animals. The sixth animal in the group served as a control. This animal was banded but no active elements were incorporated into the appliance.

H. Appliance Design;

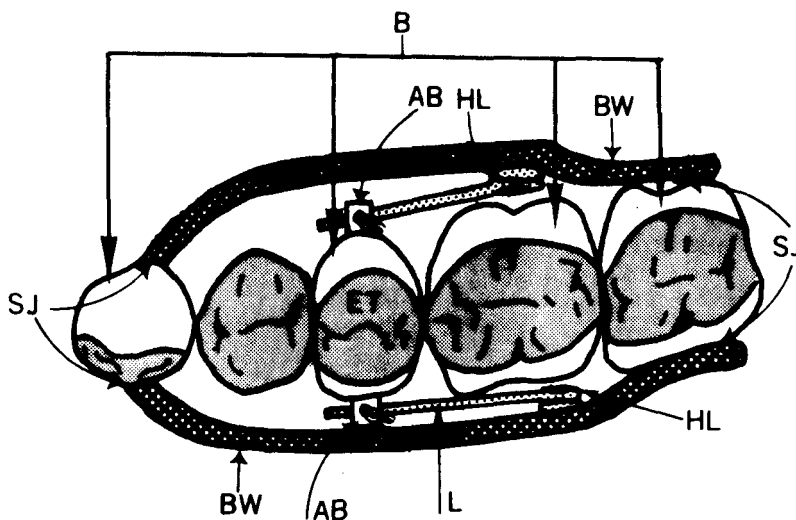
The above discussion of the design of the force system has suggested the use of an intrinsic system acting in a vertical direction. Ideally, the action line of this extrusive vertical force should coincide with the long centerline of the tooth. This would be impractical, however,

because of the irregularity, size, and shape of the tooth root structure. Because of this factor two balanced forces must be applied; one on the buccal side and one on the lingual side Figure 3.

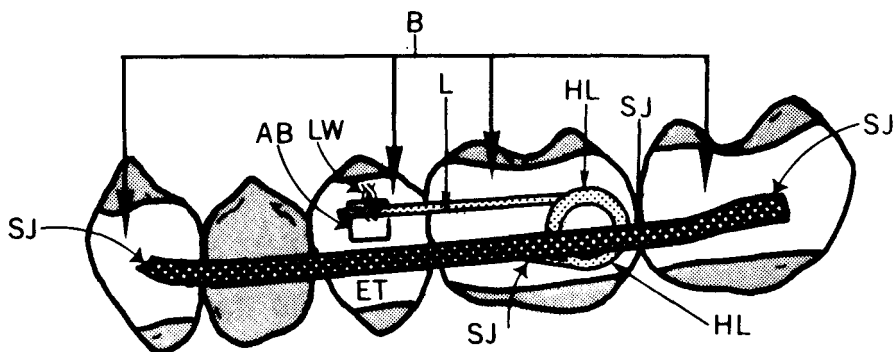
This requirement necessitates constructing a substantial base or frame work along each side of the premolar tooth that is to be extruded. Since the reciprocal forces would be intrusively directed, the adjacent teeth on the ipsilateral side of the arch should serve as adequate anchors for the proposed framework.

Accordingly, bands were planned for the canine, the second premolar, first molar, and second molar. It was then determined that heavy round wires could be used to bridge the space between the canine band and the two molar bands, thus giving a structure near the premolar to which the active elements of the appliance could be attached.

It was noted above that two balanced extrusive forces, needed to obtain pure extrusion, would be applied on the buccal and lingual surfaces of the second premolar tooth. These forces are readily obtained by the use of two horizontal helical loop springs. The plan was to solder one leg of one loop to the lingual bridging wire and to ligate the other leg of the loop to an angle bracket on the lingual surface of the



OCCLUSAL ASPECT



BUCCAL ASPECT

AB	ANGLE BRACKET
B	BANDS .004 INCH DIAMETER
BW	BRIDGING WIRE .036 INCH DIAMETER
SJ	SOLDER JOINT
L	LEG OF HELICAL LOOP
HL	HELICAL LOOP
ET	EXPERIMENTAL TOOTH
LW	LIGATURE WIRE .010 INCH DIAMETER

FIGURE 3

APPLIANCE DESIGN

band on the premolars. This same method of attachment was planned for the other loop on the buccal side of the premolar. The result of this design should be to provide balanced extrusive forces on two sides of the second premolar.

I. Appliance Construction:

The appliances for this experiment were fabricated on Vel-Mix stone models which were prepared from rubber base impressions. The teeth to be banded were carefully trimmed around the gingival margins in order to facilitate the fitting and adaptation of the bands. The molars, premolars, and canines were banded with .004 inch diameter stainless steel banding material. A passive maxillary lingual arch and a similar buccal arch of .036 inch diameter round wire were made for each appliance. These arch wires were soldered to the bands on two second molars and canines, but were free of the second premolars on the right side and the remaining teeth. Two die-formed angle brackets were soldered on the buccal and lingual surfaces of the second premolar band. The foregoing steps were done for each of the six experimental animals and this completed the fabrication of the passive elements of the appliances.

The active elements of the appliances consisted of two units each of horizontal helical loops of green Elgiloy wire. A 0.011 inch diameter wire was used for the 25 gm., and 50 gm. forces, and a 0.014 inch diameter wire for the 75 gm., 100 gm. and 150 gm. forces. The helical springs were made of 1-1/2 turns of wire formed as horizontal expansion loops which effectively develop forces in a vertical plane to extrude teeth. One horizontal expansion loop spring had one leg soldered to the lingual bridging wire and its free leg was ligated to the angle bracket on the lingual surface of the premolar band. The other horizontal expansion loop spring had one leg soldered to the buccal bridging wire and its free leg was ligated to the angle bracket on the buccal surface of the premolar band. The length of each free leg of the helical loop springs was 7 mm. The diameter of each loop of the spring was 2 mm.

The active elements were designed to store forces having the following magnitudes: 25 gm., 50 gm., 75 gm., 100 gm., and 150 gm., respectively in five of the experimental animals. The sixth animal, serving as a control was banded in the same manner as the first five experimental animals, but no active elements were present. Each active element was tested on a load-deflection apparatus designed by

Vestevich and White (1963) Figure 4. The desired forces, spring rates, spring specifications, and the linear characteristics of all the springs used in this experiment were recorded Table II. Graphs showing the force versus the distance returned were plotted Figures 5, 6, 7, 8, 9, and 10.

Experimental design calls for extrusion of the maxillary second premolar tooth out of occlusion. The high cusps and deep interdigitation of the opposing cusps create functional interferences which must be removed. A bite-opening device was necessary to eliminate these interferences, thereby permitting the appliance forces to act effectively. A bite plane was used to open the bite three to four millimeters in the premolar region. The bite-opening device consisted of an acrylic bite plane which was attached to the lingual bridging wire and was cemented to the maxillary anterior teeth. The bite plane covered the anterior teeth from canine to canine and extended above the gingival margins of the teeth labially. In order to remove any further interferences, the tips of the cusps of the canine teeth were reduced in both arches after appliance cementation.

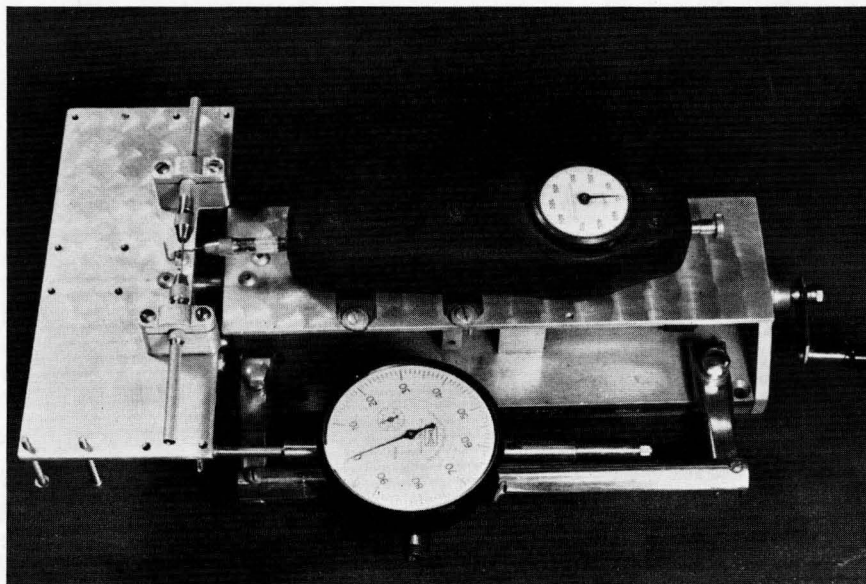


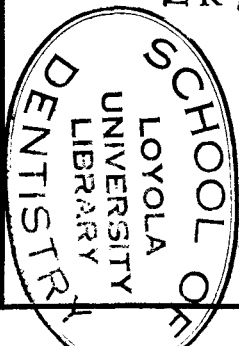
FIGURE 4

LOAD-DEFLECTION TESTING APPARATUS

TABLE II

SPRING SPECIFICATIONS FOR THE ONE AND
ONE-HALF TURN HORIZONTAL HELICAL LOOP APPLIANCE

Animal	I	II	III	IV	V	VI
Force of Activation (gms.)	25	50	75	100	150	0
Green Elgiloy Wire Diameter (inches)	.011	.011	.014	.014	.014	0
Leg Length (mm.)	7	7	7	7	7	0
Loop Diameter (mm.)	2	2	2	2	2	0
Average Spring Rate (gm. /mm.) - (Buccal and Lingual Springs)	5.85	6.0	19.6	17.4	21.4	0
Σ K gm. /mm.	11.6	11.6	39.2	34.8	42.8	0



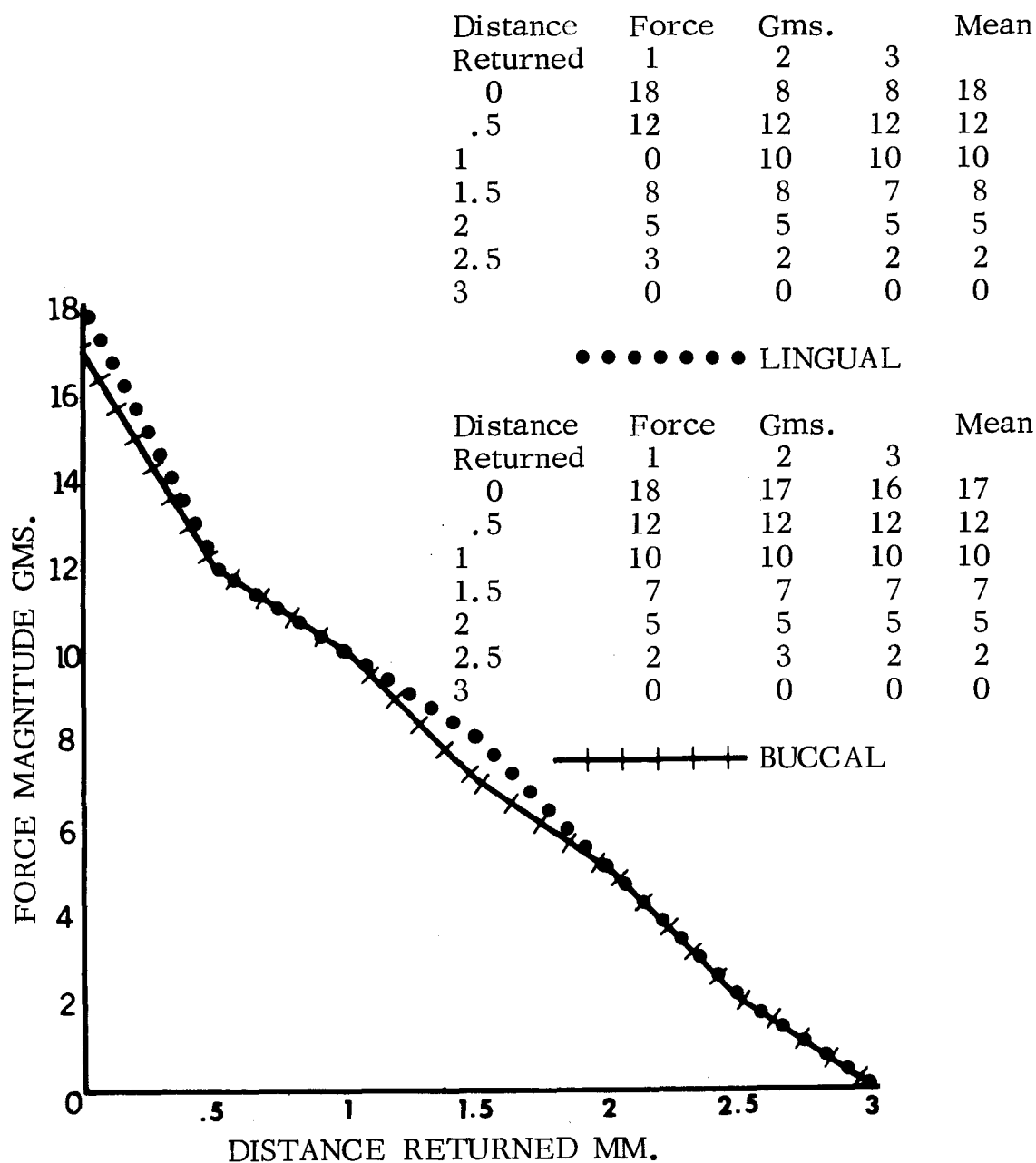


FIGURE 5

LOAD-DEFLECTION CHARACTERISTIC - ANIMAL I - 25 GMS.

Distance Returned	Force 1	Gms. 2	3	Mean
0	25	27	27	26
.5	20	22	22	21
1	17	19	19	18
1.5	12.5	15	15	14
2	10	10	10	10
2.5	9	9	10	9
3	5	8	8	7
3.5	3	3	5	4
4	2	2	2	2
4.5	0	0	0	0

..... LINGUAL

Distance Returned	Force 1	Gms. 2	3	Mean
0	28	28	29	28
.5	23	22	22	22
1	20	19	19	19
1.5	17	15	15	16
2	10	10	10	10
2.5	9	9	10	9
3	5	8	8	7
3.5	3	3	5	4
4	2	2	2	2
4.5	0	0	0	0

+ + + + + BUCCAL

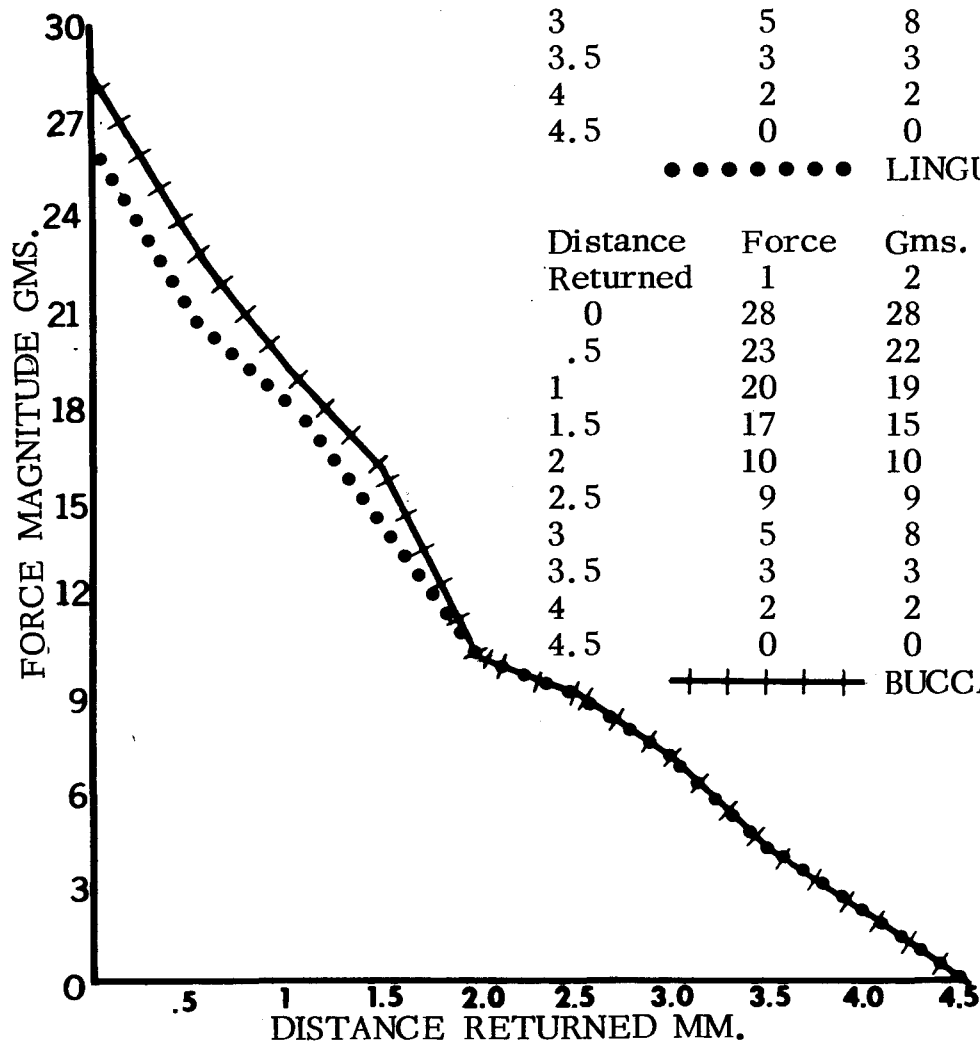


FIGURE 6

LOAD-DEFLECTION CHARACTERISTIC - ANIMAL II - 50 GMS.

Distance Returned	Force 1	Gms. 2 3		Mean
0	48	48	47	48
.5	35	35	35	35
1.0	22	21	22	22
1.5	15	12	12	12
2.0	5	5	5	5
2.5	0	0	0	0

• • • • • LINGUAL

Distance Returned	Force 1	Gms. 2 3		Mean
0	48	48	48	48
.5	35	35	35	35
1.0	22	22	22	22
1.5	15	15	12	12
2.0	5	5	5	5
2.5	0	0	0	0

+ + + + + BUCCAL

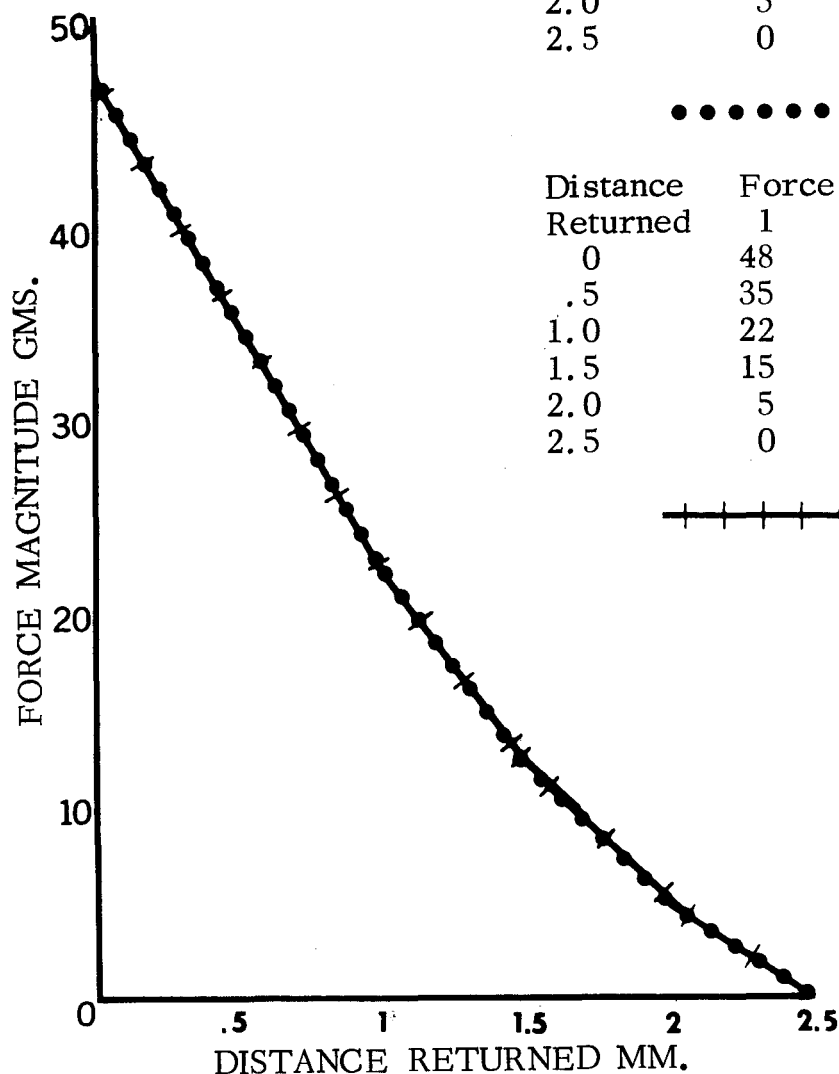


FIGURE 7

LOAD-DEFLECTION CHARACTERISTIC - ANIMAL III - 75 GMS.

Distance Returned	Force 1	Gms. 2 3		Mean
0	62	62	62	62
.5	47	48	48	48
1.0	35	33	33	34
1.5	21	22	22	22
2.0	12	12	12	12
2.5	5	5	5	5
3.0	0	0	0	0

• • • • • LINGUAL

Distance Returned	Force 1	Gms. 2 3		Mean
0	50	50	50	50
.5	40	40	40	40
1.0	32	32	32	32
1.5	25	25	25	25
2.0	20	20	21	20
2.5	12	12	12	12
3.0	9	9	5	8
3.5	0	0	0	0

+ + + + + BUCCAL

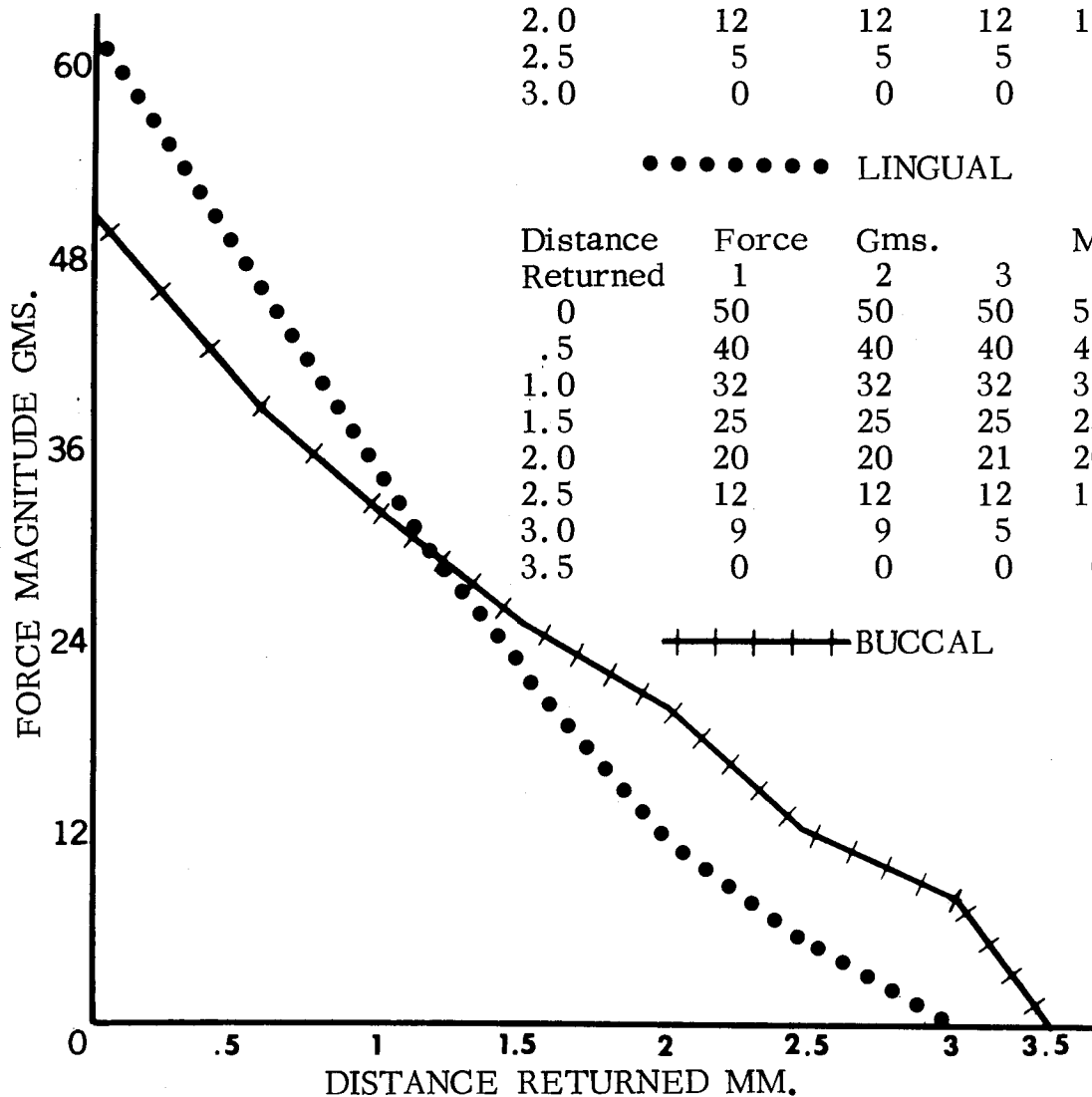


FIGURE 8

LOAD-DEFLECTION CHARACTERISTIC - ANIMAL IV - 100 GMS.

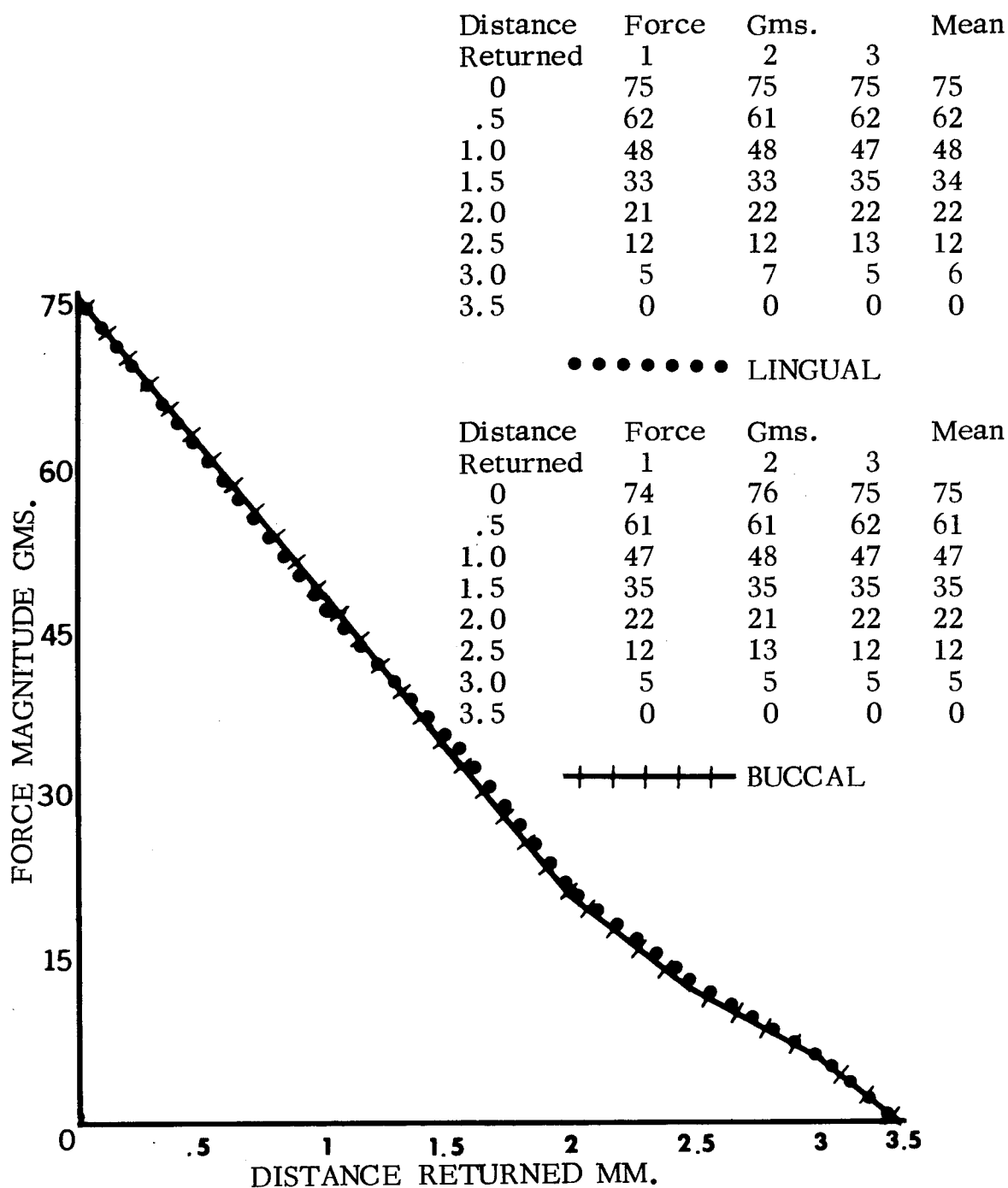


FIGURE 9

LOAD- DEFLECTION CHARACTERISTIC - ANIMAL V - 150 GMS.

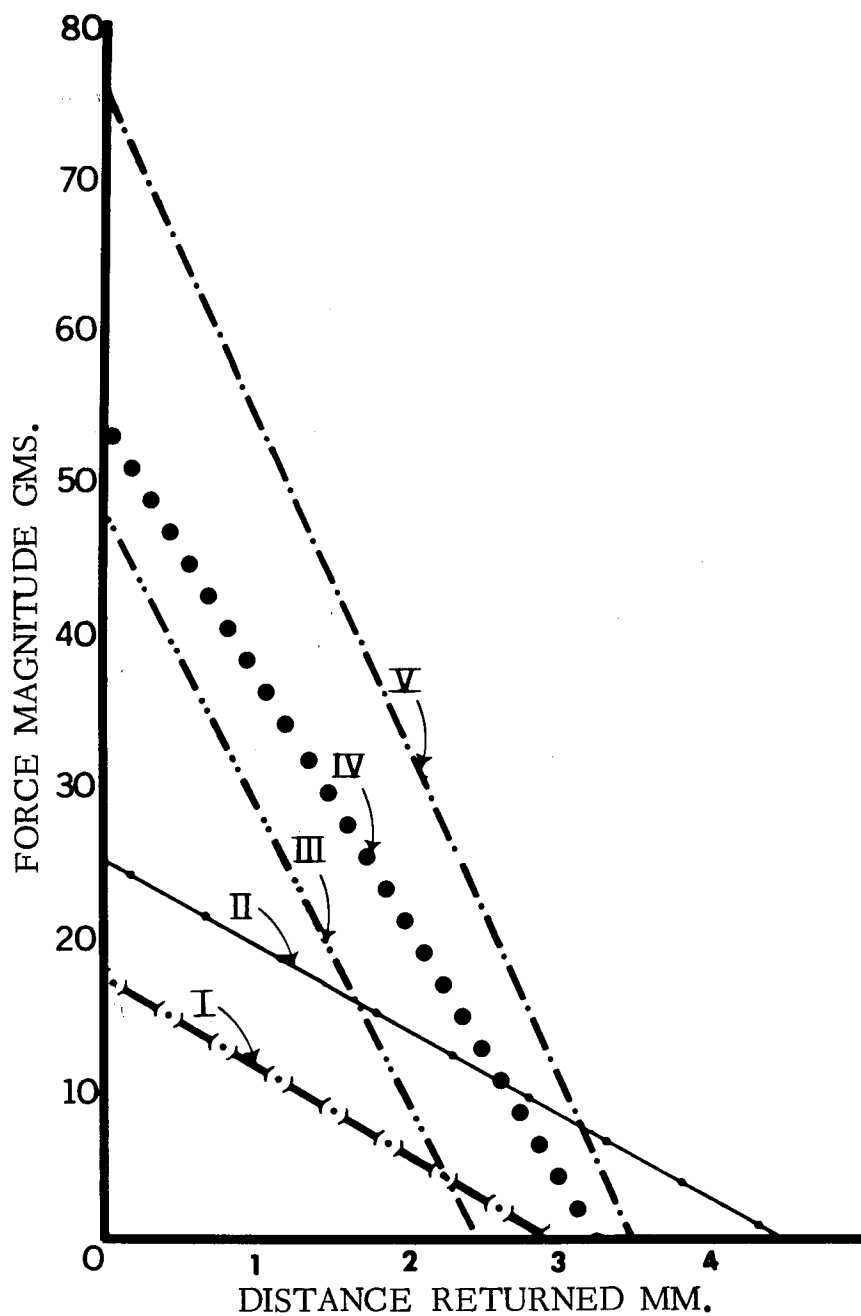


FIGURE 10

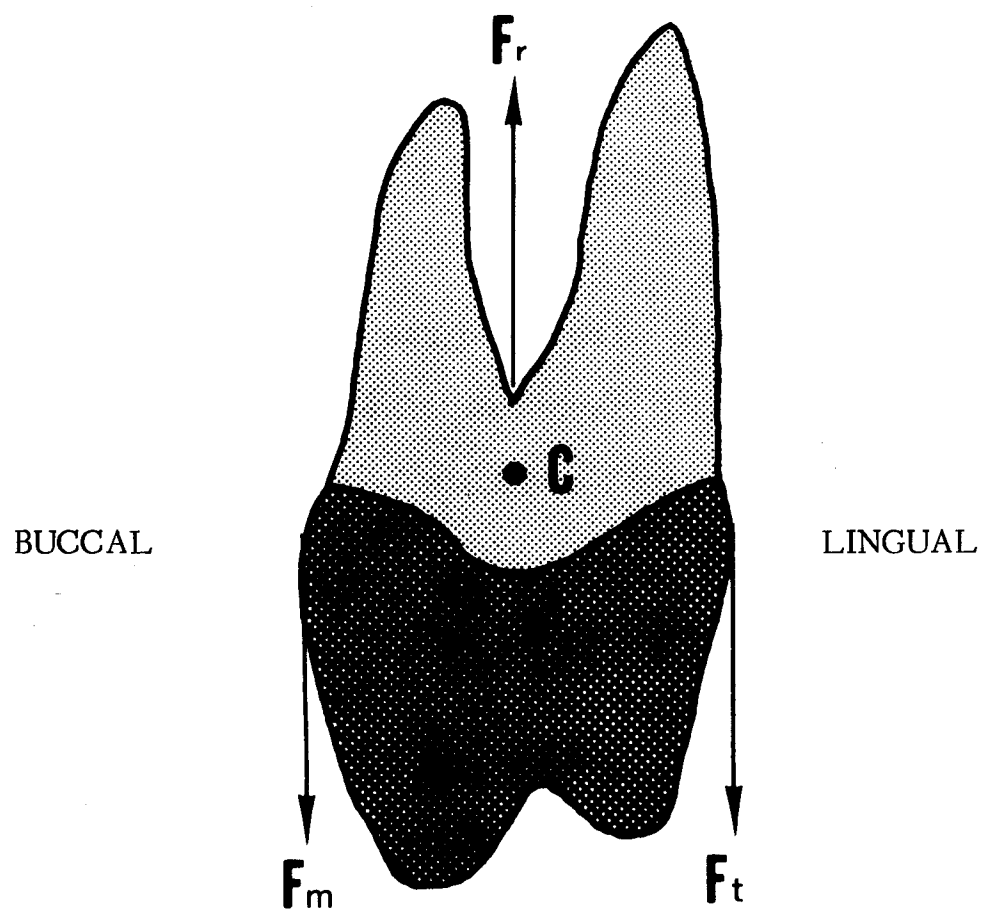
LOAD-DEFLECTION CHARACTERISTICS - ANIMALS I - V

J. Analysis of the Force System:

In order to analyze the force system used in this experiment a free body diagram will be outlined. This will demonstrate the effect of the force system on the maxillary second premolar. Figure 11 diagrammatically illustrates the buccal-lingual view of this tooth.

When a tooth is being extruded, it is being translated along its long axis. To produce pure extrusion a force should be applied along the long center line of the tooth opposite to F_r which is defined as the net resisting force. Since the exact location of the long center line is unknown, it must be estimated and the effect of the error in location will be minimized if a second point contact is made on the crown of the tooth. Any tendency for the tooth to tip buccally or lingually will be resisted by the balanced springs on each side of the tooth (F_t and F_m). There was no special provision to prevent the tooth from moving mesially to distally, but the applied force was not expected to have any horizontal components that would cause such movement.

Reciprocal forces are directed intrinsically and are applied to the canine and the two molar teeth. Since the loop is located closest to the molar teeth, the intrusive force will be heaviest on them but they are



F_r Resisting Force
 F_m Extrusive Force
 F_t Extrusive Force
C Centroid

FIGURE 11
FREE BODY DIAGRAM

large enough to resist it safely and no movement should be expected.

K. Preparation and Injection of Lead Acetate Solution;

Lead acetate, $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$, in the neutral or normal state is colorless crystals or white granules with a slight acetic odor. The pH of an aqueous solution is 5.0.

Lead acetate solution was prepared by dissolving 1 gm. of lead acetate in 250 ml. of sterile distilled water. This solution was used to prepare the required dosage, which was 4 mg. per kg. of body weight. Preparatory to general anesthesia the animal was weighed, and from the ensuing figure the correct dosage was prepared Table I.

Two common sites were used for intravenous injection. These were the saphenous (v. saphena) and the popliteal (v. popliteal) veins which are terminals of the larger femoral vein. The preferred site for injection was the saphenous vein. The rate of lead acetate injections was 3 cc. per minute. Each animal received the first lead acetate injection after appliance cementation and activation. Additional lead acetate injections were administered to each animal at the rate of one injection weekly for four weeks. The animals were sacrificed twenty-four hours following the final injection.

L. Appliance Cementation and Activation:

Preparatory to insertion and cementation of the orthodontic appliance, the animal was anesthetized. The contact points on either side of the teeth to be banded were relieved with a diamond disc so that cementation of the bands could be made with greater ease and to reduce any additional forces. Trial insertions of the orthodontic appliance and bite plane were made to ensure correct alignment and fit on the teeth and the palatal area anteriorly. Black copper cement was used to cement the appliance to the teeth. The excess cement was removed and the tips of the canine cusps were reduced to eliminate any functional interferences. The two helical loop springs on either side of the left maxillary second premolar tooth were tested with force gauges to verify the actual orthodontic forces desired. Minor adjustments could be made at this time with an orthodontic plier. The arms of these helical loop springs were then ligated to the angle brackets on either side of the maxillary second premolar band with 0.010 inch diameter ligature wires Figure 12.

To keep the animal from removing the appliances the hands and forearms were covered. To do this the forearms of the animal were shaved and painted with a tincture of benzoin to minimize irritation from

A

B

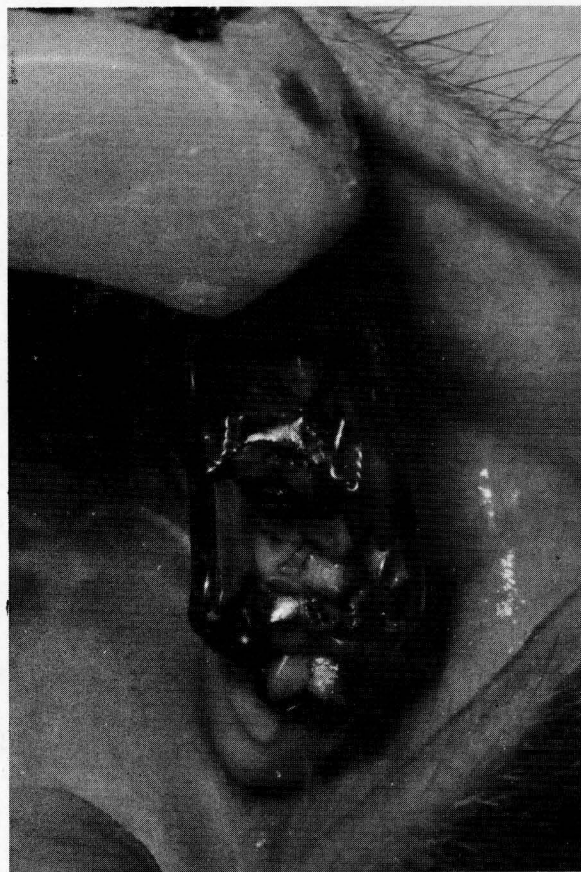
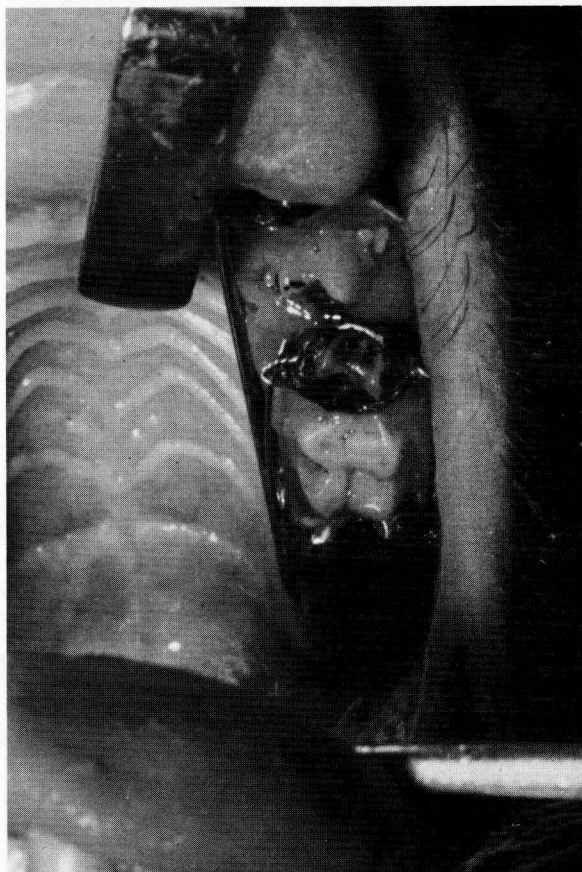


FIGURE 12

EXPERIMENTAL APPLIANCES IN THE MOUTH

- A. APPLIANCE ACTIVATED
- B. APPLIANCE DEACTIVATED

the adhesive tape. A soft knit specially designed gauze sleeve was fitted over the hand and forearm, and then wrapped with a layer of cotton. Gauze strips, impregnated with plaster, were soaked in water and wrapped around the cotton Figure 13. After setting of the plaster, adhesive tape was placed over the casts.

M. Animal Sacrifice and Perfusion:

The animals were sacrificed twenty-four hours following the fifth and final injection of lead acetate solution. All of the animals were perfused to provide adequate tissue fixation. The animals were anesthetized as described previously and prepared for the sacrifice and perfusion. A thoracic incision was made over the sternum from the left clavicle to the last rib and the skin was reflected. The sternum was removed with portions of the first through the fifth ribs. The pericardium was reflected exposing the heart. Further blunt dissection freed the arch of the aorta, the superior vena cava, and the descending aorta. The descending portion of the aorta was clamped off, and an incision was made into the wall of the left ventricle. A perfusion canula attached to the perfusion liquids was passed into the left ventricle and advanced into the aortic arch. The canula was securely tied to place to prevent any escape of

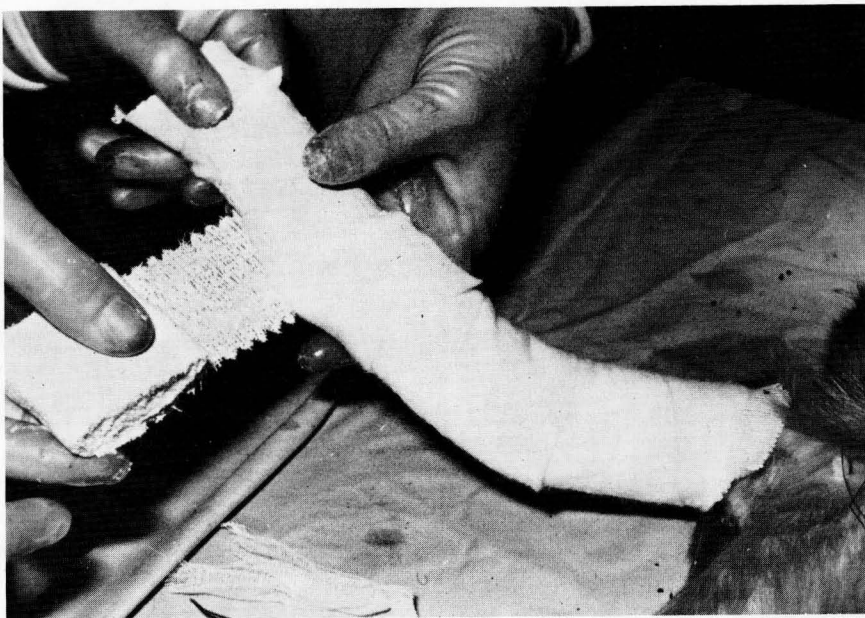


FIGURE 13
ANIMAL RESTRAINTS

the solutions, and backflow into the ventricle. The superior vena cava was cut and a ten per cent sodium citrate solution was allowed to flow into the aorta and, hence, the circulatory system by a gravity feed system. This forced the blood out from the tissues above the level of the heart. This solution was allowed to flow until a clear solution appeared on its return through the superior vena cava. The flow of sodium citrate was discontinued and was replaced with a ten per cent buffered neutral formalin solution. Formalin solution began to flow from the superior vena cava and the tissues of the head and neck became very rigid. The formalin solution was allowed to flow for ten minutes. Perfusion was now complete.

The animal was decapitated. The skin, skull cap, brain, and mandible were removed and the remaining portion of the skull was stored in a ten per cent solution of formalin for one week. The sections pertinent to the experiment were removed after fixation for decalcification.

N. Decalcification and Lead Precipitation:

Tissue blocks of the experimentally moved teeth, reference tooth, and surrounding structures were decalcified in 0.1 N hydrochloric acid. The pH was kept as near constant as possible by using eighteen liters

of decalcifying solution. Hydrogen sulphide was continually bubbled through the hydrochloric acid solution to maintain a supersaturated level of decalcifying solution. In the ensuing reaction the hydrogen sulphide reacted with the lead phosphate, which was deposited in the bone and precipitated it to insoluble black lead sulphide. Excess hydrogen sulphide was passed through a solution of sodium hydroxide and potassium permanganate to render it less noxious Figure 14.

The degree of decalcification was determined roentgenographically. Decalcification was considered complete when calcific material was no longer seen in the roentgenogram. Decalcification was completed in approximately two weeks.

O. Histologic Preparation of the Sections:

The individual sections were washed with distilled water and then placed in gelatin solutions of 10, 20, 30 per cent concentrations. These solutions contained 2.5 ml. of concentrated Lusol solution. The specimens remained in each of the respective gelatin solutions for forty-eight hours in an incubator at 37°-40° C. The specimens were then embedded in a thirty per cent gelatin solution for forty-eight hours.

To further harden the sections they were placed in a refrigerator

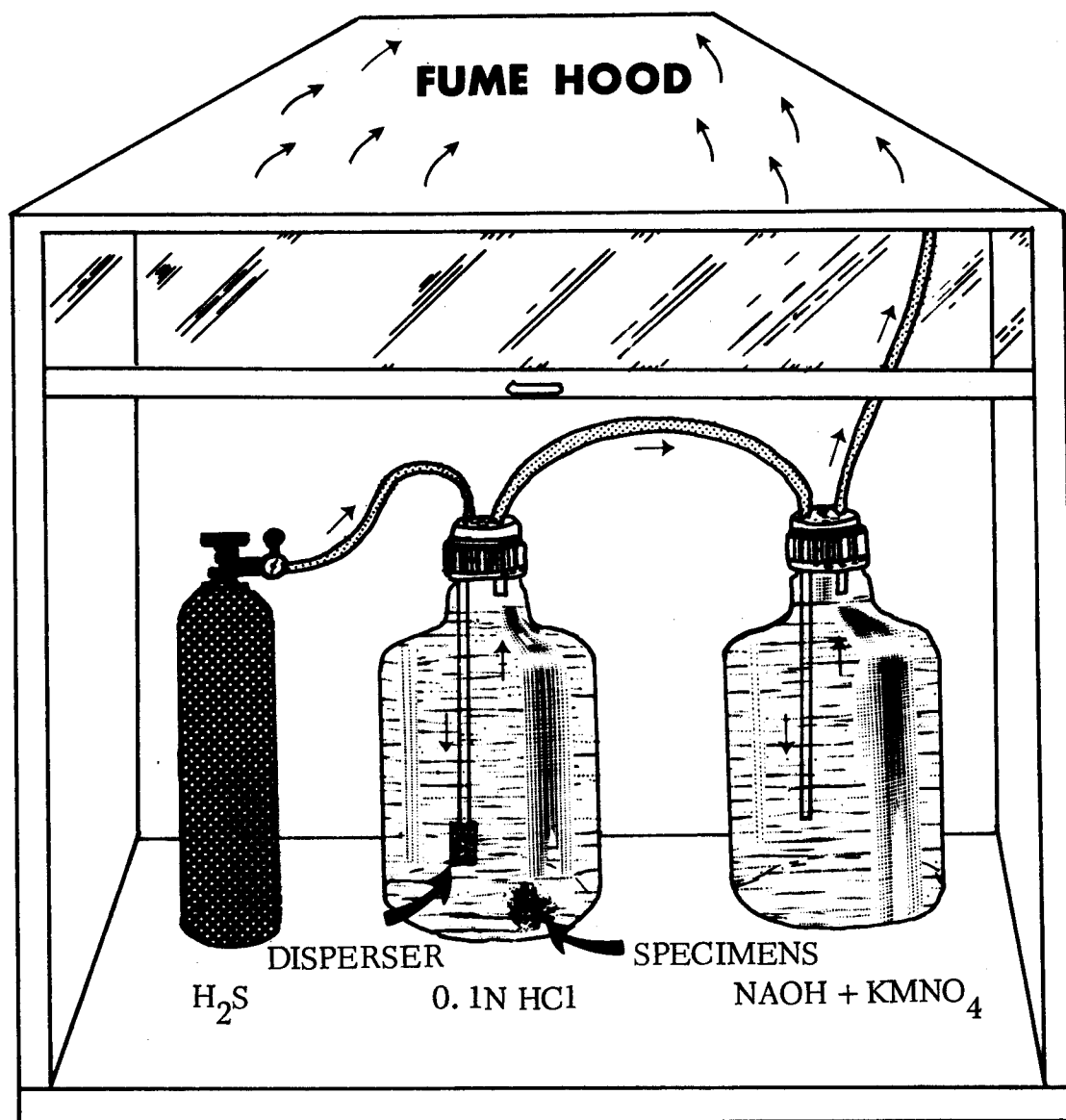


FIGURE 14

DECALCIFICATION APPARATUS

in a ten per cent formalin solution for twenty-four hours.

A cryostat or freezing microtome was used to cut the section. The block sections were frozen to -20°C . and cut mesiodistally in the long axis of the tooth to a thickness of twenty-four microns. The sections were floated in a Stender dish containing distilled water and then positioned on the glass slide. Glycerine jelly was used to mount the sections, which were then covered with a glass cover slip.

P. Method of Measurement:

The instrument for recording measurements was a micrometer slide comparator Figure 15. It consisted basically of a 32X stationary microscope with a movable stage on a weighted base support. This instrument is designed to make precision linear measurements. The stage or carriage is mounted so as to slide in very straight guided ways by means of a precision micrometer screw. The smallest units of measurement were 0.01 mm. units, but intermediate points could be estimated by interpolation.

Two cross hairs at 90° to each other indicated the center of the field and were used for alignment. The cross hairs were advanced by means of the screw and could be brought into coincidence with the

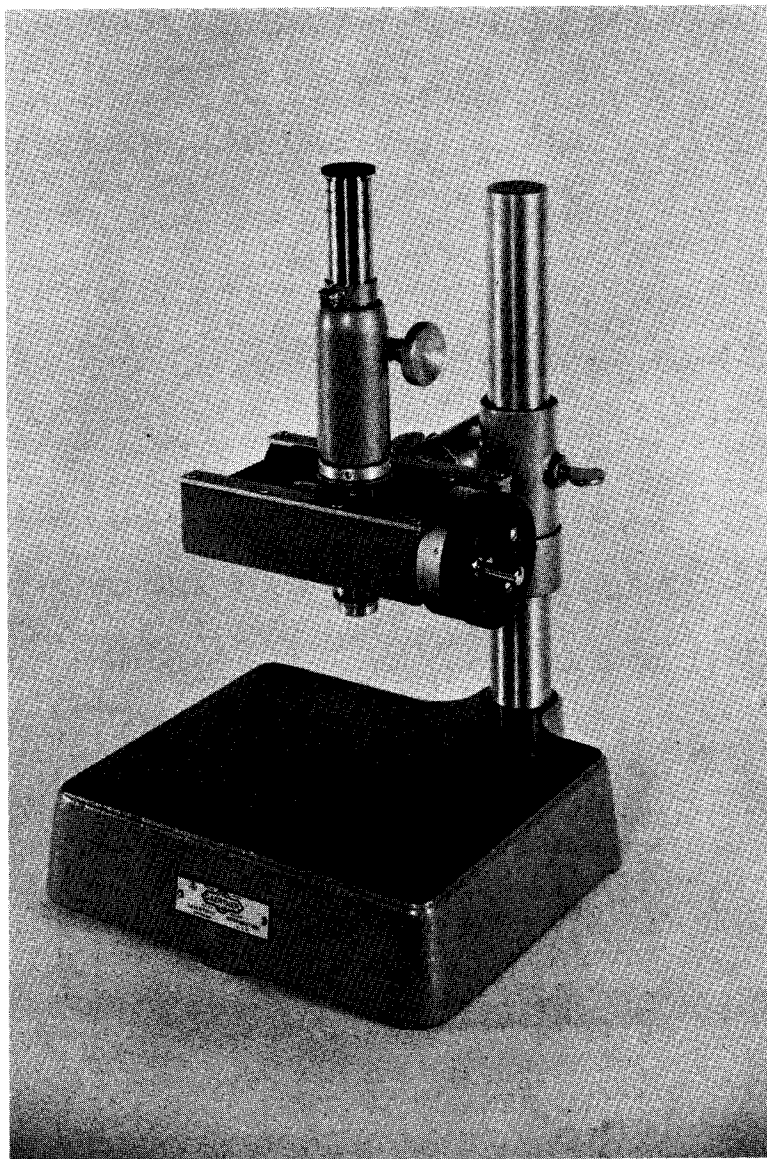


FIGURE 15
MICROMETER SLIDE COMPARATOR

reference points of the illuminated object quickly and exactly. Settings could be repeated with high consistency.

Testing for precision of the instrument was accomplished by measuring the distance between two arbitrarily placed points to the nearest 0.001 mm. Three operators measured the distance between these two points. Ten measurements made by each operator were recorded by an associate to the nearest 0.001 mm.

The ensuing data were subjected to the analysis of variance to ascertain the precision of the measuring process Table III - Part I. The important result of the analysis of variance was the measure of experimental error (the precision of the measuring process) Table III - Part II. This is contained in the mean square, $10.4 \mu^2$ estimated from 27 degrees of freedom. The standard error of measurement was .00322 mm. and the 99 per cent confidence limits on the distribution of errors were ± 0.0089 mm. This is shown in Figure 16, where each division of the horizontal scale equals one micron.

Measurements were taken at the apices of the mesiobuccal and distobuccal roots, and at the interradicular septum perpendicular to the root surfaces. The area at the mesio buccal root apex was

TABLE III
ANALYSIS OF VARIANCE FOR THE MEASURING INSTRUMENT

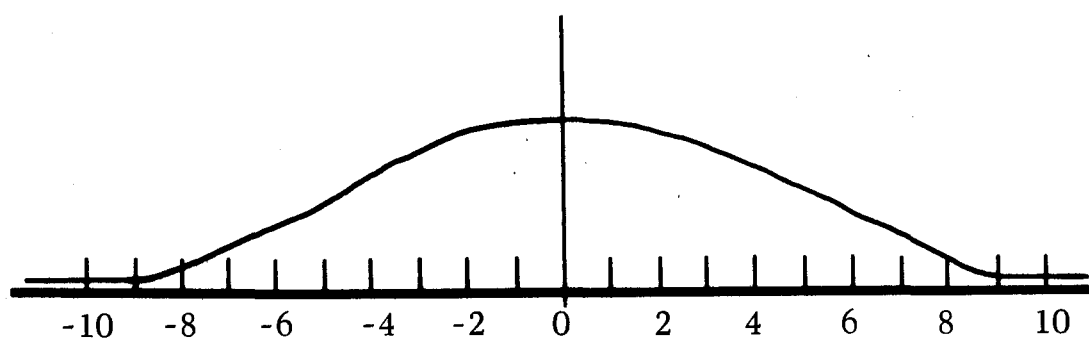
PART I

Source	D. F.	S. S.	M. S.
Operators	2	63.2	31.6
Measurements	9	70.0	7.7
O X M (Total exper. error)	18	210.8	11.7
Total	29	344.0	

It is apparent that the sums of squares for measurements and interaction can be combined to provide a valid estimate of experimental error. This yields the following table:

PART II

Source	D. F.	S. S.	M. S.	Var. Ratio	F. Ratio at .05
Operators	2	63.2	31.6	3.04	3.35
Error	27	280.8	10.4		
Total	29	344.0			



$$\text{S.E.} = \pm 3.22 \mu$$

$$99\% \text{ C.L.} = \pm 8.9 \mu$$

FIGURE 16

DISTRIBUTION OF ERRORS

identified as A. The area at the interradicular septum was identified as B. The area at the distobuccal root apex was identified as C.

The stage of the micrometer slide comparator was moved so that the cross hairs in the microscope were positioned at the junction of the newly apposed bone and the old bone, or to the first lead line. A reading was made at this point. The cross hair was then advanced to the last or fourth lead line which appeared at the junction of the newly apposed bone and the periodontal space. A second reading was made at this point. The difference between these two readings was the amount of newly apposed bone or area 1. A similar method was used to determine the width of the periodontal space, or area 2. The total, or area 3 was obtained by adding the area of the newly apposed bone and the width of the periodontal space Figure 17.

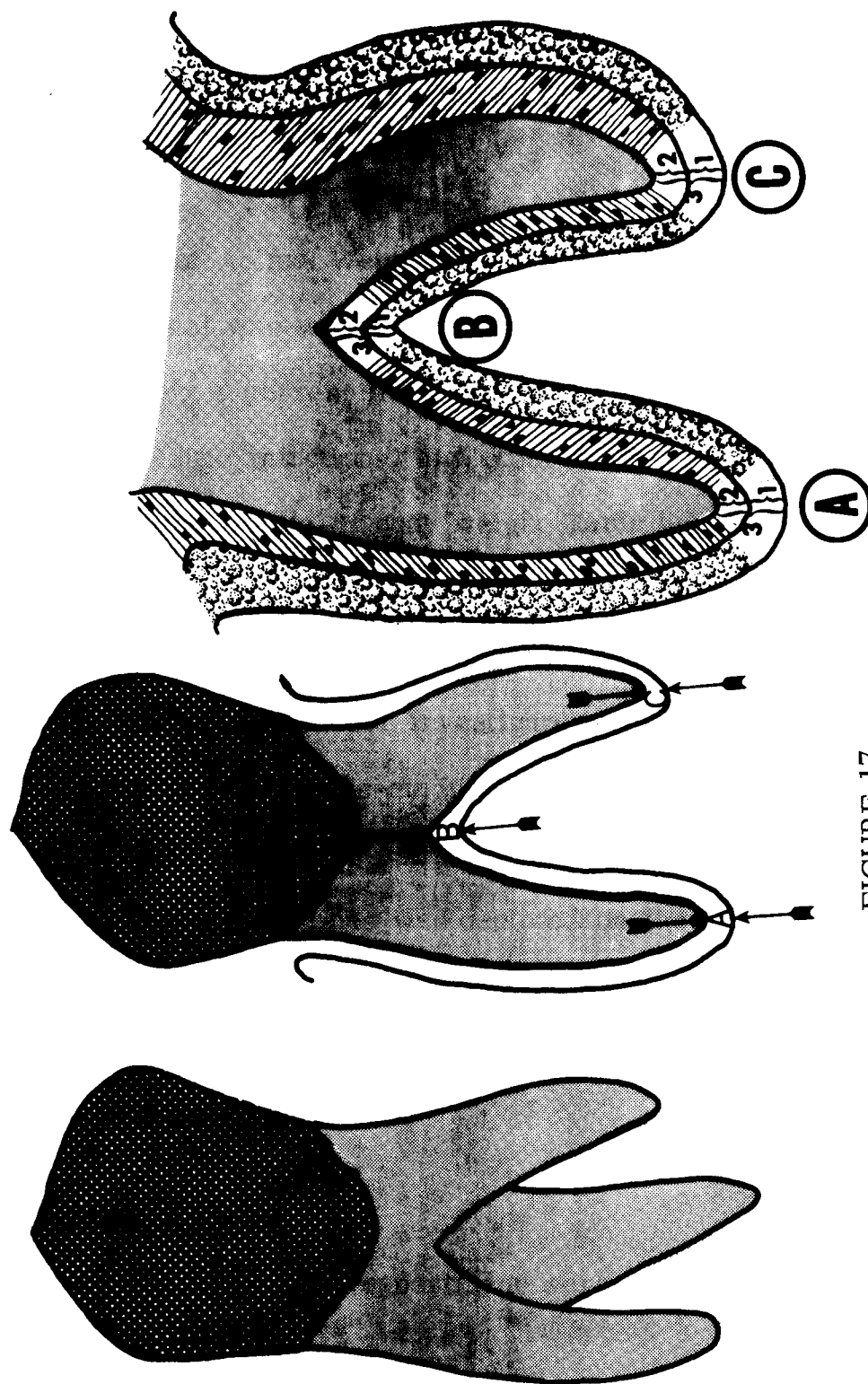


FIGURE 17

AREAS OF MEASUREMENT

CHAPTER IV

FINDINGS

A. Influence of Animal Weight Changes:

Each animal was weighed weekly during experimental treatment, thereby providing a current index to the condition of the colony. Feeding problems, parasitic infections, and other colony problems often can be detected from the trends in body weight Table I.

The analysis of variance was used to analyze the weights. The results are shown in Table IV. The ensuing experimental error appeared unreasonably large. A further investigation, therefore, was conducted to determine whether there was an interaction between Animals X Time, which might be inflating the estimate of error. An obvious lack of parallelism between the lines was depicted on the graph in Figure 18. This is indicative of a large extent of interaction between the Animals X Time. No valid estimate of experimental error could be made because of the large interaction.

B. Resume of Data:

Data taken during the experiment were described in Chapter III,

TABLE IV
ANIMAL WEIGHTS

Sources	D. F.	S. S.	V. E.
Animals	5	154.5	30.9
Time	3	19.4	6.5
Animals X Time	15	181.4	12.1
Total	23	355.3	

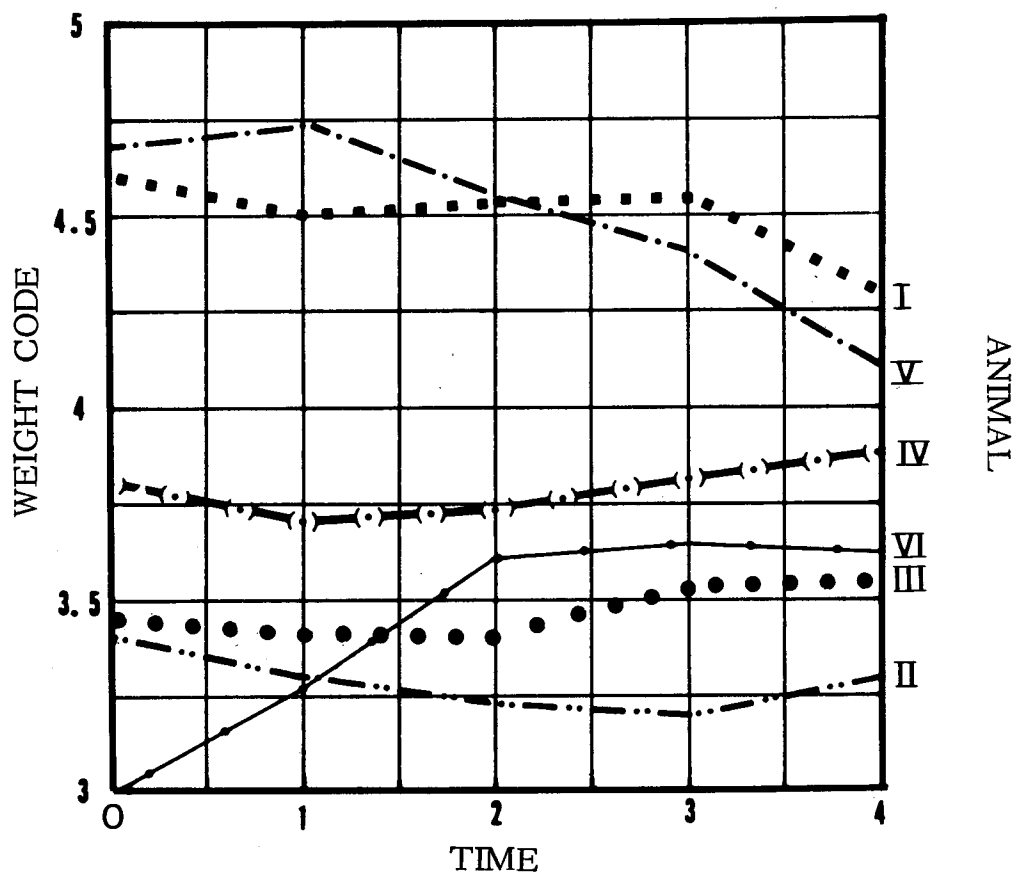


FIGURE 18

INTERACTION OF CHANGES IN ANIMAL WEIGHT

Materials and Methods. These data consisted of the following:

1. Amount of newly apposed bone during the experiment.
2. Width of the periodontal space at the end of the experiment.

A separate data sheet was kept for each animal Tables V and VI. The points in the periodontal space that were examined and measured were the apex of the mesiobuccal root, the interradicular septum, and the apex of the distobuccal root Figure 17.

The average width of the newly apposed bone was computed for the three areas of interest from the foregoing data. The average width of the periodontal space was computed for these three areas. The distance and the direction that the tooth moved could be calculated from these averages.

C. Analysis of Variance of the Apposed Bone:

Reference was made to the data sheets showing the amount of bone deposited during the experiment. It was possible to select those measurements pertaining to each of the chosen areas and to analyze these to see what significant differences could be attributed to the recognized sources of variation. The results of this comprehensive analysis are shown in Table VII. The differences between animals

TABLE V

DATA SHEET - AREAS OF MEASUREMENT FOR ANIMAL IV

Slide	Area A			Area B			Area C		
	1	2	3	1	2	3	1	2	3
1	114	45	159	98	32	130	91	68	159
2	113	46	159	95	29	124	92	67	159
3	109	53	162	96	31	127	94	64	158
4	110	49	159	95	26	121	91	70	161
5	107	54	161	98	27	125	95	62	157
6	113	47	160	98	30	128	94	64	158
7	111	48	159	98	32	130	100	65	165
8	107	49	156	97	32	129	93	64	157
9	113	44	157	91	33	124	100	66	166
10	108	54	162	92	37	129	98	56	164
\bar{X}	110.5	48.9	159.4	95.8	30.9	126.7	94.8	64.6	160.4
S.D.	2.67	3.66	1.95	2.57	3.14	3.05	3.42	3.80	3.40
S.E.	0.84	1.15	0.61	0.81	0.99	0.96	1.08	1.20	1.07

TABLE VI

DATA SHEET - AREAS OF MEASUREMENT FOR ANIMAL I - VI

Animal		Area A			Area B			Area C		
		1	2	3	1	2	3	1	2	3
I	\bar{X}	55.9	55.1	110.1	74.5	49.4	124.1	94.7	46.6	141.3
	S.D.	2.08	4.24	2.77	1.77	1.95	1.96	1.94	2.0	2.16
II	\bar{X}	35.1	40.7	4.8	107.2	35.5	143.0	50.3	50.2	101.1
	S.D.	1.91	1.56	2.14	2.81	5.70	2.74	2.74	9.15	2.64
III	\bar{X}	55.6	65.5	121.1	60.1	70.9	131.0	50.4	83.6	134.0
	S.D.	1.71	1.58	1.61	2.02	1.45	1.88	1.89	3.13	2.10
IV	\bar{X}	110.5	48.9	159.4	95.8	30.9	126.7	94.8	64.6	160.4
	S.D.	2.67	3.66	1.95	2.57	3.14	3.05	3.42	3.80	3.40
V	\bar{X}	70.1	47.3	117.4	105.2	37.2	142.9	46.7	46.7	93.4
	S.D.	2.84	3.65	2.67	2.57	7.59	2.99	1.63	1.15	2.63
VI	\bar{X}	5.5	36.1	41.6	8.5	22.5	31.0	12.0	38.9	50.9
	S.D.	.52	2.88	2.59	1.50	2.0	1.76	1.24	2.31	2.23

TABLE VII
ANALYSIS OF VARIANCE FOR THE APPOSED BONE

Source	S.S.	D. F.	Variance Estimate	Variance Ratio	F
Between Animals	140216	5	28043	5331.36	p < 1%
Between Sections	57	9	6.33	1.20	N.S.
Interaction Animals X Sections	178	45	3.95	.75	N.S.
Between Areas	13786	2	6893	1310.45	p < 1%
Interaction Areas X Sections	85	18	4.72	.89	N.S.
Interaction Areas X Animals	42203	10	4220	802.2	p < 1%
Residual Error (Experimental Error)	474 (637)	90 (153)	5.26 (4.816)		
Total	19699	179			

were highly significant and the differences between the three areas were highly significant as expected. A great interaction between the animals and areas being studied, however, was not expected. This discovery prompted some investigation of the findings. A graph was prepared (Figure 19) to demonstrate the interaction between the Areas and the Animals. The lack of parallelism in between the three plotted lines on the graph was indicative of the large interaction between these two sources of variation. The following measurements are stated in hundredths of a millimeter. The amount of bone deposited at point A in Animal I was 559. The amount of bone apposed at point A for Animal II was essentially the same, or 556. Animal III had an appreciably smaller amount of bone apposition for a value of 351. A considerably higher amount of bone apposition, 1105, was seen in Animal IV. Bone apposition was 701 in Animal V. Very little bone apposition took place in the control Animal VI, as expected. The values at points A and C respectively were 55 and 120. Animal III experienced a large amount of bone apposition at point B; 1072, but very little at point A -351. Animal V at point B had a large amount of bone apposition, 1052; and a much smaller amount at point A, 701; and far less at point G, 467.

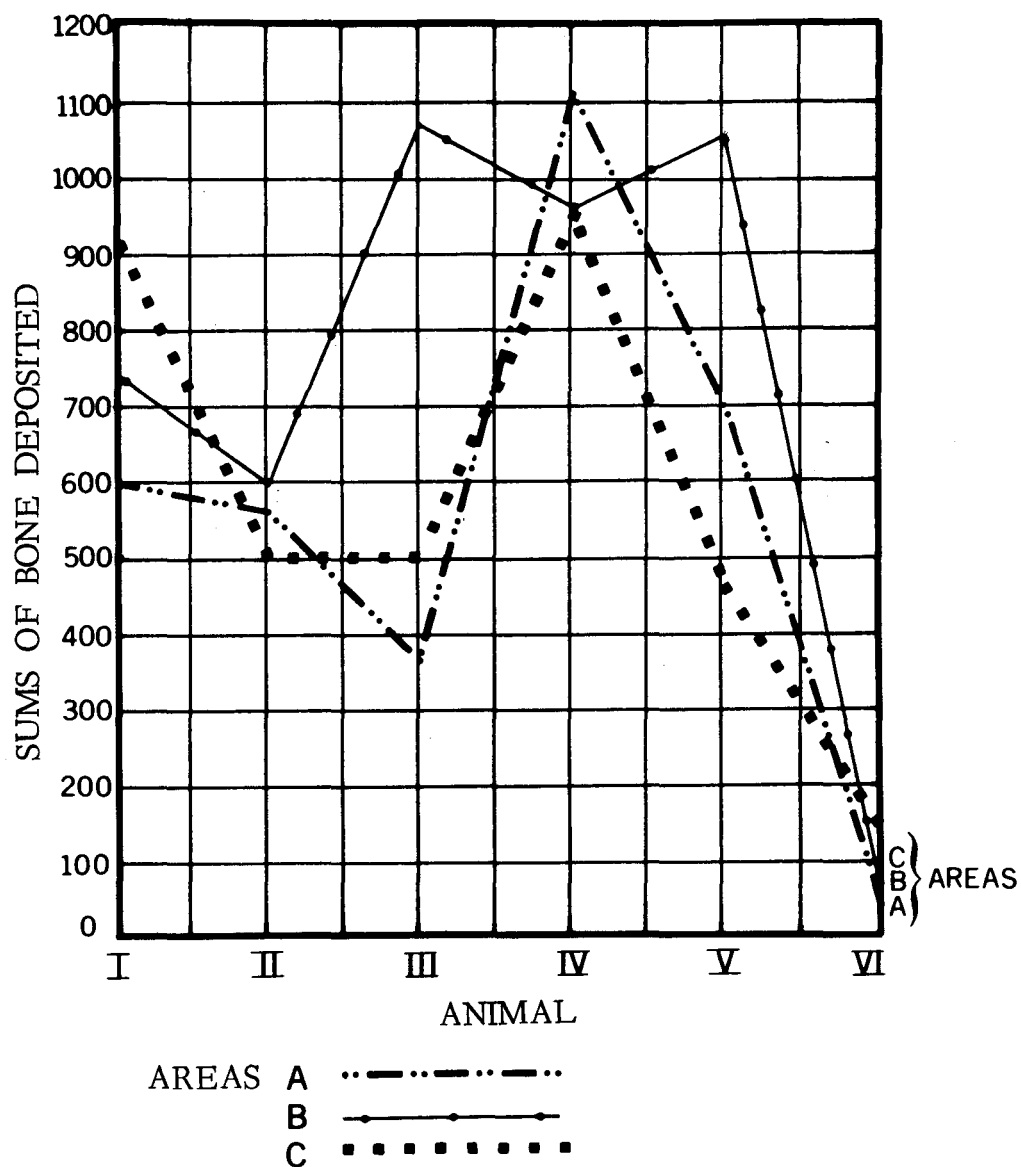


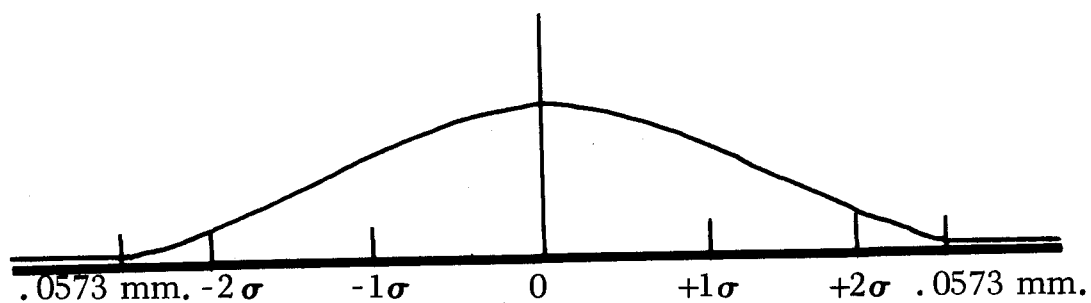
FIGURE 19

INTERACTION BETWEEN AREAS AND ANIMALS

A comparison of the experimental Animals and the Sums of Bone Apposition at various points, therefore, did not produce a parallel relationship.

Reference is made again to the results of the Analysis of Variance Table VII. The interactions between Animals X Sections and Areas X Sections are practically non-existent. They are merely independent estimates of experimental error. They can, therefore, be added to the residual error to produce 153 Degrees of Freedom, and a value of 637 for the Sum of Squares. A Variance Estimate of 4.816 was computed by dividing the sum of the squares by the degrees of freedom. This implies a standard error of measurements of 2.194 units. The standard error of measurements was 0.022 mm., since the units employed in all measurements were in hundredths of a millimeter. This means that the distribution of experimental error was approximately as illustrated in Figure 20.

With 153 degrees of freedom, the 99 per cent confidence limits were $\pm (2.2 \times 2.6)$ which equaled ± 5.73 or 0.0573 mm. This includes



S. D. = $.022 \text{ mm.}$

99% C. L. = $\pm 0.0573 \text{ mm.}$

FIGURE 20

DISTRIBUTION OF ERRORS

all sources of experimental error.

D. Tooth Movement and Center of Rotation;

Tooth movement and the center of rotation of the experimentally moved teeth were determined by applying trigonometry and plane geometry to the measurements of average root movements for each root of each tooth. Data from Animal IV will be considered to demonstrate how the calculations were made.

The mean values of the total distances from the first lead line to the tooth at areas A and C were taken from the data sheet of Table V. The mean values of the periodontal width of reference Animal VI were then subtracted from these total distances to give the estimated amount of tooth movement. The periodontal space at the apices of the reference animal at the beginning of the experiment was assumed to be about 37.5 units.

During the course of the experiment the mesiobuccal root extruded about $159.4 - 37.5 = 121.9$ units. The distobuccal root extruded about $160.4 - 37.5 = 122.9$ units. The measured distance between the mesio-

buccal and distobuccal roots was 324 units Figure 21. All the above measurements are stated in hundreths of a millimeter. The only tooth movements to be considered in this experiment will be those occurring in a mesiodistal plane. The following expression was utilized to determine the degree of tipping:

$$\begin{aligned} \arctan &= \frac{\text{Extrusion large distance - small distance}}{\text{inter-apex distance}} \\ \arctan &= \frac{1}{324} \\ \arctan &= .003086 \\ \arctan &= 0^{\circ} 10' \end{aligned}$$

The point of intercept on the horizontal line and the center of rotation was computed by using the formula:

$$\begin{aligned} \frac{\text{large distance}}{E} &= \frac{\text{Extrusion large distance - small distance}}{\text{inter-apex distance}} \\ \frac{122.9}{E} &= \frac{1}{324} \\ E &= 324 \times 122.9 \\ E &= 398.2 \text{ mm.} \end{aligned}$$

We may assume from the foregoing that the experimental tooth in Animal IV tipped $0^{\circ} 10'$ in a mesial direction and approached pure

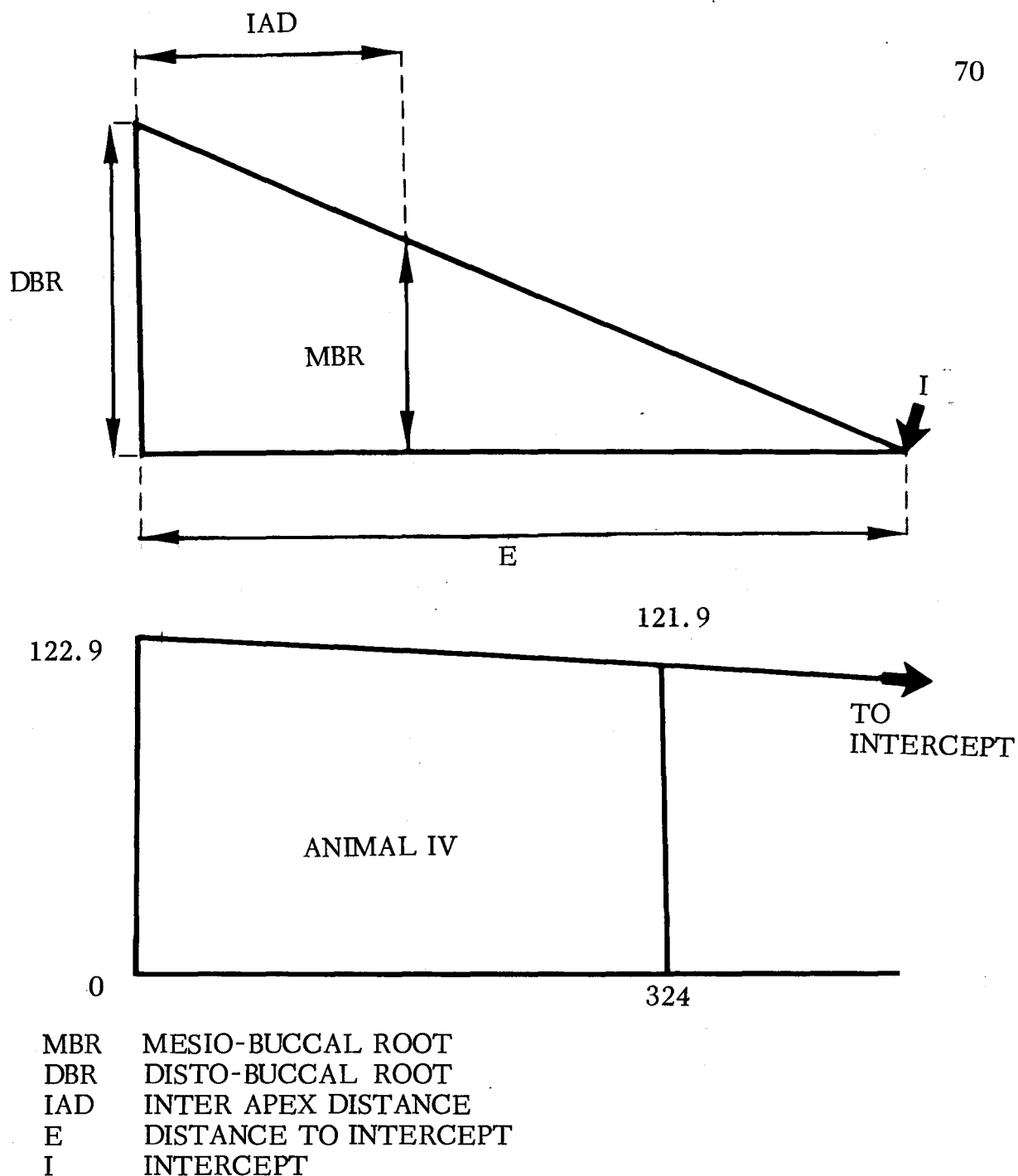


FIGURE 21

MOVEMENTS OF TEETH IN A MESIO-DISTAL PLANE

extrusion. The center of rotation was located on a line 398.2 mm. mesial to the distobuccal root apex. The foregoing procedure was followed for Animals I, II, III, V, and VI.

Animal I had an exerted force of 25 gms. and tipped $5^{\circ} 26'$ in a mesial direction. It also extruded. The center of rotation was located on a line joining the two root apices 10.92 mm. mesial to the distobuccal root apex.

Animal II with a force of 50 gms. tipped $5^{\circ} 45'$ in a mesial direction and extruded. Its center of rotation was located 6.31 mm. mesial to the distobuccal root apex on a line joining the two root apices.

Animal III had an exerted force magnitude of 75 gms. Tipping occurred $2^{\circ} 31'$ in a mesial direction with some extrusion. The center of rotation was located 21.99 mm. mesial to the distobuccal root on a horizontal line with the two root apices.

Animal V, on which a force of 150 gms. was used, tipped $4^{\circ} 43'$ in a distal direction. The center of rotation for this animal was located 9.69 mm. distal to the mesial buccal root. This tooth also extruded

The control animal, VI, had no force exerted on it. A tipping of $0^{\circ} 58'$ occurred in a mesial direction. Some extrusion took place. The

center of rotation was located on a line joining the two root apices at a distance of 5.52 mm. mesial to the distobuccal root.

The results of the foregoing computations are outlined in Table VIII. The table lists the degrees of tipping, centers of rotation and their locations.

E. Correlation Determination of Forces and Tipping:

A correlation coefficient of the experimental force magnitudes and the degrees of tipping was determined, to ascertain whether or not a significance existed. The horizontal x-axis represents the force magnitudes, and the vertical y-axis represents the degrees of tipping. The following formula was used to determine r , known as the correlation coefficient:

$$r = \frac{\sum (x-\bar{x}) (y-\bar{y})}{\sqrt{\sum (x-\bar{x})^2 (y-\bar{y})^2}}$$

$$r = -0.976$$

The significance of r was then estimated from the expression:

$$t = \frac{r \sqrt{n-2}}{\sqrt{1-r^2}}$$

$$t = 5.897 \text{ (3 D.F.)}$$

TABLE VIII
DEGREES OF TIPPING AND CENTERS OF ROTATION

Animal	Degree of Tipping	Center of Rotation from D - B Root
I	5° 26'	10.92 mm.
II	5° 25'	6.31 mm.
III	2° 31'	21.99 mm.
IV	0° 10'	398.20 mm.
V	4° 43'	-9.69 mm. (M-B Root)
VI	0° 58'	5.52 mm.

The foregoing "t" test revealed a highly significant value at the .01 level of probability. This suggests that as the experimental force magnitudes increased, the degrees of tipping decreased.

Data from the foregoing calculation were utilized to estimate a regression line. The following expression was used to determine b, the slope:

$$b = \frac{\sum (x - \bar{x}) (y - \bar{y})}{\sqrt{\sum (x - \bar{x})^2}}$$

$$b = -0.087$$

Substituting b into the equation, $y - \bar{y} = b (x - \bar{x})$, the values of the regression equation for x and y were determined:

$$y = -0.087 x + 8.836$$

$$x = 100.75 \text{ when } y = 0$$

Using the above regression equation, the following values were determined for the various experimental force magnitudes:

$$\text{When } x = 25 \text{ gms. , } y = 6.64$$

$$\text{When } x = 50 \text{ gms. , } y = 4.45$$

$$\text{When } x = 75 \text{ gms. , } y = 2.25$$

$$\text{When } x = 100 \text{ gms. , } y = 0.66$$

$$\text{When } x = 150 \text{ gms. , } y = -4.31$$

Figure 22 shows the computed regression line graphically. We may assume from the foregoing calculations that when a force of 100.75 gms. was used to extrude a tooth in this experiment no tipping occurred.

F. Bone Apposition Versus Force Magnitude:

A correlation coefficient determination was made to determine whether any significance could be attributed to bone apposition and force magnitude. The x-axis referred to the various force magnitudes exerted on the experimental animals, and ranged from 25 gms. to 150 gms. The y-values were derived from the root apices at points A and C. The total amount of bone apposed at the mesiobuccal root apex and the distobuccal apex was added and a mean value for the two was computed.

The formulas used previously for determining the values of r , and t were applied to the above data. The results of the computations revealed no significant correlation between the amount of apposed bone and the various force magnitudes applied to the experimental teeth.

G. Correlation Between Bone Apposition and Extrusion:

According to the original purpose of the experiment, it was

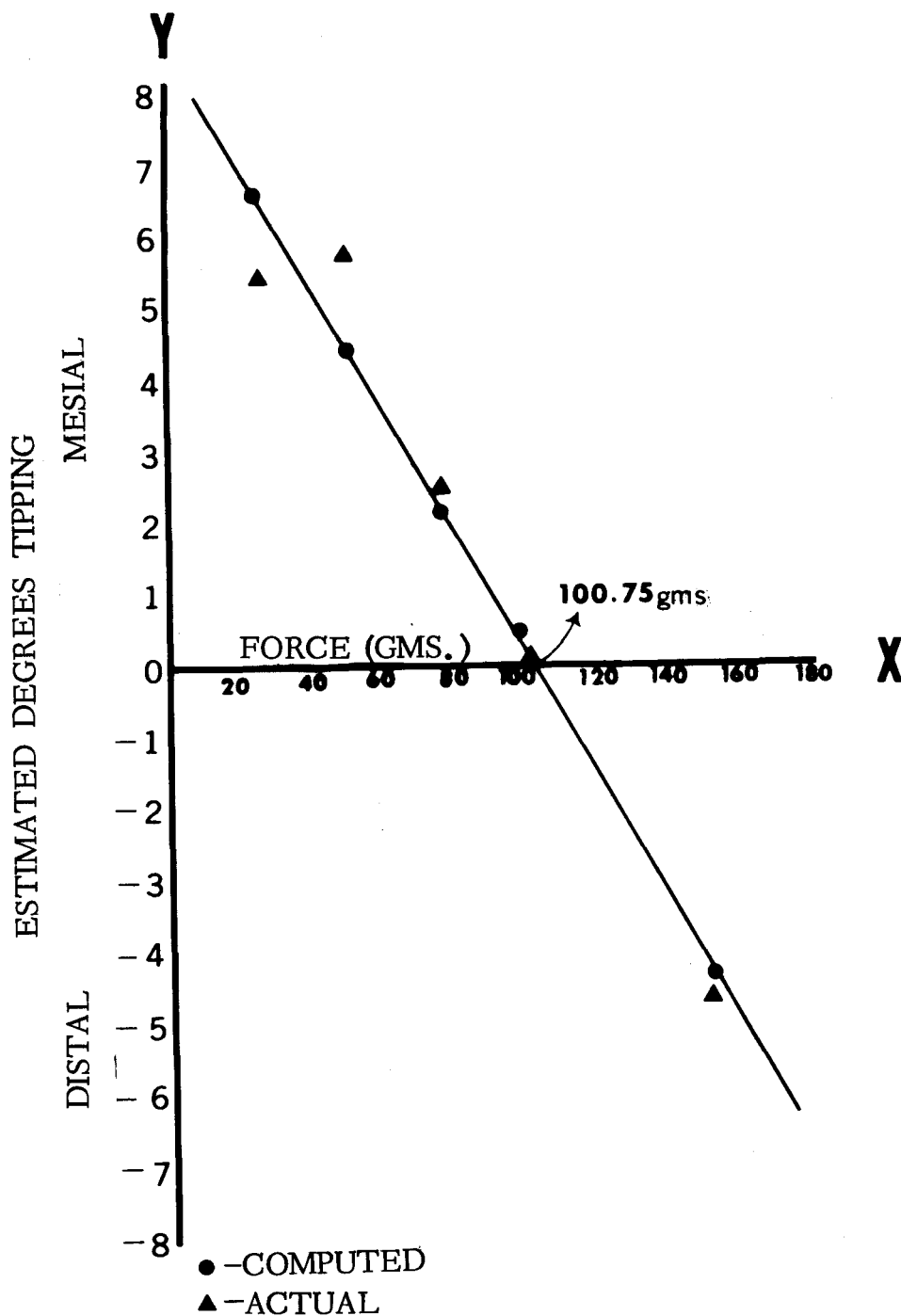


FIGURE 22

REGRESSION LINE - FORCES AND TIPPING

necessary to search for a correlation between bone apposition and extrusion of the experimental teeth.

Bone apposition was measured for each root apex. Net extrusion was estimated for each root apex. These associated values were plotted in Figure 23, and formed a scatter diagram with a recognizable trend.

The previously applied expressions for computing the values for r , and t were applied to the above data. The ensuing result of the computation were as follows:

$$r = 0.891$$

$$t = 5.544 (10 \text{ D.F.})$$

The foregoing values were valid and the "t" test denotes the high significance of the coefficient of correlation.

Additional application of the above data followed to calculate a regression line of y upon x . The equation, used previously, for determining slope or b was applied to the foregoing data. The value for b was found to be 0.9203. Utilizing additional data and equations from before, the following regression equation ensued:

$$y = .92 x + 22.8$$

$$y = 22.8 \text{ when } x = 0$$

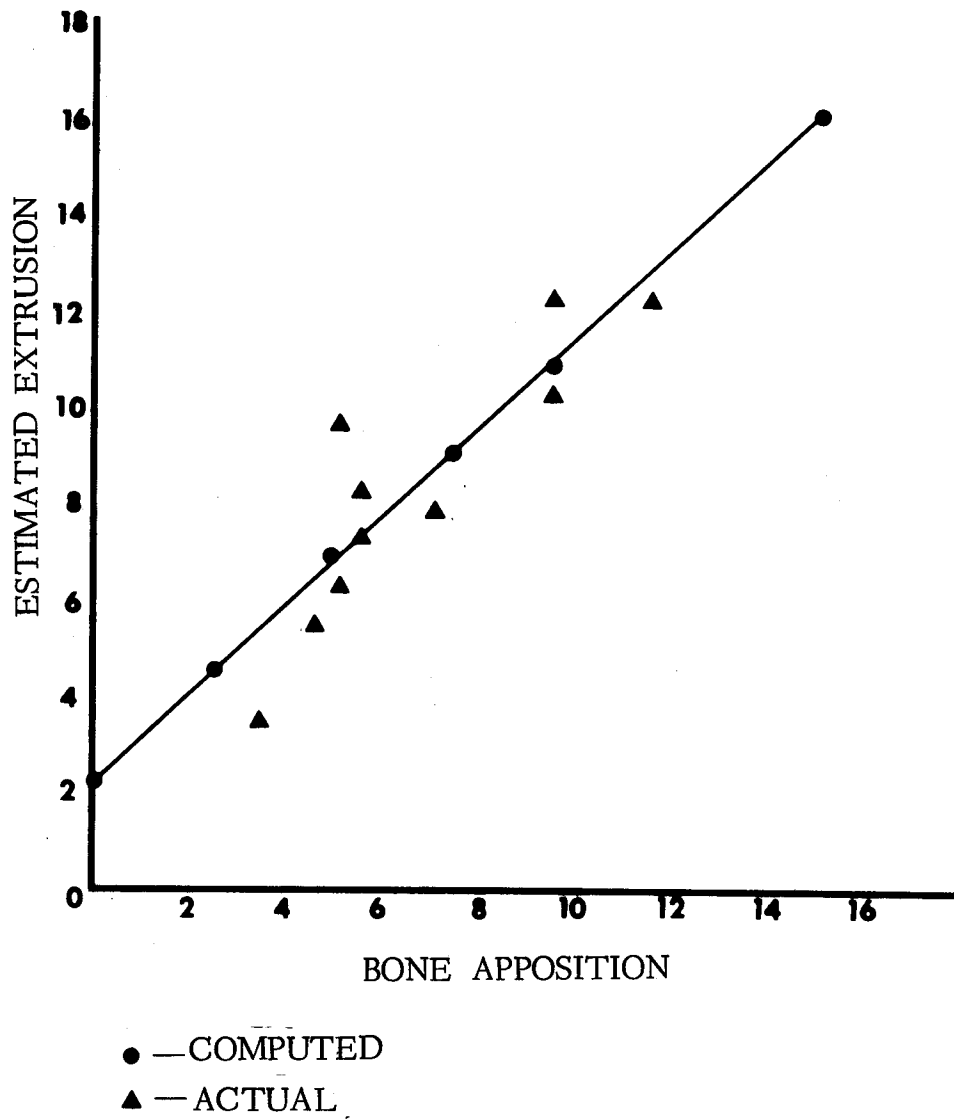


FIGURE 23

REGRESSION LINE - BONE APPPOSITION AND EXTRUSION

Substituting the various experimental force magnitudes for the value, x , the following values resulted by applying the above regression equation:

When $x = 25$ gms., $y = 45.8$

When $x = 50$ gms., $y = 68.80$

When $x = 75$ gms., $y = 91.80$

When $x = 100$ gms., $y = 114.8$

When $x = 150$ gms., $y = 160.8$

The results from the foregoing data were plotted on a graph (Figure 23), which portrayed the significant findings computed before.

H. Analysis of Variance Between Lead Lines:

Initial observation of the histologic section of the experimental animals revealed a diverse pattern of bone apposition. The course of the lead lines did not reveal a uniform, parallel pattern as expected. An irregular pattern of lead lines existed generally from area to area and from section to section. On this basis it was extremely difficult to measure changes in bone apposition as related to time, although a study was made to determine whether any relationship could be detected

Fisher's analysis of variance was used to study the relation

between the lead lines apposed each week to the time and the various sections of each root of the experiment. The results of the analysis revealed no significant difference in spacings between lines, the sections, and the spacings due to the weeks of the experiment.

The complete findings of this analysis are outlined in Table IX. The most interesting part of this table is the variance estimate due to the possible interaction between Weeks and Sections. The standard deviation due either to error or to interaction was 0.095 mm. which yields 99 per cent confidence limits of ± 0.263 mm.

I. Histologic Evidence:

Animal I

Observations were first made on the distal aspect of the maxillary second premolar root. Bone apposition occurred on the alveolar crest and along the distal surface of the alveolar wall adjoining the distobuccal root. The periodontal space appeared increased in width. The amount of bone apposition decreased upon approaching the apex of the tooth. Bone apposition had taken place in the fundus of the alveolus around the apex with osteophytes projecting into the periodontal space in an occlusal direction.

TABLE IX
ANALYSIS OF VARIANCE BETWEEN LEAD LINES

Sources	D. F.	S. S.	V. E.	Variance Ratio	F Ratio	Signif.
Weeks	3	4.2	4.2	1.54	2.96	.05 N. S.
Sections	9	15.8	1.75	1.95	2.25	.05 N. S.
W X S Interaction	27	24.4	0.90			
Total	39	44.4				

Bone apposition has occurred on the mesial aspect of the alveolar wall adjoining the distobuccal root. The periodontal space appeared widened. The amount of bone apposition increased as the interradicular septum was approached. Bone apposition had taken place on the alveolar wall adjoining the distal surface of the mesialbuccal root. The periodontal space was increased in width. Bone apposition was irregular with osteophytes projecting into the periodontal space. Lead lines were observable but they were very irregular. They followed the bone apposition pattern and the curvature of the osteophytic bone in a parallel course. Bone apposition decreased on the alveolar wall approximating the apex of the mesiobuccal root. Bone had been deposited around the apex of the mesiobuccal root with osteophytes projecting into the periodontal space Figures 24 and 25. Lead lines were again observed and followed an irregular pattern.

Bone resorption has occurred on the mesial aspect of the alveolar bone adjoining the mesiobuccal root. Howship's lacunae were observed in the alveolar wall Figure 26. The interradicular bone between the second premolar and the first premolar has resorbed resulting in a near root to root contact. The periodontal space appeared widened



RA	ROOT APEX
PD	PERIODONTAL SPACE
O	OSTEOPHYTES
A	APICAL FORAMEN

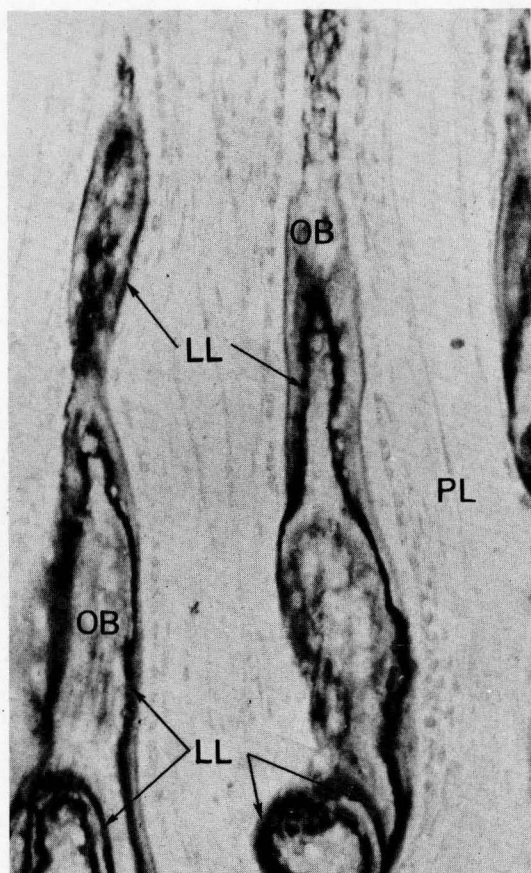
(85 X)

FIGURE 24

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

BONE APPPOSITION OPPOSITE APEX OF MESIO-

BUCCAL ROOT - ANIMAL I



LL LEAD LINES
PL PERIODONTAL SPACE
OB OSTEOPHYTIC BONE
(410 X)

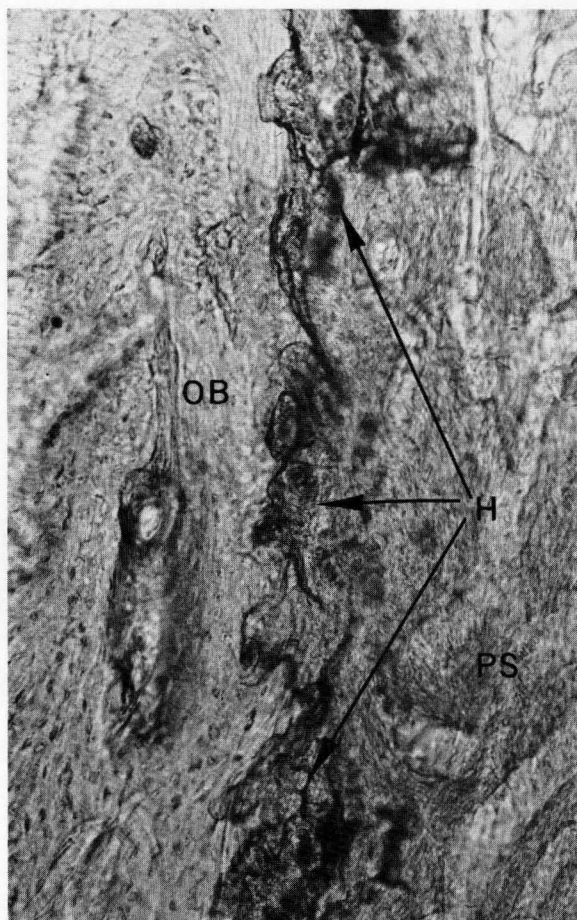
FIGURE 25

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

OSTEOPHYTIC BONE - HIGH MAGNIFICATION

OPPOSITE APEX OF MESIO-BUCCAL ROOT

ANIMAL I



H HOWSHIP'S LACUNAE
OB OLD BONE
PD PERIODONTAL SPACE
(210 X)

FIGURE 26

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

HOWSHIP'S LACUNAE IN THE ALVEOLAR WALL - MESIO-

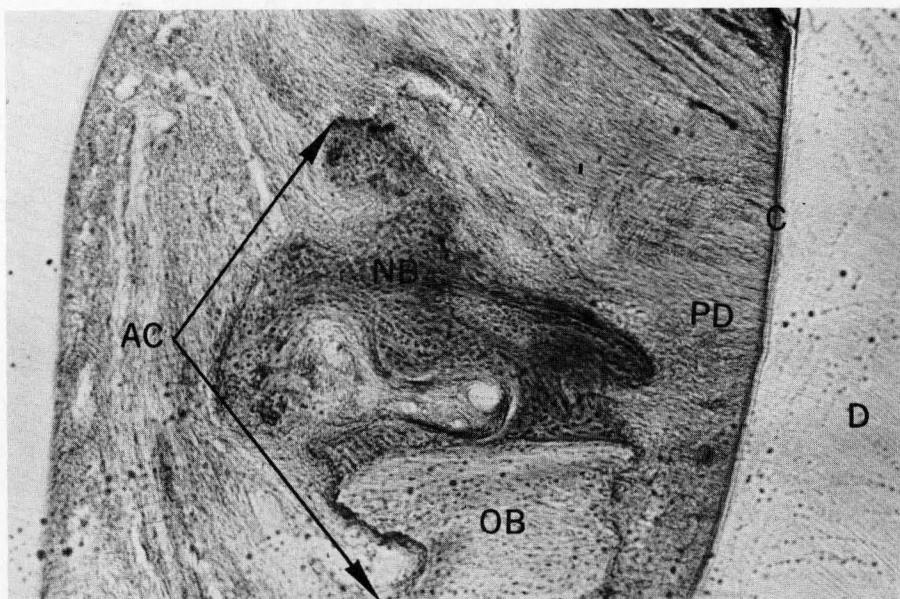
BUCCAL ROOT - ANIMAL I

further up the mesial surface of the mesiobuccal root. This was due to osteoclastic resorption as Howship's lacunae were observed along the entire length of the mesial alveolar wall. Bone apposition, however, had taken place on the alveolar crest.

Animal II

Bone apposition has occurred on the alveolar crest Figure 27. The periodontal space did not appear to be increased in width. Bone apposition has occurred along the alveolar bony wall opposite the distobuccal root. Bone apposition has occurred at the area of the fundus. This bone is osteophytic and arranged along the long axis of the tooth. The lead lines in this area, while clearly visible, were very irregular in outline. Bone apposition has occurred along the mesial aspect of the alveolar wall opposite the distal buccal root, and also on the interradicular septum. The periodontal space on this side appears increased in width.

The periodontal space along the distal wall of the mesiobuccal root appears normal in width. Bone apposition has occurred in a wedged shaped manner, being greatest at the interradicular septum (Figure 28) and tapering off towards the apex of the tooth, where some



D	DENTIN
C	CEMENTUM
PD	PERIODONTAL SPACE
NB	NEW BONE
OD	OLD BONE
AC	ALVEOLAR CREST

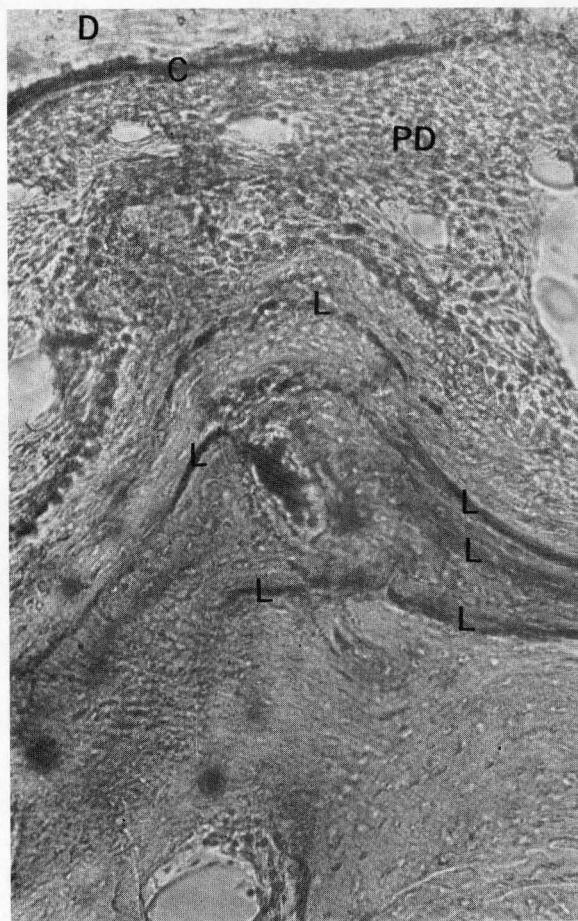
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FIGURE 27

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

BONE APPPOSITION ON THE ALVEOLAR

CREST - ANIMAL II



L LEAD LINES
PD PERIODONTAL SPACE
D DENTIN
C CEMENTUM
(210 X)

FIGURE 28
EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION
LEAD LINES IN NEW BONE AT INTERRADICULAR
SEPTUM - ANIMAL II

resorption had taken place. Bone apposition has taken place in the region of the fundus, but not to the extent as was found in the area of the fundus of the distobuccal root. Bone apposition has occurred on the mesial aspect of the alveolar bony wall opposite the mesiobuccal root near the apex and the periodontal space appeared increased in width. Areas of resorption exist opposite the middle of the mesiobuccal root on the mesial aspect. The periodontal space along the entire length of the mesiobuccal root of the mesial aspect appeared increased in width. Bone apposition has occurred on the alveolar crest.

Animal III

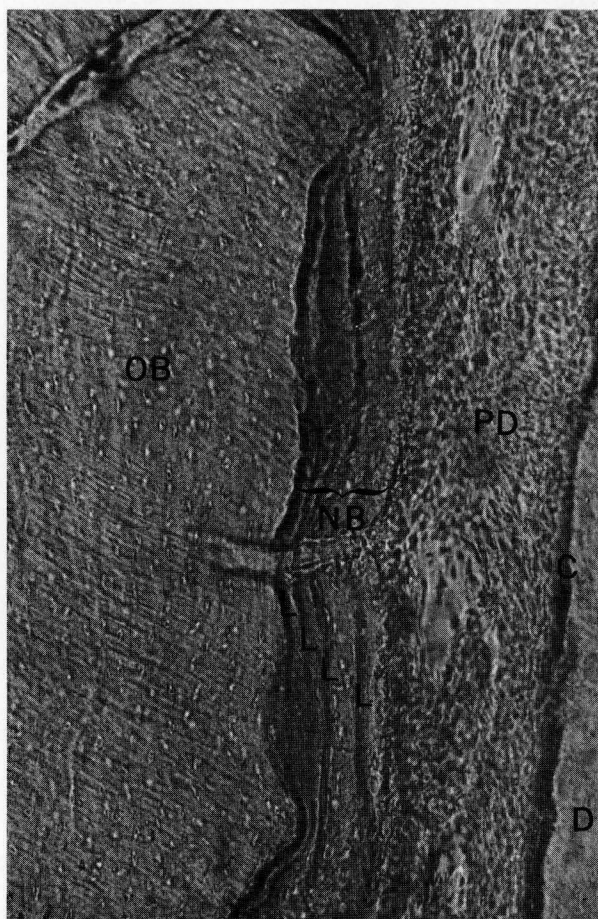
Observations in this section were first made on the mesiobuccal root. Bone apposition has occurred on the alveolar crest and appeared rounded. The periodontal space appeared increased in width and there was evidence of bone apposition on the distal aspect. The periodontal space was also increased in width on the distal aspect of the distobuccal root. Bone apposition, formed into osteophytes, were seen projecting into the periodontal space towards the root apex. The lead lines were very faint and brown in color, in contrast to the grayer appearing old bone. This contrast permitted the old bone to be easily distinguished from the

most recently apposed bone.

Bone apposition has taken place along the alveolar wall opposite the mesial aspect of the distobuccal root. Bone apposition is very irregular and osteophytic in nature Figure 29.

Osteophytic bone apposition has taken place along the alveolar wall adjoining the distal surface of the mesiobuccal root. The new bone laid down was regular all the way down to the apex. The periodontal space was increased in width. Bone apposition has taken place around the apex with osteophytes again projecting into the periodontal space, and arranged in the direction of the long axis of the tooth at the fundus. Bone apposition has taken place around the apex at the mesial aspect of the alveolar bony wall opposite the mesiobuccal root at the fundus. The amount of bone apposition decreased as the middle of the root was approached occlusally. Bone resorption then commenced at this site opposite the middle of the mesial aspect of the mesiobuccal root, and continued occlusally to the alveolar crest. Bone apposition, however, again took place on the alveolar crest.

There was a small area of resorption on the mesial aspect of the mesiobuccal root near the alveolar crest Figure 30.



NB	NEW BONE
OB	OLD BONE
PD	PERIODONTAL SPACE
L	LEAD LINES
D	DENTIN
C	CEMENTUM

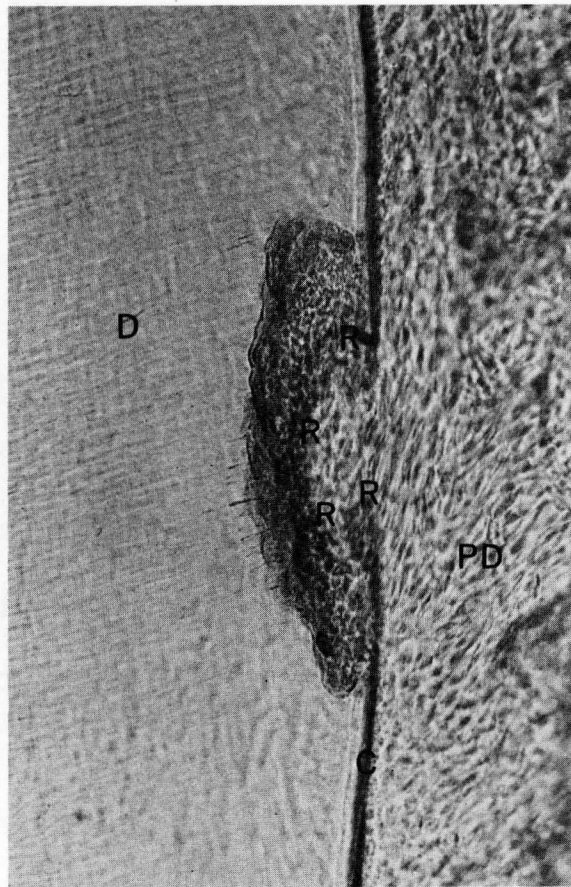
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FIGURE 29

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

LEAD LINES IN NEW BONE - MESIAL ASPECT OF DISTO-

BUCCAL ROOT - ANIMAL III



R RESORPTION
D DENTIN
C CEMENTUM
PD PERIODONTAL SPACE
(210 X)

FIGURE 30

EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION

RESORPTIVE AREA - MESIAL ASPECT OF MESIO-BUCCAL

ROOT - ANIMAL III

Animal IV

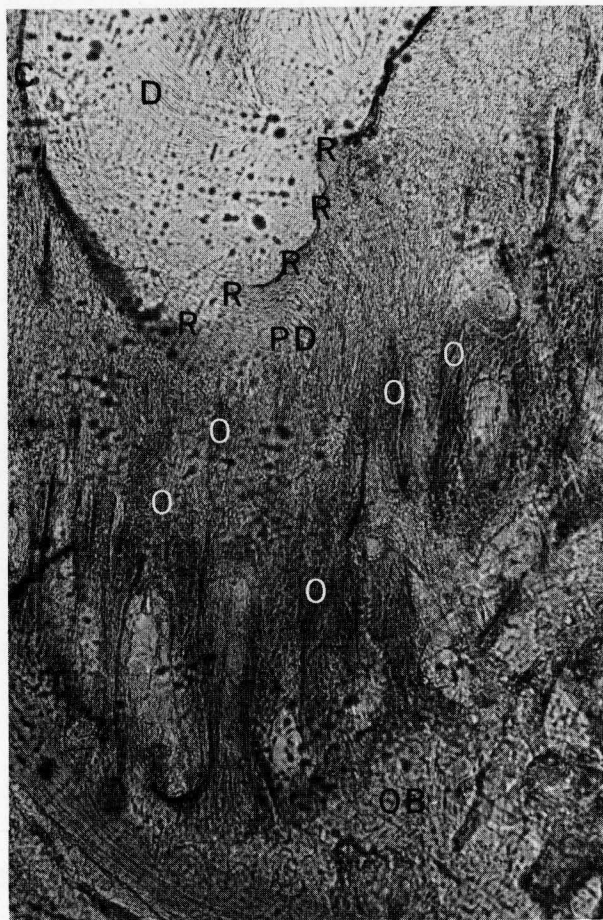
Bone apposition has occurred on the alveolar crest. The most recently deposited bone in this specimen appeared to be a more heavily brown stained color than the bone in the sections previously described. Bone apposition has occurred in a wedge-shaped manner along the distal alveolar wall and tapers off toward the middle of the root where resorption has occurred. Resorption has taken place all the way down towards the fundus where bone apposition has occurred. A large resorptive defect exists in the middle portion of the distobuccal root. The periodontal space has increased in width. Bone deposition has occurred along the distal aspect of the interradicular septum. The periodontal space appears to be increased in width. The periodontal space width is greater than the two areas previously described on the mesial aspect of the interradicular septum. The amount of bone apposition is also increased. The amount of bone apposition that has taken place around the fundus of the mesiobuccal root appears to be about equal in amount to that seen on the distobuccal root. The bone is apposed in a regular manner and is osteophytic in appearance. The osteophytes are arranged in the long axis of the tooth. Lead lines are

present but they are irregularly outlined. Root resorption has taken place in the area of the fundus on the mesial aspect of the mesiobuccal root Figure 31. Bone apposition has occurred on the alveolar crest.

Animal V

The amount of bone apposed around the apex of the distobuccal root is less than the amount apposed at the apex of the mesiobuccal root. The differences in bone apposition around the apices of the mesiobuccal and distobuccal alveolar bony walls suggest that the tooth has tipped in a distal direction.

The most recently apposed bone is very irregular and osteophytic. Bone deposition has occurred along the mesial aspect of both roots to a greater extent than the distal aspect. Root resorption has occurred on the distal aspect of the mesiobuccal root at the apex. The lead lines are seen running nearly parallel following the curvature of the osteophytic bone. Bone is apposed on the alveolar crests. Bone apposition has taken place on the interradicular septum. The bone apposition occurring opposite the root apices at the fundus is irregular and has osteophytes projecting into the periodontal space in the direction of the long axis of the tooth. The newly deposited bone is a heavily



D DENTIN
R RESORPTION
O OSTEOPHYTES
PD PERIODONTAL SPACE
OB OLD BONE
C CEMENTUM
(85 X)

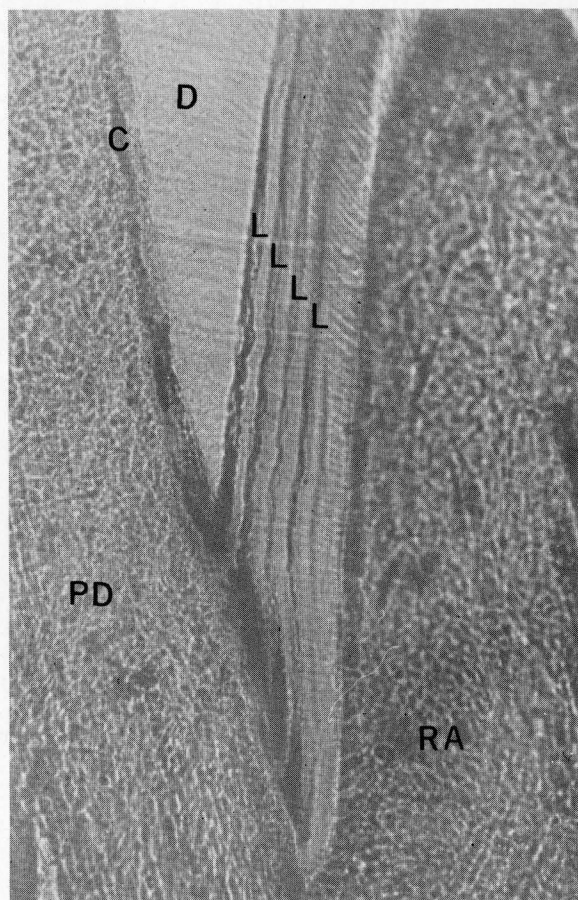
FIGURE 31
EXPERIMENTALLY EXTRUDED TOOTH - SAGITTAL SECTION
ROOT RESORPTION ON MESIO-BUCCAL ROOT APEX
ANIMAL IV

stained brown color.

Animal VI

This animal was the control. The teeth were banded exactly as Animals I, II, III, IV, and V, except no active forces were applied to the teeth. The periodontal spaces at the mesiobuccal and distobuccal roots appeared to be uniform in width. Bone apposition had occurred on the alveolar crests. The amount of bone apposition on the alveolar bone opposite the apices of this tooth at the areas of the fundus appeared to be about equal. Bone deposition has occurred on the distal alveolar bony wall opposite the mesiobuccal root. Bone resorption has taken place on the mesial aspect of the alveolar bony wall opposite the distobuccal root. Howship's lacunae are dispersed along this mesial aspect of the alveolar bony wall. Lead lines are observable but are faint and irregular in outline. Bone is apposed on the interradicular septum. All of these factors indicate this tooth was in the process of extrusion.

The lead lines in the dentin are very regular and nearly parallel at the root apex of the reference tooth Figure 32. The lead lines in the new bone opposite the root in the alveolar wall are also very regular and nearly parallel in contrast to the previous animals Figure 33.

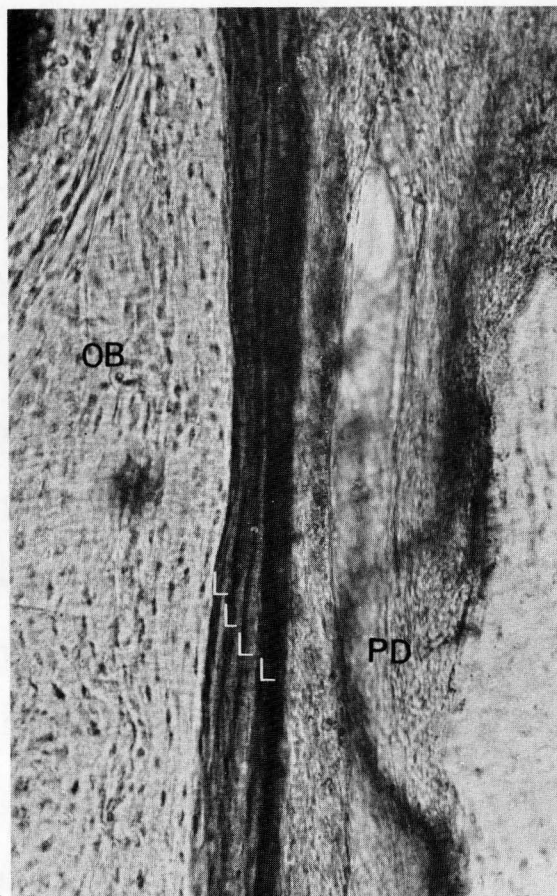


D DENTIN
L LEAD LINES
RA OPEN ROOT APEX
PD PERIODONTAL SPACE
C CEMENTUM
(210 X)

FIGURE 32

REFERENCE TOOTH - SAGITTAL SECTION

LEAD LINES IN THE DENTIN - ANIMAL VI



OB OLD BONE
L LEAD LINES
PD PERIODONTAL SPACE
(210 X)

FIGURE 33

REFERENCE TOOTH - SAGITTAL SECTION

LEAD LINES IN THE NEW BONE - ANIMAL VI

CHAPTER V

DISCUSSION

Down through the years many workers have employed ingenious methods for applying forces to teeth of their choice and measuring the resulting motions that were exhibited by these teeth. Sometimes the magnitudes of the forces applied were known, and at other times the forces were mere speculations. Sometimes the distances teeth moved were determined with a fair degree of accuracy, and at other times it was stated that these teeth moved without specifically stating whether they tipped, translated, extruded, or intruded. The over-all process of applying various types of forces to teeth, observing resulting movements of these teeth, and then endeavoring to evaluate the type of motion that occurred has been one method that has been pursued by many investigators for over fifty years.

Within the past few decades, a method to measure the apposition of bone surrounding teeth being moved orthodontically was first conceived and described by Okada and Mimura (1938, 1940, 1941, 1942). This method employs lead acetate injections given at definite intervals during periods when orthodontic forces were applied to teeth. The expectation was that

lead phosphate would be deposited in a thin layer at the time of each injection while new bone was being apposed and this would identify a layer of bone distinguishable from bone previously laid down. It would be possible ultimately to section the teeth and then count and perhaps even measure the number of lead phosphate lines that had been laid down within the newly apposed bone.

After studying initially the work of Okada and Mimura and subsequently that of Okada and Asoda (1958), it was decided that an experiment would be devised in which incremental apposition of bone might be delineated by the lead phosphate lines. This consisted basically of using a series of monkeys and providing them with carefully designed and tested orthodontic appliances, which would deliver balanced extrusive forces of known magnitudes to a chosen tooth. These monkeys would be injected with lead acetate solution at appropriate time intervals according to the method of Okada and Mimura. The animals would ultimately be sacrificed to permit one to study the resulting lines deposited in the newly apposed bone surrounding the experimentally moved teeth.

The use of this new method involved adapting certain technics,

which in themselves were not new, but which required specific investigation in order that their applicability to the problem might be determined. This might be more accurately expressed as the necessity for a determination of the precision of measurement that could be made when one studied the changes in width of the periodontal space, and amount of the newly apposed bone. To make this determination, a micrometer slide comparator (Figure 34) was used in a planned study. Three different operators were asked to measure the distance between two definite points on a slide so that a standard error of measurement could be computed. The resulting standard deviation of the distribution of experimental errors (.00322 mm.) meant that 99% confidence limits were approximately $\pm .0089$ mm. It was previously known that the least count of the measuring instrument was 0.01 mm. Through interpolation this least count was extended down to one micron or .001 mm. What has been determined is the ability to set the cross hairs at a given point. Since it is necessary to perform such a setting twice in order to obtain a measure of distance, the actual precision of measurement is at least .00456 mm. The 99% confidence limits are

$\pm .0126$ mm.

The measuring instrument itself was a microscope with a traveling stage. The stage was adjusted by means of a horizontal micrometer screw which was graduated so that readings could be taken at 0.01 mm. and interpolation could be made to 0.001 mm. The least count of the system was regarded as 0.01 mm., and therefore, our expressions for precision of measurement were in terms of ± 1.26 times the smallest graduation of the micrometer. This is not too small or too large an error. It is one that seems to be very good for the type of work that was required of it.

We have frequently referred to the expression "precision of measurement" without having mentioned anything about the accuracy. Precision can be defined as the measure of ability to replicate or duplicate measurements, whether they be erroneous or absolutely accurate. That detail of replication and ability to repeat the reading is the feature that is implied by the word precision. Accuracy may be defined as the ability to obtain an absolute value, if such exists. It means the measure of deviation from some absolute standard if

there be one in the field in which one is trying to make measurements. In the present field measurements were made in terms of fundamental physical quantities which were distance (millimeters), time (weeks), and weights of the animals (kg.). It was necessary to know the latter in order to compute the correct amount of lead acetate to be administered as a percentage of the animal's body weight in kgs. The cgs system, modified for application to this particular problem, was actually the system of fundamental units that was being used here.

It is of interest to compare the accuracy and precision attained by previous workers in their efforts to measure the movement of teeth. Storey and Smith (1952) made measurement directly on the experimental subject with a needle point caliper for calculating the magnitude and direction teeth moved. In a recent study by Jeffry and Kostiwa (1965), tooth movements were measured on casts. Lerch and Arai (1966) used a radiographic method to record changes in tooth position, stating that the 99% confidence limits on the location of their reference point formed a square measuring 0.37×0.37 millimeters. This error is

moderately large considering that distances smaller than a millimeter are to be accurately measured. The method employed in our research is unique since it utilized a definite reference line from which measurements were taken with a very precise and accurate micrometer slide comparator.

Contemplation of the technic of Okada and Mimura, led to the question of lead toxicity and whether such possible toxicity might affect the pattern of bone deposition. The LD_{50} dose of lead acetate, according to Solmann, is 0.2 gm. per kg. of body weight. The dosage administered to the animals in this study was only 4 mg. per kg. of body weight every week. The recommended daily dosage, according to Okada and Mimura is 1-2 mg. per kg. of body weight. The dosage used in this experiment was, therefore, well within the tolerable limits suggested. Signs of lead poisoning did not appear during the course of the experiment.

Quantitization of the histological evidence when analyzed statistically has disclosed some information requiring discussion, Table VII. The difference between the Animals was anticipated, since the animals had varying extrusive forces of known values. We may assume that in each animal, therefore, a stated amount of force was developed

and thereby created a certain amount of bone apposition. A difference between the Areas was not expected because pure extrusion would have shown a uniform apposition of bone at the chosen areas of measurement; hence no significant difference would have occurred in the areas. The fact that the mesial roots generally moved less than the distal roots suggested a mesial tipping.

The only changes in tooth movement that were considered in this study were those occurring in a mesiodistal plane. The findings of the tooth movement and centers of rotations are outlined in Chapter IV, D. These findings revealed extrusion with tipping had taken place in the teeth of the experimental animals. Pure extrusion was approached in only one experimental animal. This was considered to be a significant accomplishment because pure tooth extrusion is difficult to achieve. Jeffry and Kostiwa in their attempts, produced extrusion with tipping. Another observation was that mesial tipping occurred in all of the experimental teeth excepting one; this tooth tipped in a distal direction.

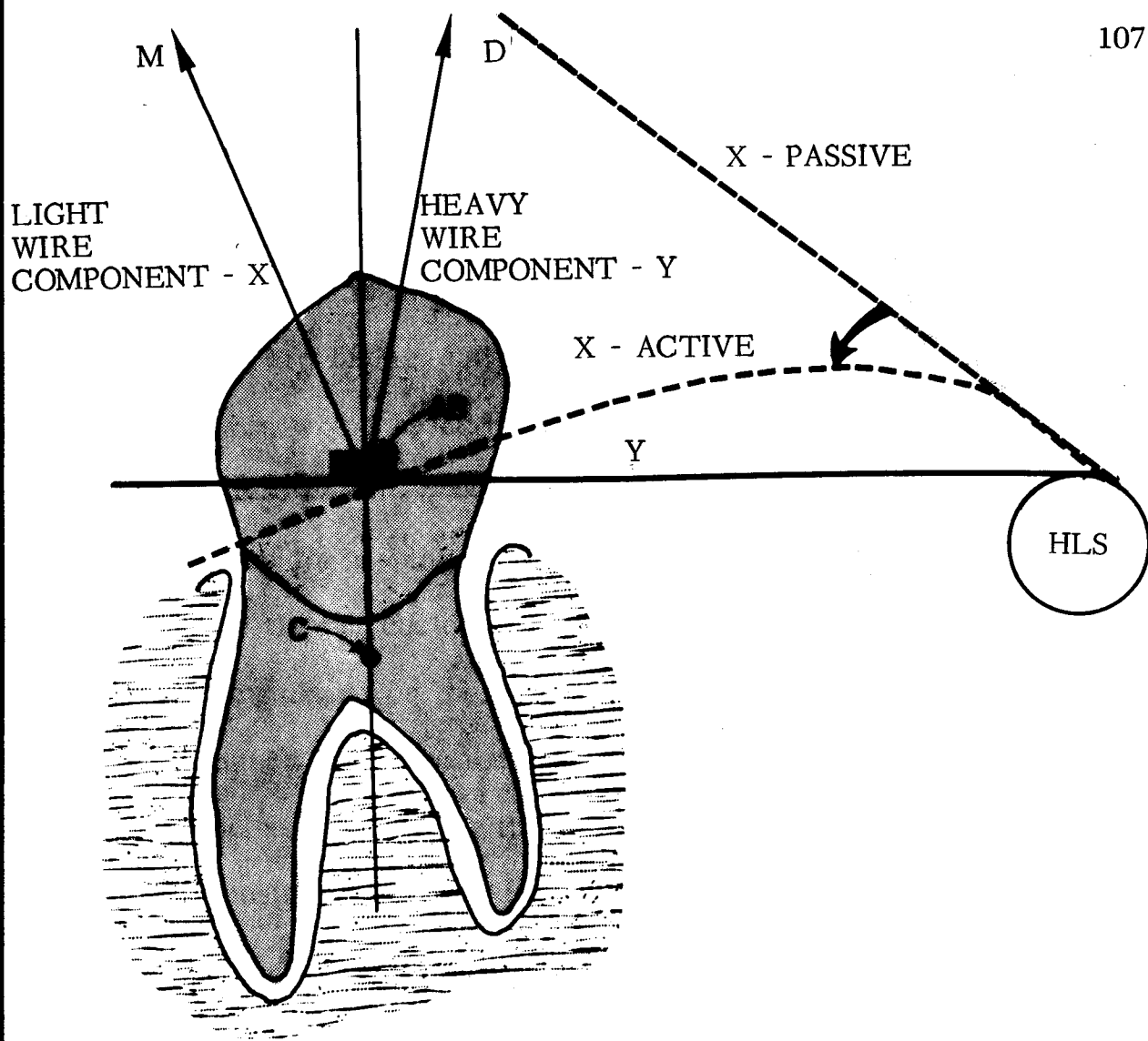
The correlation between the application of experimental force magnitudes and the degrees of tipping was highly significant. This suggested that as the experimental force magnitudes increased, the

degrees of tipping decreased. The plotted regression line led us to believe that no tipping occurred when a force of 100.75 gms. was used to extrude a premolar tooth of a monkey in this study. Although this regression line (Figure 22) was considered significant statistically, this author believes that the force of 100.75 gms. must not be regarded as an accurate or optimal force for producing extrusion.

The mechanical conditions existing in this experiment must be examined:

1. Part of this relationship (Figure 22) may be explained by the fact that the forces were developed in Animals I and II by a wire of small diameter (0.011 in.) which would have a tendency to bend when activated thus changing the action line and consequently causing the tooth to tip forward or mesially. As the force was increased in Animals III, IV, and V, the diameter of the wire was increased (0.014 in.) and, therefore, the wire did not bend as much. Hence, a more truly extrusive movement was achieved progressively. See Figure 34 for a diagrammatic description of above extreme cases.

2. Appliance design called for the helical loop springs to be placed in such a position so as to prevent breakage from biting and other oral



C	CENTROID
AB	ANGLE BRACKET
X	LOW MAGNITUDE SPRING
Y	HIGH MAGNITUDE SPRING
M-D	MESIAL-DISTAL
HLS	HELICAL LOOP SPRING

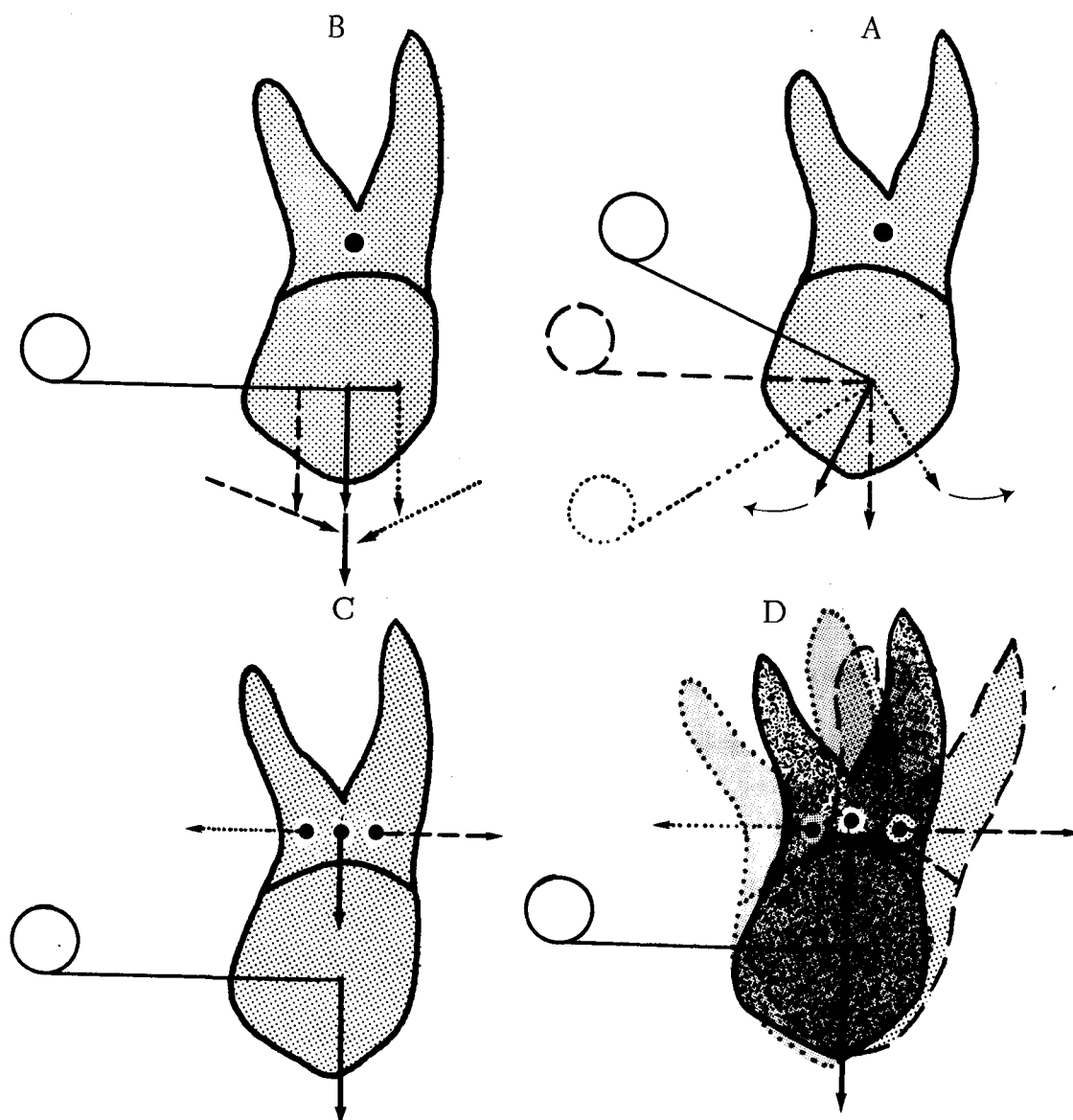
FIGURE 34

RELATION OF TIPPING TO FORCES

functions or habits Figure 35 A. For example, the coil may have been placed more occlusally in Animal IV than Animal V. The tendency would then be for the force to be more gingival and the resultant force, therefore, would be acting in a mesial direction (Animal I - IV). Conversely, if the roots were higher and the loop was more gingivally placed, the resultant force would be acting in a more distal direction (Animal V). This reasoning, based on the directions of the resultants, may be another reason for tipping.

3. The tipping could have been influenced by bracket placement. Either the angulation or the mesiodistal position of the bracket could have determined the direction of a tipping moment, Figure 35 B.

4. Another reason for the occurrence of tipping could have been due to the position of the effective centroid. The position of the effective centroid in relation to the crown would vary for several reasons. If the force on the tooth was acting vertically, it could vary in relationship to morphologic differences in the positions of the roots. In addition, if the distobuccal root in one of the experimental teeth is a very large one and the mesiobuccal root is smaller, then of course this would tend to shift the centroid towards the distal Figure 35 C.



- A CHANGE IN DIRECTION OF FORCE
 B CHANGE IN POINT OF APPLICATION
 C SHIFT OF CENTROID DUE TO MORPHOLOGY
 D SHIFT OF CENTROID DUE TO CHANGE IN AXIAL INCLINATION

FIGURE 35

TIPPING ASSESSMENT

5. A final possibility for tipping may have been due to an initial axial inclination of the experimental tooth. If the tooth is inclined mesially and a vertical or extrusive force were applied to it, tipping would occur in a distal direction. Conversely, if the tooth was inclined distally, the extrusive force would tend to tip the tooth mesially Figure 35 D.

The foregoing reasons for tipping of the experimental teeth appear to be more valid than the simpler assumption that a lighter force tipped the tooth mesially and the heavier force tipped it distally. Ideally, if one were to apply true and purely controlled extrusion at the exact point of centroid then no tipping should occur, but because of the intrinsic and extrinsic factors this is not wholly practical.

The large interaction between the Animals and the Areas being studied (apices and interradicular septum) was not expected. Figure 19 shows a series of three graphs representing the amounts of bone deposited at the chosen areas plotted against the numbers of the experimental animals. The large statistical interaction is verified by the lack of parallelism between these three lines. From this lack of parallelism we see that one cannot predict the amount of bone deposited in a given

area simply by knowing the magnitude of the extrusive force.

The interactions between Animals X Sections and Areas X Sections were practically non-existent. Therefore, they were just additional estimates of experimental error.

A correlation study was made to determine whether any significance could be attributed to the average amount of apposed bone at the two apices and the experimental force values. The correlation coefficient, r , and the "t" value from the resulting data expressed no significant correlation between the amount of apposed bone and the various force magnitudes applied to the experimental teeth. This was a situation anticipated from Figure 19.

It has long been known that when a tooth is moved in a mesial or distal direction that there is a resorption of bone on the pressure side and an apposition of bone on the tension side. In this experiment there was little opportunity for bone resorption but there was mainly the opportunity for bone apposition. This was especially true at the fundi opposite the apices. It was desirable to know whether there was a close correlation between the distance that the teeth extruded and the amount of bone added at the apices.

As shown in Figure 23, there appeared to be a linear relation between the motion of the teeth and the amount of bone added. The regression line was tested for the significance of its coefficient of correlation with the finding of a high degree of correlation between the amount of extrusion and the amount of apposed bone at the apices.

One of the goals of this experiment was to be able to discern clearly the demarcation of the lead lines within the newly apposed bone and to relate it to increments of time. Initial observation of the histologic sections of the experimentally moved teeth revealed a diffuse pattern of bone apposition. The lead lines were observable but they were very irregular. They did not follow a uniform, parallel pattern as expected. The irregularity of the lead lines existed generally from area to area and from section to section; therefore, changes in bone apposition as related to time increments could not be accurately measured.

The distances between the lead lines at a chosen area where some uniformity in outline existed, were measured on ten sections. These data were subjected to an analysis of variance to determine whether any relationship existed between the distances separating the

lead lines and the weekly injections of the lead acetate. The ensuing results revealed no significant difference between the sections and the four week period of the experiment Table VIII.

A gross general examination of the histologic sections revealed varying degrees of bone apposition and resorption. The periodontal spaces of the experimentally moved teeth varied in their widths. This was more evident at the root apices and interradicular septum. Osteophytic bone deposits were clearly demonstrated opposite the root apices at the fundus. The osteophytes appeared to be projecting into the periodontal space in the direction of the long axis of the tooth. This observation concurs with that of Huettnner and Whitman (1958). They observed that new bone spicules formed in the alveolar area formerly occupied by the apical end of the tooth. They also found that the periodontal fibers changed to follow the line of movement. Oppenheim (1933) found that newly formed spicules of bone were arranged parallel to the direction of force; namely, parallel to the stretched fibers of the periodontal membrane.

New bone appeared to have been apposed along the distal aspects of both roots more often than on the mesial aspects. The apical crests

disclosed evidences of newly apposed bone. Marshall (1931), studying extrusion of teeth, observed that bone proliferation occurred on the alveolar crests.

Staining of the newly opposed bone was diffuse and colored brown. Lead lines were observed in the new bone and ran nearly parallel following the curvature of the osteophytic bone. Definite lines of demarcation existed between the newly apposed bone and the old bone.

In the first animal there was distinct evidence of bone apposition on the distal aspects of the experimental tooth and at the fundus of the alveolus opposite the root apices suggesting that extrusion and tipping occurred in a mesial direction. There was some evidence of "jiggling" due to the general increase in width of the periodontal spaces on both the mesial and distal aspects of both roots. Extrusion, therefore, did not occur in a purely vertical direction but in a "jiggling" manner.

The experimental tooth in the second animal was extruded and tipped mesially. This is seen by the great amount of bone deposition around the apex of the distobuccal root and because of bone resorption on the distal aspect of the mesiobuccal root near the apex. The small increase in the width of the periodontal space suggests that very little

"jiggling" has taken place.

The experimental tooth in the third animal underwent extrusion as seen by bone apposition around the fundus opposite both root apices. A generalized increase of the periodontal width occurred. We may assume that this tooth was extruded with very little tipping in a mesial direction. A small resorptive area exists in this tooth on the mesial aspect of the mesiobuccal root near the alveolar crest. In view of the fact that very little or no tipping took place it must be presumed that an unusually high degree of tension of the periodontal fibers occurred at this site.

More bone apposition has occurred in the fourth animal at the alveolar crests than those previously described. The bone apposition at the apical areas and interradicular septum is also increased. Bone deposition appeared to take place uniformly on both sides of the roots. This is to be expected where a pure extrusion occurs. The roots are conically shaped and as they extrude there is a narrower portion of root lying in the remaining areas. More bone would, obviously, be expected to be apposed on both sides.

More bone was apposed around the fundus opposite the apex of the mesiobuccal root than the distobuccal root on Animal V than

was seen in the remaining animals. Bone apposition has occurred generally along the mesial aspect of the roots. We may assume from the above general description of bone apposition that this tooth has extruded more on its mesial than distal side. This suggests that this tooth tipped distally whereas the others tipped mesially.

Bone apposition also took place around the root of the tooth in the control animal, but to a much lesser degree. The periodontal space appeared uniform in width. Lead lines were irregular but distinct. This tooth approached pure extrusion with no obvious tipping. This tooth extruded because it was taken out of function when a bite plane was cemented to the anterior teeth.

We may assume from the experiment that the extrusion of multi-rooted teeth should be done with low force magnitudes even though greater magnitude forces might move the teeth faster. A force is not optimal if it causes damage even though its magnitude may seemingly be small.

The most obvious changes to the alveolar bone during orthodontic movement occurs first on the tension side, according to Weinmann and Sicher (1955). The tensions stretching the periodontal fibers

create modified bone. This modification, unfortunately, does not create similar bone changes as those seen in normal bone remodeling.

Osteophytic repairative bone, rather, is seen everywhere in the periodontal space. The osteophytes are bony outgrowths arising as thin trabeculae from a bony surface and are generally at right angles to this surface. The osteophytes represent immature, coarse fibrillar bone, the outlines of which were shown by the lead phosphate.

Osteophytes develop very rapidly, hence, the bony matrix is laid down in such a way that the lead lines appeared irregular and diffuse in many areas. A typical picture was an uneven brown staining with a dispersion of darker brown-black lines throughout. The lead acetate injections were given during the laying down of large amounts of bony matrix undergoing calcification. The insoluble lead phosphate, therefore, became irregularly deposited throughout this matrix, and could be easily distinguished from the older gray colored bone. It was the presence of this irregular outline of the lead lines that prevented the accurate measurement of the incrementally apposed bone.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A. Summary:

The purpose of this study was to observe the time rate of bone apposition resulting from the application of extrusive forces of known magnitudes to a maxillary second premolar of a *Macaca rhesus* monkey. The principles of high-deflection and low-magnitude forces were applied to the design of the force system to develop these extrusive forces. The ensuing intrinsic system, acting in a vertical direction, applied a balanced pair of extrusively directed forces to the buccal and lingual surfaces of the experimental tooth. An adequate structure was designed to resist the reciprocal intrusively directed forces and moments. The experimental tooth was out of normal functional occlusion.

Five separate magnitudes of force were selected ranging from 25 gms. to 150 gms. These forces bracketed an estimated range of empirical values of 80 - 90 gms. for the extrusion of a monkey premolar tooth.

Lead acetate was used as a vital stain because it is more sensitive to changes in calcification tide than other vital stains. Lead

lines, in the form of insoluble lead phosphate were deposited in the new alveolar bone. Although these lines were not regular, smooth, uniformly spaced curves, they served to distinguish the old bone from the newly apposed bone.

Precise readings from chosen areas of the periodontal spaces were taken and recorded by means of a special microscope which was designed to make linear measurements. Vertical changes only in the mesiodistal plane of the experimental teeth were evaluated. Histologic changes were reduced to numerical values and were analyzed statistically. Efforts were made at all times to derive a measure of the precision of the whole measuring process in order to guarantee reliability and dependability.

There was extrusion in all instances but there was also a small amount of tipping in all instances because of many uncontrollable factors.

Due to the irregular formation of bone spicules and the lack of any smooth lines in the apposed bone it was not possible to plot the actual time rate of bone apposition.

The experimental animal with the 100 gm. force applied to it displayed nearly pure extrusion and the fastest movement. From an

experimental point of view this might be considered ideal in the extrusion of multirooted teeth in monkeys, however, this force magnitude was considered excessive because of the large amount of root resorption exhibited. We may assume from this that high magnitudes of force should be avoided.

The use of lead acetate as a vital stain in this experiment did not produce any discernible signs of lead toxicity when maintained within the tolerable limits suggested by Okada and Mimura.

B. Conclusion:

1. The foregoing study utilized a unique method for observing and assessing the experimental extrusion of the maxillary second premolar of a *Macaca rhesus* monkey. This experimental method has good possibilities for determining and evaluating this tooth movement, but the heavier forces are to be avoided and even lighter forces should be used to yield less resorption and more easily measured increments of bone. The time intervals should be greater than one week to allow more space between the lead lines.

2. The force system developed controlled tooth movement which approached pure extrusion. The result of this design provided balanced

extrusive forces on two sides of the maxillary second premolar tooth. It is believed that lighter forces and better balancing should be achieved to obtain more nearly pure extrusion.

3. Lead acetate vital staining was used successfully to show the increments in newly apposed bone. Clear lines of demarcation between the newly apposed bone and the old bone were observed in the chosen areas. This method permitted a precise microscopic measurement of tooth movement.

4. Tooth movement and the center of rotation of an experimentally moved tooth can be determined by applying trigonometry and simple geometry to the measurements of average root movements for the mesiobuccal and distobuccal root of a given tooth.

5. It is believed that tipping can be reduced by bracket positioning, a study of tooth morphology and axial inclination, and an estimate of centroid position in relation to the bracket position.

6. No significant correlation could be found between bone apposition and force applied because of the difficulty of measuring the irregular increments of apposed bone.

7. In future studies of a similar nature a measuring microscope

which could be positioned orthogonally instead of in only one direction would be advisable.

8. This study has developed a more sensitive and precise method of assessing tooth movement and apposition of bone than has been reported, but it has much opportunity for improvement.

9. High-deflection and low-deflection magnitudes, as described by Jarabak and Fizzell (1963), should serve as a basis for designing a better force system to effect pure extrusive tooth movement.

10. The prompt and abundant appearance of osteophytic bone filling in the periodontal space on the tension side produced the irregular lines that prevented accurate measurements.

11. When a tooth is extruded by an excessive orthodontic force, the distribution of the bone changes is different from that found in spontaneous eruptive movement; new formation of bone along the alveolar crest and at the fundus of the alveolus differs in the two processes.

12. Detailed cytological and histological studies should be carried out to determine the exact processes involved in vital staining and other techniques.

13. As workable techniques are developed, studies should be made

of the effect of staining on longevity of the experimental animal, the behavior of the animal after the lead acetate injections, as well as the longevity of the lead lines.

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APPROVAL SHEET

The thesis submitted by Dr. John S. Theodorou 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.

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DATE

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