A Study of Factors Affecting Healing of Developing Periapical Lesions in Immature Teeth of Dogs

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A STUDY OF FACTORS AFFECTING HEALING
OF DEVELOPING PERiapICAL LESIONS IN
IMMATURE TEETH OF DOGS

by

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DEDICATION

To my "best buddy" and wife, Ann, whose love, understanding, and especially patience allowed me to continue my educational pursuits.
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VITA

The author, James Edward McCormick, was born in Owen Sound, Ontario, Canada on the first of July, 1946.

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CHAPTER I

INTRODUCTION

Dentistry has evolved into a dynamic field with new techniques being introduced to better facilitate the treatment needs of our patients. The focal infection theory has finally been discarded and replaced by sound scientific and biological principles. The age old cure of extraction to relieve a toothache has been replaced by sophisticated methods to maintain the patient's natural dentition. The long overdue general recognition of preventive dentistry has been well accepted by the dental profession and public at large.

In the past fifty years, there have been revolutionary changes in each phase of dentistry. The periodontist no longer utilizes the painful pushback procedure of alveolar denudation or other "mutilectomy" techniques. The importance of matching soft tissue and bony contours is now well accepted. New surgical techniques such as the apically repositioned flap or bone and soft tissue grafts have been introduced to better facilitate the patient's needs.

Orthodontics has introduced direct bonding brackets to aid aesthetics and home care. New types of wires are used which are easier to manipulate and which can be tempered to provide more spring action. The torque and activation forces are being incorporated into the bracket design rather than the arch wire while cephalometrics is aided by computer analysis. In addition, adult orthodontics is becoming routine and greatly
improves the functional and psychological outlook for these people who
previously were condemned to extraction and prosthetic replacement.

Prosthodontics has incorporated a thorough understanding of the
role of occlusion into their treatment regimen. The introduction of
fully adjustable articulators has been a major contribution. Overden­
tures have greatly aided the retention of removable prostheses while new
impression materials have been discovered which have improved dimen­
sional stability for longer periods of time.

Operative dentistry has modified the cavity design principles of
G.V. Black to minimize reduction of sound tooth structure. New re­
storative materials such as composite resins have been introduced and
the acid-etch technique has improved temporary therapy for fractured
teeth.

Oral surgery has developed surgical techniques to improve tooth
to jaw or jaw to cranial base relationships. Maxillary osteotomy pro­
cedures are now being used to reposition segments or all of the upper
jaw while mandibular osteotomy procedures are used to correct retro­
gnathic or prognathic jaw relationships.

However, innovations of endodontic methods have not been so nu­
merous. Endodontic pioneers such as Coolidge, Blayney and Sharp in
the early twentieth century stressed the importance of thorough canal
preparation followed by hermetic sealing of the root canal in the apical
region. These principles, reinforced by sixty years of success, remain
as the foundation of modern endodontics.
For many years, the treatment of pulpally involved immature teeth with periapical involvement has been a perplexing problem to the dentist. The introduction of the apexification technique has been an exciting innovation to simplify therapy and greatly enhance the degree of success (1). This technique, being relatively new, is subject to differences of opinion. Controversy concerning the materials used and the underlying mechanism of repair has led to numerous studies of root end induction to further understand this dynamic technique. The purpose of this study is to further clarify some of the conditions by which hard tissue repair occurs at the apices of pulpless immature teeth.
CHAPTER II

REVIEW OF THE LITERATURE

NORMAL TOOTH DEVELOPMENT AND ROOT FORMATION

Schour, Massler, and Brescia (2) described tooth development beginning as a proliferation of basal cells in the oral epithelium when the embryo is five to six weeks old. This forms an ectodermal thickening in the region of the future dental arches and is called the dental lamina. Tooth buds develop by rapid cellular proliferation at discrete points along the dental lamina which produces knobs of ectodermal cells extending into the underlying mesenchyme. Unequal growth in the tooth bud results in a shallow invagination on its deeper surface and the enamel organ becomes evident with the inner and outer enamel epithelial layers surrounding the stellate reticulum and the stratum intermedium. With continued cellular proliferation, the bud stage of the enamel organ passes through the cap and bell stages.

The organizing influence of the inner enamel epithelial cells causes the underlying mesenchymal cells to proliferate and condense into the dental papilla (the future dental pulp). After lining up opposite the cells of the inner enamel epithelium, mesenchymal cells differentiate into odontoblasts and lay down an organic matrix which calcifies to form dentin as the odontoblasts recede from the enamel organ. Once the first layer of dentin has formed, the ameloblasts move away from their position and leave behind an organic matrix which when mineralized forms enamel.
After enamel and dentin formation has reached the future cemento-enamel junction, the development of the root begins. The inner and outer enamel epithelium come together at a point termed the cervical loop to form Hertwig's epithelial root sheath. The root sheath then forms the epithelial diaphragm by bending inwards to a horizontal plane which narrows the wide cervical opening of the tooth germ. Skillen (3) believed the enamel organ extended apically as a stimulative layer to root development. Orban (4) stated the plane of the epithelial diaphragm remains relatively fixed while the epithelium proliferates coronally, resulting in a lengthening of the epithelial root sheath. Diab and Stallard (5) substantiated this spatial stability concept in an autoradiographic analysis using tritiated thymidine on the developing teeth of rats. Skillen (3) described the cells of the root sheath as being very rudimentary in character. They activated root dentin formation but lacked the physiologic properties necessary for the secretion of enamel. Schour, Massler, and Brescia (2) stated the epithelial proliferation stimulates mesenchymal cells in the dental papilla to differentiate into odontoblasts and form root dentin. Diab and Stallard (5) found a direct correlation between the number of root sheath cells preparing to divide and the process of odontoblastic differentiation. The radioactive index of the root sheath cells rose rapidly when dentin was first formed and dropped to zero when root dentin formation stopped. Kenney and Ramfjord (6), using isotopes to study root formation in rhesus monkeys, agreed that the root sheath plays a role in odontoblastic differentiation.
The next stage of root development involves cementum deposition on the root surface. Connective tissue cells from the surrounding dental sac differentiate into cementoblasts. Gottlieb (7) theorized Hertwig's sheath must degenerate prior to cementum formation and that no cementum will form in areas where the epithelial root sheath remains on dentin. Schour, Massler, and Brescia (2) stated that connective tissue elements of the surrounding dental sac proliferate into the epithelial root sheath dividing it into a network of epithelial strands. When these connective tissue cells come into contact with the outer surface of the root dentin, they differentiate into cementoblasts and deposit a layer of cementum onto the root dentin. Owens (8) speculated that as the connective tissue cells come in contact with the cells of the inner layer of Hertwig's sheath they differentiate into cementoblasts. Diab and Stallard (5) found that cementum formation was more closely related to the developmental stage and function rather than the state of cellular activity in the root sheath. They found that completion of the root by cementum formation in some instances took place only after the disintegration of the root sheath, while in other cases cementum formed over the root sheath which trapped epithelial cells between the cementum and dentin. Their conclusion that cementum formation is dependent neither on the presence nor the absence of an epithelial root sheath has been substantiated by Kenney and Ramfjord (6). Skillen (3) stated that cementum formation begins when the teeth are in occlusion due to the stimulation caused by the forces of mastication. He felt this masticatory stress will also
have a decided influence on the manner in which the apex is finally completed. Owens (8) believed that cementum formation begins during tooth eruption when the root is about two-thirds formed and there is a need for more substantial tooth attachment.

Schour, Massler, and Brescia (2) noted that proliferation of epithelium in the diaphragm lags behind that of the pulpal connective tissue in the later stages of root development and apposition of dentin and cementum results in the final narrowing of the apical foramen. Owens (8) stated that pulpal cells trapped in the dentin give rise to a bone like tissue, osteodentin, which contributes to the highly cellular root apex. Although it is generally agreed that root formation is completed about 3 years after the eruption of teeth, Friend (9) concluded that this process takes considerably longer.

Bernick, et al., (10) described how the connective tissue elements of the dental sac mature into the periodontal ligament. Schour, Massler, and Brescia (2) reported that remnants of the epithelial root sheath persist as the epithelial rests of Malassez in the periodontal ligament. However, Diab and Stallard (5) and Kenney and Ramfjord (6) showed that labelled cells of the epithelial diaphragm did not migrate into the periodontal ligament. The function of the epithelial rests is now surrounded by speculative conjecture.

**TREATMENT METHODS FOR IMMATURE TEETH**

Zeldow (11) recommended four available methods of treatment for pulpally involved and pulpless immature teeth. If the pulp remains vital,
he recommended using a pulpotomy technique to allow normal root development to continue. However, if the pulp is necrotic, conventional root canal treatment, endodontic surgery, or apexification procedures may be used for immature teeth.

A pulpotomy procedure is the treatment of choice for a young incompletely developed tooth with a vital pulp (1,12,13,14,15,16,17). This technique will allow the healthy radicular pulp and Hertwig's epithelial root sheath to continue to function in order to complete the development of the immature root apex (11,18). A wide variety of materials have been used in the pulpotomy method to obtain continued root growth. Successful results have been reported using formocresol (1), calcium hydroxide (12,19,20,21,22,23,24,25,26,27,28,29,30,31), zinc oxide and eugenol (32,33,34), a mixture of zinc oxide-metacresyl acetate-camphorated parachlorophehnol (35), iodoform Chlumsky paste (36), and glycerite of iodine paste (36). Torneck (37), performing vital pulpotomies on immature monkey teeth without using any medication, reported that root formation may continue but this growth is irregular and retarded. Furthermore, subsequent breakdown and necrosis of the remaining pulpal tissue can lead to apical abscess formation and root resorption.

Bodenham (21) and Krakow, et al., (23), stated that pulp extirpation and conservative root canal treatment after root maturation are not routinely needed. They suggested that definitive root canal treatment should only be performed when a subsequent pathological condition is detected by radiographic examination or appropriate clinical tests such as tenderness to percussion or sinus tract formation. However, chronic
inflammation may remain in the pulpal tissue and cause resorption and ap­
position on the canal walls, and pulpal calcifications may slowly render
these canals non-negotiable to endodontic instrumentation (38). Fischer
(39) has shown that teeth having pulp chambers obliterated by hard tis­sue appear to be homogenous on radiographs, but in fact contain vital
pulpal remants. He stated that this remaining connective tissue is more
susceptible to infection or necrosis which will ultimately lead to peri­
apical pathosis. While studying pulpotomy procedures in immature pre­
molar teeth of dogs, Vojinovic (36) found that after apical closure,
pulpal necrosis could still occur and lead to destructive processes in
periapical and bifurcation areas. With these unfavorable sequellae in
mind, pulpotomy is only regarded as a temporary measure which leaves a
healthy radicular pulp to permit completion of the incompletely devel­
oped root. Pulp extirpation and conservative endodontic treatment are
recommended when root development is complete (11,12,15,19,26,28,30,31,
32,33,34,35,36,38,39).

If the pulp is necrotic, one of the other three methods outlined
by Zeldow (11) must be employed. The first technique involves bio­
mechanical cleansing and obturation of the root canal. The obturation
of the root canal space has been accomplished by using Diaket (41,42,43)
oxidized regenerated cellulose and amalgam (44,45), or gutta-percha and
a sealer (15,46,47,48,49). Various techniques for the complete oblitera­
tion of the root canal space with a hand-rolled or otherwise customized
gutta-percha point have been described (34,50,51,52).
Boyle (53) explained the healing process as a deposition of cementum onto the root end to cover the root filling material and obliterate any lumen still present at the apex. He stated this process will only occur in the absence of infection. Moodnick (28) reported that since granulation tissue is a necessary precursor for healing, the removal of the bulk of necrotic tissue, medication, sterilization, and obturation of the root canal will result in a high percentage of successful cases. However, the problems associated with treating immature teeth by conventional endodontic methods are well known. Friend (9) and Duell (41) noted that root development in the labio-lingual plane tends to lag behind development in the mesio-distal plane (see diagram page 11). The divergent or blunderbluss canal has an apical dimension wider than the root canal and the narrowest portion of the root canal is in the cervical region of the immature tooth. Furthermore, according to Ingle (54), the incompletely developed canal wall is not fully calcified which makes it very porous. As a result, the wide open, sometimes divergent, apex makes it very difficult to obtain a hermetic apical seal which is one of the basic requirements for successful endodontic therapy (51).

Zeldow's (11) second method of treating non-vital immature teeth utilized surgical intervention and has been widely advocated in the past. Maxmen (55) and Ingle (56) described the post resection canal filling technique utilizing a ball burnisher at the apex as a matrix against which gutta-percha may be packed. A warm burnisher is then used to remove excess gutta-percha and to seal the apex. Patterson (51) and Sommer, Ostrander, and Crowley (52) preferred to fill the canal first with gutta-percha
MESIO-DISTAL AND LABIO-LINGUAL VIEWS OF CANAL

MORPHOLOGY AT VARIOUS STAGES OF ROOT DEVELOPMENT

(1) DIVERGENT WALLS* (2) PARALLEL WALLS*

(3) TAPERING WALLS* (4) MATURE APEX*

* as seen on intraoral periapical radiograph
before surgically exposing the apex to remove excess gutta-percha with a warm burnisher. This method will reduce surgical exposure time and minimize post-operative sequellae. Grossman (51) and Law (26) advocated filling the canal with gutta-percha before performing a root resection at a level which ensures an apical seal. Another popular surgical technique mentioned by numerous authors (19,34,43,54,58,59) involved obliterating the canal with gutta-percha followed by the surgical approach of apicoectomy and placement of a reverse amalgam restoration.

However, numerous reports (30,48,60,61,62,63) discuss the disadvantages of the surgical technique. From the patient's viewpoint, this method is the least desirable as it is an emotionally traumatic and uncomfortable procedure for a young patient. The surgical procedure may not be well tolerated and patient management can be a problem in a young apprehensive individual. Due to the difficulty in obtaining an adequate apical seal against the thin friable dentinal walls, a root resection is often necessary to obtain a greater bulk of root structure for the placement of the reverse alloy filling. This procedure will terminate root development and possibly create an unfavorable crown root ratio and hence a guarded prognosis for the treated tooth. Anatomic reasons may limit this procedure to anterior teeth, and if the periapical lesion is extensive, profound anaesthesia may be difficult to maintain throughout the entire procedure. Despite a period of initial bone apposition, long term follow up of some surgical cases reveals resorption and subsequent periapical rarefaction. Cooke and Rowbotham (60) speculated that these failures may be related to the permeability of cementum in young teeth.
Ideally, the problem of the blunderbuss apex could be solved by changing the morphology of the apical portion of the root to allow for routine obturation of the root canal with gutta-percha. Zeldow's (11) third method of treating immature pulpless teeth was the induction of biological apical continuation or apexification. There are two basic approaches to this treatment regimen. The canals are cleansed as well as their irregular walls permit, and intracanal drug therapy may be used in addition to obtain favorable conditions in the root canal. The first approach, as advocated by Frank (58), involved filling the canal to the apex with a paste dressing. On the other hand, Ostby (64) induced bleeding into the apical portion of the canal while the coronal portion of the root canal was filled with gutta-percha. When apical closure has occurred using either of these methods, the canal can be sealed conventionally using condensed gutta-percha.

Many investigators (11,28,33,34,58,60,61,65,66,67,68) who have reported successful clinical cases of induced apical closure seemed to feel that elements of Hertwig's sheath were responsible. They assumed that the formative activity of Hertwig's sheath can be preserved in pulpless teeth in spite of pathological changes in the periapical tissues. Then, once the infection has been eliminated and more favorable conditions have been created in the root canal, Hertwig's sheath will resume its role in root apex formation.

Subsequent histological investigations (18,69,70,71,72,73,74) have indicated that Hertwig's sheath does not play a role in root end induction of necrotic immature teeth. The origin of the newly formed apical barrier
is the transformation of undifferentiated mesenchymal cells of the sur­rounding periapical tissue into hard tissue forming cells such as cement­oblasts (18,69,70,72,73,75,76,77,78,79,80,81,82).

HISTORICAL PERSPECTIVE OF APEXIFICATION

Several reports in the literature are of historical interest in the development of the apexification procedure. In 1929, Applebaum (83) described two cases of cementum plug formation at the apices of immature teeth in spite of marked infection present in these teeth. Easlick (84) in 1943 reported a vital pulpotomy procedure using a zinc oxide-glycerite of iodine paste in an immature permanent incisor which had been fractured and thus exposed by trauma. Seven months later, completion of root development was seen. Johnson (85) in 1945 packed the apical portion of a pulpless immature root with bone-like salts in a gelatin base. Complete calcification of the root canal was seen on radiographs taken five months later. In 1950 Israel (47) first treated the initial symptoms to make an acutely abscessed immature incisor comfortable. He then filled the coronal half of the root canal with gutta-percha and silver cement. Twenty months later when the apex was almost closed, he refilled the canal completely with gutta-percha using a lateral condensation technique. Herbert (86) in 1959 placed a polyantibiotic paste in the apical root canal of two immature teeth with periapical radiolucencies. Radiographs taken four years later showed that the periapical radiolucencies had disappeared and apical calcification had occurred.

In 1960 Cooke and Rowbotham (60) showed it was possible to obtain
either continued root formation or an apical closure by calcific repair in apparently pulpless teeth. Their treatment procedure involved thorough canal debridement followed by canal disinfection with tricresol formalin or beechwood creosote. When the tooth was free of symptoms, they introduced an antiseptic paste of zinc oxide, iodoform, cresol, and thymol to within two or three millimeters of the apex. They reported continued success in the stimulation of apical closure, but noted that continued root development after pulpal death was atypical in form and less pointed than the corresponding normal tooth.

Ostby (64) was an advocate of the role a blood clot plays in periapical healing. In 1961, he published a report of a traumatized pulpless incisor tooth with a large radiolucent lesion around an undeveloped apex. Following canal debridement and sterilization, overinstrumentation and laceration of the periapical tissues induced bleeding and subsequent blood clot formation in the root canal. The coronal half of the canal was filled with gutta-percha and a sealer. A one year recall radiograph revealed that the radiolucency was gone and apical closure had occurred. Ostby explained that the initial fibrin clot served as a matrix for ingrowing granulation tissue which in turn was gradually transformed into fibrous connective tissue. This process started at the foramen and proceeded into the root canal. The formation of granulation tissue resulted in the resorption of surrounding canal walls followed by the deposition of cellular cementum as the transformation into fibrous tissue took place.

Following the same line of reasoning, Moodnick (28) in 1963 proposed that the simple removal of the bulk of necrotic tissue, medication and
sterilization of the root canal, and the obturation of the root canal with gutta-percha a few millimeters short of the apex would provide healing at the apex. He reported eighty percent success in fifty cases treated in this manner. He pointed out that granulation tissue is a necessary precursor of healing and that radiolucent periapical lesions as a result of pulpal inflammation consist of granulation tissue which will undergo repair once the bulk of canal irritants are removed.

Ball (65) in 1964 presented a case report of a necrotic immature central incisor which was cleansed and treated with a phenol-camphor dressing on a shortened paper point. When the acute phase had subsided, he inserted a radiopaque polyantibiotic paste into the root canal and five months later the apex had achieved full development. The polyantibiotic paste, instead of an antiseptic paste, was used to avoid the possibility of chemical irritation to the periapical tissues.

In 1964, Kaiser (87) presented reports of root end induction in non-vital permanent teeth. He was the first to report that calcium hydroxide had the capacity to induce physiologic closure of immature pulpless teeth.

Crabb (88) in 1964 used a mixture of calcium hydroxide and distilled water to achieve apical closure in immature teeth. He stated that mechanical cleansing of the root canal is of major importance in the success of root canal treatment.

Friend (42) in 1966 treated eighty-seven necrotic teeth with open apices by first providing symptomatic therapy until the teeth were comfortable and a negative culture obtained. Then he filled the canals with
Diaket and placed a permanent restoration. He reported continued root growth or calcification across the end of the root filling material in twenty of the eighty-seven cases treated.

In 1966 Rule and Winter (68) achieved sealing of immature apices with calcified material after filling the canal with a resorbable iodoform paste or a polyantibiotic paste for seven months. They suggested the final gutta-percha root filling could then be inserted one to two millimeters short of the apex. One year recall radiographs revealed closure of the root apex by calcified material.

Bouchon (89) in 1966 treated the acute symptoms of an abscessed incisor in an eight year old boy. Following canal sterilization, Walkoff's iodoform paste was inserted halfway to the apex. Apical closure was seen fifteen months later and conventional root canal therapy with gutta-percha and a sealer was performed. A one year recall radiograph showed complete healing.

Based on the osteogenic potential (90,91,92,93) and antibacterial properties (94) of calcium hydroxide, Frank (58) in 1966 gave a course of therapy to resume apical development based on normal physiological patterns. The root canal could then be obliterated by conventional lateral condensation techniques. He noted that the prime effort was to reduce the canal contaminants by biomechanical instrumentation and medication followed by the partial reduction of the root canal space with a resorbable paste seal of calcium hydroxide and camphorated parachlorophenol. He has noted four basic patterns of apical development: (a) normal development continues; (b) an obliterated apex results without
any change in the size of the root canal; (c) despite the lack of radiographic evidence, an instrument inserted into the canal will feel a definite stop which ensures that a thin calcific bridge has formed; and (d) a radiographically demonstrable calcific bridge forms slightly coronal to the apex (see diagram page 19). Any of these four results is considered successful as it permits obturation of the root canal with gutta-percha and a sealer. Frank (61) and Frank and Weine (95) re-emphasized the importance of this technique and extended its usefulness to nonsurgical treatment of the perforative defect of internal resorption.

Day (96) in 1967 treated a pulpless incisor of a ten year old girl until he obtained a negative culture. He then filled the canal with calcium hydroxide paste and a six month recall radiograph revealed a calcified barrier at the apex. He then filled the canal conservatively with gutta-percha.

In 1967 Michanowicz and Michanowicz (67) treated immature pulpless teeth by first treating the acute symptoms and obtaining two successive negative cultures. Then they placed a paste of calcium hydroxide and sterile water at the apex with a plugger followed by filling the remainder of the canal with gutta-percha and a sealer. They presented several cases illustrating root end closure of immature teeth so treated.

The use of the apexification technique has been advocated in the recent dental literature (31,97,98). Successful case reports have been given using calcium hydroxide mixed with various vehicles including camphorated parachlorophenol (20,33,63,79,95,99,100,101,102), methyl cellulose (66,103,104), water (40,105,106,107,108), Ringer's solution (18,103,
SUCCESSFUL CLINICAL RESULTS OF APEXIFICATION PROCEDURES

(a) normal root maturation

(b) apex closes but canal retains divergent configuration

(c) clinical barrier but no radiographic evidence

(d) radiographic barrier short of apex
Successful reports of apexification on immature human teeth have also been published using a zinc oxide-metacresol acetate-camphorated paramonochlorophenol mixture (35), iodoform Chlumsky paste (82), and a tricalcium phosphate resorbable ceramic (112). Nevins (113) has reported the induction of hard tissue formation within the root canal of a human pulpless immature tooth using a collagen-calcium phosphate gel. Barker and Mayne (114) reported three cases of natural apexification in the absence of any treatment subsequent to trauma. Shusterman (115) presented the unsuccessful long term result of the reverse filling and replantation of an avulsed immature incisor.

HISTOLOGICAL REPORTS OF APEXIFICATION IN HUMAN TEETH.

The first report on the histological nature of the newly formed apex of a human tooth was published by Heithersay (66) in 1970. The development of a vertical fracture necessitated extraction of this tooth which had been successfully apexified previously. The newly formed apical barrier was a mixture of pulpal tissue remnants, and regular and irregular interglobular dentin covered by thick layers of cellular and acellular cementum with attached periodontal membrane. Cvec (18) and Citrome (116) speculated that the surviving pulpal remnants were responsible for the reparative dentin formation seen in this tooth.

Holland, Souza and Russo (74) in 1973 induced root end closure using a mixture of calcium hydroxide and iodoform in six teeth for thirty days before extracting these teeth for orthodontic reasons. Histological examination of the apical barrier revealed a mixture of osteodentin and
Klein and Levy (76) in 1974 presented a case report of a successful root end induction procedure using a mixture of calcium hydroxide and metacresol acetate. Then the canal was filled with gutta-percha and sealer but a gross overfill led to a subsequent root resection. Histological examination of the root apex revealed a mixture of cellular and acellular cementum.

Cvec and Sundstrom (117) in 1974 studied the histological nature of the apex of twelve human teeth extracted for orthodontic or prosthetic reasons following root end closure using a paste of calcium hydroxide and saline. The apical barrier consisted of cementum-like tissue as well as the presence of calcified areas similar to that induced by calcium hydroxide implanted in the subcutaneous tissues of rats as reported by Mitchell and Shankwalker (90). A similar finding was reported by Ham, Patterson, and Mitchell (72,73) in root end induction experiments on immature teeth in primates.

Piekoff (79) in 1976 described a case report of the successful root end closure of an abscessed immature incisor using a mixture of calcium hydroxide and camphorated parachlorophenol. The tooth was extracted and examined histologically following fracture of the tooth which occurred during post and core preparation. The calcified cap was highly irregular and showed intermingling of a number of tissues including bone and cementoid.

In 1977 Holland, Mello and Nery (118) extirpated the pulps of twenty mature vital human teeth and inserted a calcium hydroxide paste.
The teeth were extracted from two to eighteen days later. They showed that calcium hydroxide maintains the vitality of the apical pulp stump and induces apical closure by a cementum-like hard tissue deposition. However, Weinstein and Goldman (119) performed a similar procedure on five adult monkeys but they waited for up to three hundred and thirty days before examining the apical tissues histologically. They found there was no apical bridging by calcified tissue, and thirty-seven of forty teeth treated showed periapical inflammation and granuloma formation. They concluded the metabolism of the mature apex is different from the immature apex and that calcified tissue will not form at the mature apex in response to calcium hydroxide as it will in the immature apex.

Vojinovic (82) in 1977 reported on the histological nature of the apices of four immature human teeth extracted for orthodontic or prosthetic reasons which previously had root end induction procedures by a variety of methods. Two of these teeth were treated with iodoform Chlumsky paste: one tooth had a vital pulp extirpation subsequent to trauma while the other had a necrotic pulp and acute apical periodontitis. Histological examination of these two teeth revealed identical apical barriers composed of a conglomerate of cementum and immature bone with small cavities filled with undifferentiated soft tissue. Another tooth with an acute apical periodontitis was treated by an induced blood clot which led to the formation of a calcified apical barrier composed of irregular dentin. They concluded the structure of the apical barrier does not depend on the kind of paste used to fill the canal nor on the degree of pathological changes present in the periapical tissues. The fourth tooth had a mortal
extirpation of the pulp using arsenic and no further apical development occurred.

Due to the difficulty in performing controlled studies on humans for histological examination of block sections or extracted teeth, a series of investigations on animals were performed to learn more about the mechanism and nature of the apexification process. Monkeys and dogs have been used as experimental models.

APEXIFICATION STUDIES IN PRIMATES

The first primate study was published by Steiner and Van Hassel (120) in 1971. They selected the rhesus monkey as an experimental model since it is closely related to man and the experimental results would be more comparable to actual clinical practice. In order to test their experimental apexification procedure under the most unfavorable situation likely to be encountered clinically, the pulps of the experimental teeth of five rhesus monkeys were macerated and innoculated with \textit{Streptococcus faecalis} and the occlusal access cavities were sealed with a temporary restoration. Three months later, periapical radiolucent lesions had developed. Then the teeth were debrided and a paste filling of calcium hydroxide and camphorated parachlorophenol was inserted into the root canals. The monkeys were sacrificed nine months later and an apical barrier was present in eight out of nine experimental teeth. Histological examination showed that the apical hard tissue bridge was cementum while serial sections gave the impression that cementum formation proceeded from the periphery of the apex to the center of the root in a series of
decreasing concentric rings. Complete apical closure did not occur as there was still continuity between the original root canal and the periodontal ligament.

Dylewski (70) in 1971 overinstrumented eight immature incisors of a single rhesus monkey and immediately placed a dressing of calcium hydroxide and camphorated parachlorophenol. Histological examination seventy-one days later revealed granulation tissue repair of bone defects and peri-apical destruction. Instead of a continuation of normal root development guided by Hertwig's sheath, the repair process occurring at the apex was characterized by proliferation and differentiation of the apical connective tissue into a calcified material identified as osteodentin. Dentine tubules were not seen and the growth pattern was trabecular and continuous with the predentin on the canal wall. Complete apical closure was not seen in any of the experimental teeth. Dylewski found it was not possible to correlate the histological repair process with the radiographic findings. This finding agreed with Spedding, Mitchell, and McDonald (121) who showed that radiographic interpretation of calcified repair is misleading.

In 1972 Ham, Patterson, and Mitchell (72,73) reported on the comparison between a calcium hydroxide-camphorated parachlorophenol mixture and an induced blood clot as apexifying agents in four young monkeys. The experimental teeth were left open to saliva for three days and then sealed for two months to create chronic infection and periapical lesions before the root end induction procedures were started. Apical bridge formation four months later was seen more often and in greater amounts with the
calcium hydroxide mixture. However, both methods showed incomplete apical closure composed of cellular cementum and no evidence was found of Hertwig's sheath guiding this apical development. They found if pulpal necrosis was incomplete, a more normal type of root continuation could be expected. They also reported that a negative culture gave a much better prognosis for continued apical development since no teeth with positive cultures showed apical closure. A vital dye was used after filling the teeth to demonstrate calcified tissue formation after treatment of the immature teeth.

Attempting to determine the biological effects of endodontic procedures on developing incisor teeth, Torneck, et al., (37,80,122,123) conducted an extensive series of experiments on young monkeys as follows.

In 1970 Torneck and Smith (37) described the effect of partial and total pulp removal of five immature incisor teeth in a female monkey. They performed a total pulpectomy on three teeth and partial pulpectomy on two teeth. They sealed the access cavities with amalgam and sacrificed the animal about one year later. Following histological examination of the experimental teeth, they concluded that root formation after total pulpectomy may occur but this growth is irregular and retarded. When most of the pulp was removed, the alveolar bone of the fundus tended to grow into the apical end of the canal. Any calcific bridging of the apex was related to the ingrowth of bone rather than the deposition of dental hard tissues. The presence of pulpal tissue in the apical part of the root canal indicated the regenerative capacity of this tissue as well as the difficulty in removing all pulpal remnants during
debridement procedures. Here there was an acceleration in the rate of foraminal closure by irregular dentin without a proportionate increase in root length. Subsequent breakdown and necrosis of these pulpal remnants led to apical abscess formation and root resorption. The partial pulpectomy procedure in two teeth resulted in later necrosis of the remaining vital tissue and periapical abscess formation. There was no evidence of root end closure after partial pulp removal.

In 1973 Torneck, Smith, and Grindall (80) showed that it was possible to create periapical lesions in the immature incisors of four monkeys by leaving the teeth open to salivary contamination for time periods ranging from seven to ninety-five days. Despite evidence of rather severe and extensive disease in the periapical tissues, some potential for continued root formation and apical closure remained. The source of the repair tissue was related to residual odontogenic cells of the pulp and periapical tissue which grew into the pulp space. The apical barrier which formed was an irregular deposition of dentin, cementum and bone. As a result of the Torneck study, Citrome (116) speculated that the monkey has great recuperative powers and may not be a suitable animal model for apexification studies.

In a subsequent study in 1973, Torneck, Smith and Grindall (122) left immature incisors of six monkeys open to salivary contamination for periods of fourteen to ninety-two days. Then the teeth were instrumented, irrigated, dried, and medicated with camphorated parachlorophenol before sealing with amalgam. The experimental teeth were examined histologically after time periods varying from fourteen to sixty-three days. They found
a lesser degree of postoperative root formation and apical closure when medicating the canals and sealing the access cavities as compared with leaving the canals open to saliva as done in a previous study (80). They stated that when the pulp and periapical tissues of an immature tooth are severely injured and infected, a purulent exudate may form. Leaving the tooth open will provide a pathway for drainage of this exudate. However, sealing this canal in the presence of this exudate, or factors resulting in its formation, can be detrimental rather than beneficial to the apexification process.

In a fourth study by Torneck, Smith, and Grindall (123) in 1973, thirteen immature incisors in five monkeys were left open to salivary contamination for varying periods of time from thirty-nine to 196 days. Following biomechanical preparation, a dressing of calcium hydroxide and camphorated parachlorophenol was placed in the root canals and the access cavities sealed with amalgam. Histological examination from forty-nine to 194 days later showed apical barriers composed of irregular dentin, cellular cementum and bone were present in ten out of thirteen experimental teeth. Furthermore, the root end closure was more advanced than when no treatment was provided (80) or when medication and a temporary seal was used (122). Hence, the use of a calcium hydroxide and camphorated parachlorophenol mixture as a temporary paste seal will accelerate the apexification process. However, they showed that the presence of a closed apex is not indicative of a normal periodontium. Moderate to severe inflammation which remained in the periodontal space was related to residual debris in the main canal and necrotic tissue in the
spaces and crevices of the apical bridge.

Narang and Wells (77) in 1973 implanted decalcified allogeneic bone matrix into surgically prepared cavity preparations in the apices of monkeys teeth. They showed new cementum formation within the canals and indicated that an implanted material which is acceptable to the host will cause cementogenesis in the tooth apex. Nevins (78) points out that this material is difficult to prepare so that it will conform to the shape of the root canal for an apexification procedure.

Myers and Fountain (124) instrumented immature incisors of four monkeys and left them open to salivary contamination. Then they reinstrumented the teeth and filled them with either an induced blood clot, whole blood, or saline before closing these teeth with a cavit seal. They found that ingrowth of connective tissue into the root canal space of monkeys did not occur as Ostby (64) had shown in dogs. They felt this was due to residual infection in the dentinal tubules which was toxic to tissue growth. Six immature cuspids which were left open to salivary contamination for the duration of the experiment showed apical closure or bridging by a cementoid-osteoid type of tissue. Like Torneck (80), they showed that apexification in immature monkey teeth will occur in the presence of salivary contamination.

Koenigs, et al., (125), in 1975 simulated the conditions of an open apex by overinstrumenting through the apex of twenty mature teeth in four monkeys. They filled the apical three millimeters of the canals with a tricalcium phosphate resorbable ceramic. The rest of the canal was filled with laterally condensed gutta-percha followed by a temporary
seal. Histological examination two to twenty-four weeks later revealed incomplete apical barriers had formed which resembled cementum. Regeneration of the periodontal ligament occurred and a minimal inflammatory response was seen. They concluded there was no trapped debris in the apical bridge when using this ceramic which differed from Torneck's (123) results using a calcium hydroxide mixture. Driskell (126) has used this ceramic as a tissue implant in experimentally created bone defects in dogs. As the ceramic resorbs, new tissues can proliferate and calcify to replace the ceramic. This process is accompanied by a remarkably low inflammatory response.

In 1975 Nevins, et al., (78), used polyethylene tubes with one open end of three millimeters diameter to simulate an open apex. These tubes were filled with a gel composed of native calf skin collagen fibrils and mineral solutions and implanted into the subcutaneous connective tissue of rats. Histological examination after eight weeks showed that the collagen-calcium phosphate gel was capable of inducing cellular differentiation and mineralized scar tissue formation.

In 1976, Nevins, et al., (127), conducted a short term three month study of immature teeth filled with collagen-calcium phosphate gel in four monkeys. They showed the gel acts as a resorbable substrate and induces hard tissue ingrowth to effect a physiologic closure of the root canal space. Histological examination revealed that this tissue was a vascularized cellular osteoid or cementum with pulpal remnants and osteodentin. A periodontal ligament was usually formed at the apex and no ankylosis between tooth and bone was observed. During this study,
Donlon (128) took blood samples from the monkeys every four weeks to see if the gel induced a humoral immune response. Gel diffusion and hemagglutination tests showed that no antibodies were produced in response to the collagen-mineral gel. He explained that this may not be a lack of response by the immune system, but rather an active process of T-cell suppression of B-cell function.

Nevins, et al., (129), in 1978 conducted a long term primate study comparing the collagen-calcium phosphate gel with calcium hydroxide. They used six monkeys and sacrificed three at three months and the other three at ten months. They found an equal rate of success since five out of seven using calcium hydroxide and fifteen out of twenty-one teeth using the gel showed continued apical development. Unlike the gel, calcium hydroxide did not induce hard tissue ingrowth into the root canal space. They postulated that the collagen fibers are chemotactic for host fibroblasts and also form a microscaffold capable of supporting cellular migration. The mineral crystals may serve as a nidus and seed connective tissue ingrowth which contributes to its ultimate mineralization.

Heide and Kerekes (130) performed pulpectomy procedures on eight immature monkey incisors. They immediately inserted a calcium hydroxide paste and a temporary seal. Three months later, five out of the eight experimental teeth showed an apical barrier of hard tissue.

APEXIFICATION STUDIES IN DOGS

The first study using dogs as experimental animals was performed by Camp (131) in 1968. He compared the rate of apical development in
young dogs' teeth using two different pastes and found that a mixture of calcium hydroxide and camphorated parachlorophenol was slightly more effective than a paste of calcium hydroxide and distilled water.

Torneck and Tulananda (132) in 1969 created experimental abscesses in mature dogs' premolar teeth by extirpating the pulp and leaving these teeth open to saliva for twenty to one hundred and eighteen days. Histological examination of these teeth showed that osteodentin was present in the apical portion of the tooth and this represented an attempt to effect hard tissue repair in teeth whose pulps had not been completely removed or destroyed. They showed that it was very difficult to completely remove all pulp tissue during pulpectomy procedures in dogs' premolar teeth. They also provided evidence that no repair occurred in regions where pus accumulated or where the inflammatory exudate was intense.

In 1971 Holland, et al., (133) exposed immature dogs' teeth to the oral environment following a pulpectomy procedure. They filled the teeth with calcium hydroxide alone or in combination with iodoform. In eighty percent of the experimental teeth, a bridge of hard tissue formed which was composed of cementum and/or irregular dentin. The treatment pastes worked equally well.

Binnie and Rowe (134) in 1973 studied the effects of calcium hydroxide and water, calcium hydroxide and blood salts, and Grossman's root canal sealer on the periapical tissues of pulpless teeth of young beagle dogs. Sixty eight premolar canals were opened and the pulps removed. Twenty-eight canals were filled immediately with one of the
three materials while the remaining forty canals were left open to saliva for one week before filling thirty of these canals with one of the three materials. The teeth were examined histologically at periods ranging from one to sixteen weeks. Calcium hydroxide alone was found to produce the fewest and least severe inflammatory responses in the periapical tissues and the highest percentage of successful apical closure. Furthermore, they found the percentage of successful treatments was similar for both the immediate and delayed calcium hydroxide groups. They stated that calcium hydroxide and water seemed particularly effective in infected environments. They speculated that it could be due to some bactericidal effect of calcium hydroxide in the tissues. Binnie and Rowe (135) also showed that the use of calcium hydroxide does not stimulate epithelial proliferation in the periodontal space of dogs' premolars. Calyxyl and Grossman's root canal sealer were less successful in inducing apical closure and were associated with a higher incidence of severe periapical inflammatory response.

In 1975 Mager (136) compared the effects of calcium hydroxide with resorbable tricalcium phosphate to close experimentally created open apices in dogs' teeth. After five months, an acute inflammatory reaction with no evidence of apical closure was present in the teeth treated with the ceramic. This result is vastly different from Koenigs' (125) study using monkeys. In the calcium hydroxide treated teeth, a mild chronic inflammation was present and calcification was observed at the perforation sites.

In 1974, Vojinovic (36) removed the pulps of young dogs premolars.
Some of the canals had iodoform-Chlumsky paste added immediately while the other canals were left open to saliva for one week before inserting the same paste. Histological examination of these teeth from forty-five to two hundred and sixty-five days later revealed that the root ends had closed but in overall length they were shorter than the untreated contralateral teeth. The apices were closed by an irregular calcified mass of cementum, osteodentin, and immature bone. This tissue was a product of the formative activity of cells originating from the successive differentiation of fibroblasts in the young granulation tissue in the periapex. He stated that the structure of the apical part of the root depends primarily on the presence or absence of the radicular pulp.

In 1975 Vojinovic (137) compared the root end induction qualities of calcium hydroxide with the iodoform-Chlumsky paste in pulpless immature premolars of six dogs. After the treatment pastes were inserted, half the dogs were sacrificed two months later while the other three dogs were sacrificed seven months later. He found that calcium hydroxide was more effective since apical closure was more rapid and complete. The apical barrier formed using calcium hydroxide was more compact, larger, and penetrated further into the apical part of the root canal. The iodoform-Chlumsky paste was a chronic irritant as revealed by the larger number of lymphocytes, plasma cells, and occasional mast cells seen adjacent to the paste.

England and Best (71) in 1977 removed the pulps of forty immature premolars in seven dogs. One half of the teeth were sealed with cavit
after being exposed to saliva for one week while the remaining twenty teeth were left open to salivary contamination for the duration of the experiment. The dogs were sacrificed seven to eleven weeks later for histological examination of the experimental teeth. Apical closure by a calcified bridge of cellular cementum occurred in eighty-six percent of the open group and fifty percent of the closed group. Like Torneck (122), he felt the smaller incidence of success in the closed group was due to the lack of a pathway for drainage of an inflammatory exudate which accumulated in the root canal and periapical tissues and impeded the apexification process. He also showed that apical closure in immature dogs' teeth will occur in the presence of periapical lesions without the insertion of a treatment paste. He concluded that host resistance could be much greater in dogs than in humans.

Citrome (116) in 1977 compared the effects of calcium hydroxide, collagen-calcium phosphate gel and the formation of a blood clot as inducers of root end closure in immature pulpless teeth in two dogs. Following the removal of pulpal tissue and the insertion of the treatment pastes, a vital dye was used to identify post-operative calcifications. He found that all teeth treated with calcium hydroxide showed apical calcifications three months later with a mild if any inflammatory reaction present. However, the teeth treated with an induced blood clot or the collagen-calcium phosphate gel showed severe inflammatory reactions and little evidence of apexification. He pointed out that all materials tested so far in monkeys have been successful, but not so in the dog. He concluded that the dog was a more sensitive animal and
hence the appropriate animal model for apexification studies.

THE ROLE OF pH IN INFLAMMATION

The acidity or alkalinity of a solution is an expression of the relative proportions of hydrogen and hydroxyl ions present. Sorenson (138,139) in 1909 related the concentration of hydrogen ions in a solution according to a scale running from zero to fourteen with the midpoint seven being neutrality, i.e., where there are equal concentrations of hydrogen and hydroxyl ions. This scale defined pH as the negative log of the hydrogen ion concentration in grams per litre (i.e. pH = -log₁₀ [H⁺]). Hence the pH of pure water is seven. By adding an acid to a neutral solution, the concentration of hydrogen ions increases and the pH decreases. By adding a base to a neutral solution, the number of hydroxyl ions increases with a corresponding decrease in the hydrogen ion concentration. The net effect is an increase in pH and the solution becomes alkaline.

According to Menkin (140), normal tissue pH in humans is slightly alkaline with a range of 7.2 to 7.4. Wolpert, et al., (141) indicated that the normal tissue pH in mongrel dogs varies from 7.1 to 7.2 and parallels serum pH. Glinz and Clodius (142) have stated that tissue pH measurement can be used as a circulatory test to determine whether the oxygen supply to tissues is sufficient for normal aerobic metabolism. In aerobic metabolism, glucose or glycogen is transformed into pyruvic acid which enters Krebb's cycle to supply further sources of energy. However, insufficient blood supply results in a relative lack of oxygen
and tissue cells will be forced into anaerobic metabolism. Anaerobic glycolysis converts pyruvate into lactic acid which results in an increased lactate concentration in tissues (141). Blood stasis with increased carbon dioxide concentration is an additional factor adding to the tissue acidosis. Goldstein (143) reported that poor diffusion properties of necrotic tissue will limit access to growth substances and elimination of growth products. Furthermore, phagocytic cells called to the area will undergo a respiratory burst of glycolysis during the ingestion of particles and decrease the pH within the phagocytic cells (144). Some phagocytic cells, especially polymorphonuclear leukocytes, will succumb as the pH of an exudate falls (140) and release their acidic contents to further lower the pH. All of these factors are important in the acute inflammatory process.

The acute inflammatory reaction is a dynamic phenomenon which is initiated at a normal alkaline tissue pH. According to Menkin (140), in an acutely inflamed area there is a blockage or damage to vascular channels. Due to decreased tissue perfusion, cellular energy is obtained by anaerobic glycolysis which results in the formation of lactic acid. This lactic acid production is mainly responsible for the changes in local pH of the exudate. The polymorphonuclear leukocytes are the first cells to arrive in the early stages of inflammation. Menkin reported that as the pH drops below 6.8, the polymorphonuclear leukocyte succumbs and is replaced by the macrophage. As the pH drops below 6.5, all leukocytes either die or display signs of severe cell injury which results in pus formation. He concluded that acute inflammation may be
considered a function of the hydrogen ion concentration. However, an old focus of pus may have an alkaline pH. Menkin stated:

"in a very old focus, e.g., as seen in a cold abscess or in an old inspissated suppurative lesion, the pH of such material may actually be alkaline in nature."

This is because proteolysis is also a cardinal feature of inflammation. He stated that breakdown of proteins and the production of amines and ammonia will eventually result in an alkaline suppurative focus in chronic inflammation.

When an acute inflammatory focus enters into the repairative phase, there is an increase in the local vascularity of the inflamed area and the pH of the exudate again becomes neutral or slightly alkaline. In animal studies, Menkin (140) has shown that if an alkaline pH is maintained in a wound, granulocytes will migrate to the site and resolution will be rapid.

Mitchell and Shankwalker (90) and Yoshiki and Mori (145) have confirmed the ability of calcium hydroxide to initiate ectopic calcification. However, Pisanti and Sciaky (92,93) have shown that the calcium in the hard tissue barrier was derived from the serum and not from the calcium hydroxide paste used. Numerous investigators (27,66,96,97, 145,146) suggest that the high hydroxyl ion concentration may be the factor that induces calcification. Furthermore, Tronstad (147) states that the success of calcium hydroxide in counteracting inflammatory resorption may be due to its high pH. Tronstad, et al., (148) report that
the optimum condition for hard tissue resorption is an acidic pH where acid hydrolases are active and demineralization takes place. An increase in pH will neutralize lactic acid and inactivate acid phosphatase. They stated that an alkaline pH and the presence of calcium ions may activate alkaline phosphatase which has been proposed to play a role in hard tissue formation and the repair process.
CHAPTER III

MATERIALS AND METHODS

Six healthy five month old purebred beagle littermates, each weighing between 7.1 and 9.3 kilograms, were used in this study. The animals were obtained, treated and maintained at the Loyola University Medical Center Animal Research Facility. The dogs were ear marked "QL", "QM", "QP", "QQ", "QR" and "QT" to aid identification and kept in two cages, the three males in one and the three females in the other cage. The animals were under continuous veterinary supervision and maintained on a diet of standard laboratory meal and water ad libitum.

RADIOGRAPHIC SURVEYS

Preoperative radiographs were taken to verify that apical closure was not completed prior to treatment. Subsequent films were made during each operative session and at the time of sacrifice. Following the appropriate sedation or anaesthesia of the experimental animal, lateral jaw radiographs were taken using a portable hand-held X-ray generator giving sixty kvp. at twenty milliamperes using a 0.2 second exposure time and an X-ray source-to-film distance kept close to twenty centimetres. Occlusal ultra-speed dental X-ray film* was used and developed for twenty seconds in Insta-Neg**, fixed for forty seconds in Insta-Fix**

** Microcopy, Culver City, California.
and washed with water for five minutes in a portable light-tight developing box.

**SEDATION AND ANAESTHESIA**

Each animal was premedicated with an intramuscular injection of one cc. of Inovar Vet* per seven to nine kilograms of body weight to sedate the animals and make them more manageable.

At the same time the tranquilizer was administered, the dogs received subcutaneous injections of one cc. of Atropine Sulfate Injection U.S.P.** (0.5 mg./ml.) to limit salivary flow and facilitate operative procedures.

When anaesthesia of surgical depth was required following sedation, a foreleg was shaved with electric shears, the large anterior vein located, and an intravenous injection of Sodium Pentobarbital Injection*** (65 mg./ml.) was administered. This immediate acting barbiturate was injected slowly to obtain adequate anaesthesia as determined by the loss of pedal and corneal reflexes and passage through the excitatory stage to deep, regular respiration. The average dosage needed to achieve this level was approximately two cc. and provided about one to one and one-half hours of working time. Maintenance doses of one cc. were given as needed.

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** Med Tech, Inc., Elwood, Kansas.
*** W.A. Butler Company, Columbus, Ohio.
PREOPERATIVE PREPARATION

The animals were obtained at an age of 120 days to ensure that the premolar teeth were not fully developed. When a radiographic survey was taken on one of these dogs at the age of 126 days, the primary premolars were still present and the permanent premolars were seen in an early pre-eruption stage. Subsequent visual and radiographic examinations followed the loss of primary premolars and eruption of the permanent premolars. Radiographs taken when the dogs were 170 days old (Figure 1) verified that the fully erupted permanent premolars still had incompletely developed root apices and that the operative phases of this study could begin.

The preparation of a broth containing a known concentration of Streptococcus faecalis microorganisms was prepared in order to deliver a known number of organisms to each experimental tooth. A human isolate of this organism, maintained in a refrigerated tube with a solid media slant of Trypticase Soy agar, was supplied by the Loyola University Dental School Department of Microbiology. A sample of this culture, sent to the Loyola University Hospital, was identified as a gamma hemolytic variety of Streptococcus faecalis. An inoculate of this culture was transferred to a flask containing Brain-Heart Infusion broth and incubated for fourteen hours. Then, the optical density of 0.24 of this broth culture was determined using a spectrophotometer and a Brain-Heart Infusion broth blank. Successive dilutions of this broth culture in sterile water were prepared and equal volumes from each dilution tube spread out onto Trypticase Soy agar plates. These plates
were incubated for twenty-four hours at 37°C. The number of colonies per plate was determined and the dilution factor applied to determine the concentration of microorganisms in the fourteen hour broth culture. A solution containing Streptococcus faecalis organisms having an optical density of 0.24 was determined to contain $2.63 \times 10^8$ organisms per milliliter.

The day before the initial operative procedure on each dog, a flask containing Brain Heart infusion broth was inoculated with Streptococcus faecalis organisms and incubated overnight for fourteen hours. The broth culture was then centrifuged and the supernatant fluid discarded. The remaining pellet of microorganisms was washed in sterile saline and centrifuged before discarding the supernatant fluid. This procedure was repeated three times to remove any remaining media solution. The final pellet of microorganisms was resuspended in saline and diluted until an optical density of 0.24 was obtained against a saline blank. This saline solution now contained $2.63 \times 10^8$ Streptococcus faecalis organisms per milliliter and could be used for inoculation during the first operative procedure on each dog treated that day. This method was repeated each day during the initial operative procedures.

**INITIAL OPERATIVE PROCEDURE**

Due to the length of time needed for the first treatment session, two animals were operated on each of three consecutive days. All animals received identical therapy. The first two animals, at the age of 169 days, were premedicated, weighed, prepared for treatment and anaesthetized
to the desired effect as described previously. Radiographs were taken followed by a clinical periodontal examination in order to obtain initial records. A mild gingivitis, typical for this age and breed of dog, was present, but there was no evidence of calculus deposits or periodontitis. The maxillary and mandibular second and third premolar teeth were used which provided a total of sixteen wide open root apices in each of the six animals.

The maxillary right second and left third premolars, termed the "control" teeth, had no operative procedures performed in order to observe normal root development. The mouths of the animals were held open with a spring loaded mouth prop and coronal access was gained into the pulp chambers of the remaining experimental teeth using a sterile #556 carbide bur in a high speed handpiece. The length of the canals was determined radiographically with endodontic files in place as described by Weine (1).

A pH determination of the periapical tissue immediately adjacent to the apical foramen was taken using pH microelectrodes (see page 44) and a standard laboratory pH meter. During this procedure, the glass standard M1-408 microelectrode*, which was protected by a bevelled stainless steel sleeve, was inserted into the root canal to the level of the apical foramen in order to establish contact with periapical tissues. The calomel reference M1-402 microelectrode*, its' tip coated with

PHOTOGRAPH OF pH MICROELECTRODES (actual size)

LEGEND

G - Glass standard microelectrode
C - Calomel reference microelectrode
EKG gel* to aid conduction, was placed on the buccal attached gingiva in the region of the tooth being tested. The pH was measured following a one minute period of stabilization for the pH meter. Calibration of the electrodes prior to, and following, insertion of the electrodes was performed using standard buffers of pH four, seven and ten.

Under radiographic control, the pulps were removed using barbed broaches, Hedstrom files, and copious sterile saline irrigation. Great care was taken to remove as much of the pulpal tissue as possible to the level of the radiographic apex without damaging the thin, friable dentinal walls of the root canal. Then, paper points were inserted to control hemorrhage and dry the canals.

In order to infect each of the experimental teeth with an equal number of *S. faecalis* microorganisms, twenty-five microliters of the freshly prepared saline and *S. faecalis* solution were drawn into a fifty microliter pipette and inserted into the root canals. Hence, each tooth was infected with approximately 675 *S. faecalis* microorganisms. Cotton pellets were placed in the pulp chambers and the teeth were double sealed with a stop of base plate gutta-percha followed by Class I amalgam restorations. A radiographic survey was taken before the animals were returned to their cages. Before beginning the second operative session, a period of one week was allowed for the spread of periapical infection.

* Redux Creme, Hewlitt-Packard Medical Electronics, Waltham, Mass.
SECOND OPERATIVE PROCEDURE

The dogs were again treated in groups of two on three consecutive days. Following appropriate anaesthesia, the premolar teeth were examined for tooth mobility and sinus tract formation. The restorations were removed from the appropriate experimental teeth and a pH reading was taken of the tissue slightly beyond the root apex. The root canals were copiously irrigated with sterile saline, carefully instrumented with endodontic files to remove any remaining soft tissue, and dried with sterile paper points.

A thick acidic paste of methylcellulose, sterile water, and hydrochloric acid was mixed to a pH = 3 while an alkaline paste of methylcellulose, sterile water, and sodium hydroxide was mixed to a pH = 11. The pH of these pastes was verified by a pH meter before they were packed into five cc. disposable syringes. The acidic paste was injected into the maxillary right third premolars and the mandibular left second premolars while the basic paste was inserted into the maxillary left second premolars and the mandibular right third premolars. No attempt was made to keep the paste within the confines of the root canal. Sterile cotton pellets were inserted into the pulp chambers and the access cavities were sealed as before with gutta-percha and amalgam. Radiographs were taken and each dog received an intraperitoneal injection of the vital dye Procion Red H-8B* (100mg. per kg. of body weight) dissolved in ten cc. of sterile saline. This fluorescent vital dye was used to demonstrate

* Polysciences, Inc., Warrington, Pennsylvania
formation of any calcified tissue added to the apex after treatment of these teeth.

The pattern of control and experimental second and third premolar teeth in each dog is summarized in the following table:

<p>| | | | | |</p>
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| 2 | 2 | 3 | C - control
| A | C | B | I - infected control
| B | I | A | I - infected and acidic treated
| B | I | A | I - infected and basic treated
| 3 | 2 | 3 | Total of six dogs
| 3 | 2 | 3 | Total of sixteen canals per dog

**SACRIFICE PROCEDURE**

The six dogs were sacrificed at four different post-treatment intervals: one female at one week; two males at one month; one male at three months; and two females at six months.

The animals were anaesthetized, radiographed, and examined for mobility, sinus tract formation, or exfoliation of the treated teeth. The restorations were removed from all the experimental teeth and a pH reading was taken of the apical region. Each animal was sacrificed by giving an intravenous injection of five cc. of Beuthanasia-D*, a highly concentrated solution of sodium pentobarbital, specifically designed for rapid and painless euthanasia of animals within ten seconds after administration of the drug. The soft tissue was dissected from the surrounding

* Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California.
bone and jaw sections containing the experimental and control teeth were removed using an electric reciprocating bone saw and immediately placed in a ten percent neutral buffered formalin solution for fixation.

**HISTOLOGICAL PREPARATION**

Unnecessary hard tissue was removed with high speed burs to facilitate tissue fixation. The formalin solutions were changed every twenty-four hours for the first few days and the specimens remained in formalin for at least two weeks. The jaw sections were then removed from the fixative and rinsed under running water for twenty-four hours.

The specimens were decalcified for four to five weeks in a solution made up of equal parts of solutions of fifty percent formic acid and twenty percent sodium citrate until they were radiographically radiolucent and of a rubber-like consistency. They were trimmed with a razor blade into blocks containing the premolar teeth and decalcified for several more days to allow better penetration. The individual blocks were dehydrated in increasing concentrations of alcohol, embedded in paraffin, and seven micron sections were cut and mounted on glass slides. The sections were deparaffinized, hydrated, and alternately left unstained for fluorescent microscopy and stained with hematoxylin and eosin or Masson's trichrome connective tissue stain for light microscopic examination. A complete radiological and histological examination and evaluation of the reactions of the teeth and associated periapical structures was conducted.
The dogs did not seem to suffer any ill effects from the operative procedures and all animals appeared healthy with no serious weight loss throughout the study.

**CONTROL TEETH**

Two maxillary premolars in each animal, which served as vital control teeth, displayed no adverse clinical signs throughout the study. These teeth were not mobile and did not have any associated draining sinus tracts.

Radiographic findings (Table I), in all animals at the beginning of the study and in the one week animal at sacrifice, revealed that the control teeth had open apices and the walls of the root canals were widely divergent from the middle third of the root to the apex (Figures 1, 2, and 3). The one month animals at sacrifice showed complete closure of the apex in one canal, partial closure in four canals, while three canals still had blunderbuss configurations (Figure 4). The apical development of the distal root tended to lag behind that of the mesial root. The apices of all control teeth were completely formed in the three and six month animals at sacrifice (Figures 5 and 6).

Histological examination of the control teeth in the one week animal revealed an open apex, a healthy periodontal ligament, epithelial
attachment, and surrounding alveolar bone (Figures 7, 8, and 9). The pulpal tissue consisted of loose connective tissue with a layer of odonto­blasts lining the dentinal walls. Hertwig's epithelial sheath was clearly seen as a two-cell wide band of epithelium running at right angles to the long axis of the tooth toward the center of the tooth to form an apical diaphragm. In the one month animals, similar findings were evident in two of the four control teeth. The other two teeth showed partial apical closure and a mild pulpal hyperemia and congested blood vessels were seen in the dental pulp (Figure 10). The three and six month animals' control teeth had completed apical development. The periodontal ligament was narrower and composed of dense fibrous connective tissue. Reversal lines were evident in the cribriform plate. Mild hyperemia was present in the pulpal tissue. The apical arborization of the root canal was clearly evident with the main canal breaking up into a complexity of fine channels which radiate peripherally through the apical cellular cementum (Figures 11 and 12). An absence of an inflammatory response was evident in the periapical tissues of the control teeth throughout the duration of this study.

**INFECTED-ONLY TEETH**

Two mandibular premolars in each animal, which served as infected pulpless control teeth, had the pulps extirpated and were infected with *S. faecalis* for the duration of the experiment.

The periapical tissue pH values (Table II) were recorded in all experimental teeth at various intervals during the experiment. The
average initial periapical tissue pH (Table III) of the infected-only teeth in each animal ranged from 7.05 to 7.21 with an overall average of 7.12. The average periapical tissue pH at sacrifice ranged from 6.88 to 7.15 in the individual animals with an overall average of 7.04.

Clinical results (Table IV) show that all of the infected-only teeth had a mobility of two or greater after the first week of infection to the end of the experiment. The six month animals had exfoliated all of the infected-only teeth while the rest of the animals still retained these teeth at the time of sacrifice (Table V). There were eight out of sixteen teeth after one week of infection and three out of twelve teeth at the time of sacrifice that had draining sinus tracts (Table IV).

Radiographic findings (Table I) showed that all infected-only teeth had periapical and bifurcation radiolucent lesions present after one week of infection to the end of the study. These lesions did not noticeably increase in size radiographically after one more week as seen in the one week animal at sacrifice (Table VII). However, all other radiolucent lesions did increase in size radiographically with increasing post-operative sacrifice intervals. There was no evidence of apical closure in any of the infected-only teeth (Table I).

Histological evaluation of the infected-only teeth in the one week animal revealed severe degenerative changes (Figure 13). No evidence of vital pulp tissue or Hertwig's sheath was demonstrated. The epithelial attachment remained and periodontal ligament was present in the coronal two-thirds of the interproximal root surfaces. However, the attachment
apparatus was totally lacking in the apical and bifurcation areas and was replaced by a proliferating sling of epithelial tissue which was two to four cells in thickness. Severe loss of periapical and furcation alveolar bone was seen and these areas had a severe acute inflammatory infiltrate (Figure 14) consisting mainly of polymorphonuclear leukocytes, with but a few mononuclear cells evident. No evidence of dentin or cementum resorption was seen. The surrounding alveolar bone was seen to be resorbing on the lesion side and bone deposition (Figure 15) was demonstrated on the non-lesion side.

In the one month animals, the loss of periodontal attachment was more extensive. There were only isolated areas of periodontal ligament attachment while the remaining areas were replaced by an epithelial sling. This epithelial proliferation was well differentiated into a stratified squamous epithelium of two to ten cells thick and rete pegs of loose connective tissue were seen extending into the epithelium (Figure 16). A basal layer or stratum germinativum and a prickle cell layer or stratum spinosum were now evident. A chronic inflammatory infiltrate (Figure 17) was seen in the subepithelial layers consisting of lymphocytes, plasma cells, and macrophages. The alveolar bone in the furcation was nearly absent (Figure 18) while bone apposition and resorption was seen on the lesion side of the periapical alveolar bone. No resorption of dentin or cementum was evident nor was any remaining evidence of Hertwig's sheath seen.

In the three month animal, severe degenerative changes were seen in histological sections (Figure 19). The epithelial attachment and most
of the periodontal ligament were absent and replaced by a well differentiated stratified squamous epithelium with rete pegs, basal layer, prickle cell layer, and parakeratosis. Furcation and interproximal alveolar bone was virtually completely absent and a chronic inflammatory infiltrate was seen in the subepithelial tissues. Neither Hertwig's epithelial root sheath nor any evidence of dentin resorption were seen at the root apex. The root canals were devoid of contents and the epithelial sling was noticed surrounding the wide open apical foramen and continuing along the sinus tract.

All of the six month infected-only teeth were exfoliated and healing of the sockets was complete. No histological evidence of apical closure or apical calcification was seen at any time in the infected-only teeth during this entire study.

INFECTED AND ACIDIC-TREATED TEETH

One maxillary and one mandibular premolar in each animal, which served as the infected and acidic-treated teeth, were infected with *S. faecalis* for one week following pulp extirpation before an acidic paste (pH=3) was inserted into the root canal and surrounding periapical tissue for the duration of the study.

Table III lists the average periapical tissue pH values at various intervals during this study. The average initial periapical tissue pH of the acidic-treated teeth in each animal ranged from 7.03 to 7.20 with an overall average of 7.12. The average periapical tissue pH of these teeth in each animal after one week of infection ranged from 6.33 to 6.76
with an overall average of 6.50. The average periapical tissue pH at the time of sacrifice ranged from 6.92 to 7.12 in the individual animals with an overall average of 7.01.

Clinical results (Table IV) showed that all of the acidic-treated teeth had a mobility of two or greater after the first week of infection to the end of the experiment. Three out of four of the acidic-treated teeth were exfoliated in the six month animals while the rest of the animals still retained these teeth at the time of sacrifice (Table V). Draining sinus tracts were present in eight of the sixteen acidic-treated teeth after one week of infection, and in five out of thirteen so treated teeth at the time of sacrifice (Table IV).

Radiographic results (Table I) showed that all acidic-treated teeth had periapical and bifurcation radiolucent lesions present after one week of infection through to the end of the study. These lesions did not noticeably increase in size after one more week as seen in the one week animal at sacrifice (Table VII). However, all other radiolucent lesions in the remaining animals did increase in size as the post-operative sacrifice interval increased. There was no radiographic evidence of apical closure in any of the acidic-treated teeth (Table I).

Histological evaluation of the acidic-treated teeth in the one week animal revealed severe degenerative changes (Figure 20). No evidence of vital or necrotic pulp tissue was seen in the root canal, but the remains of the acidic paste could be seen as a faint pink amorphous mass in some sections. Viable epithelial attachment and periodontal ligament remained
on the interproximal root surfaces of maxillary teeth but more severe
destruction was seen in mandibular teeth. Furcation alveolar bone was
nearly completely absent and severe loss of periapical bone was evident.
A sling of proliferating epithelial tissue three to five cells thick was
present in areas where the periodontal membrane was missing and the sub-
epithelial tissues had a severe acute inflammatory infiltrate consisting
mainly of polymorphonuclear leukocytes and occasional mononuclear cells.
The surrounding alveolar bone was seen to be resorbing on the side of
the periapical lesion and bone deposition was evident on the non-lesion
side.

In the one month animals, the loss of the periodontal attachment
apparatus was far more severe. One of the mandibular teeth which had
no periodontal ligament and was completely surrounded by a sling of epith-
lium was obviously ready to exfoliate. The remaining acidic-treated teeth
had only isolated areas of viable periodontal ligament attachment (Figure
22) and no evidence of an epithelial attachment. A well differentiated
epithelial sling surrounded most of the teeth and rete pegs and basal
and prickle cell layers were seen in these stratified epithelial bands.
Furcation alveolar bone was absent and only isolated spicules of inter-
proximal bone remained. The root canals were devoid of any tissue con-
tents but a collection of extravasated red blood cells was seen in the
apical region of one root and the remains of the acidic paste could be
seen as a faint pink amorphous mass in some of the roots. Small frag-
ments of the temporary filling material were also seen in some of the
canals. No evidence of Hertwig's sheath or dentin resorption was seen. There was a mild chronic inflammatory reaction seen in the subepithelial tissues around the roots which consisted of lymphocytes, plasma cells, and macrophages dispersed among the newly forming dense fibrous connective tissue. The surrounding alveolar bone showed evidence of apposition and resorption since osteoclasts and osteoblasts were seen on the lesion side of the cribriform plate.

In the three month animal, severe degenerative changes were seen in histological sections of the acidic-treated teeth. The epithelial attachment and nearly all of the periodontal ligament were absent and replaced by a stratified squamous epithelial sling with rete pegs, basal and prickle cell layers, and parakeratin on the surface. Furcation and periapical alveolar bone were nearly absent and there was a moderate chronic inflammatory cellular infiltrate in the developing lamina propria. There was some amorphous appearing paste remnants in the root canals but Hertwig's sheath and dentin resorption were not seen. One tooth had extensive epithelial proliferation around the apex with an adjacent cyst-like area (Figures 23 and 24).

Only one of the four acidic-treated teeth remained in the six month animals (Figure 25). This tooth had no epithelial attachment and only one isolated area of periodontal attachment remained which indicated this tooth would soon be exfoliated. There were no tissue remnants left in the root canal but remnants of the amorphous acidic paste could be seen in some sections. Root resorption or evidence of Hertwig's sheath were not seen. The tooth was surrounded by a well differentiated stratified
squamous epithelial sling and there was a severe chronic inflammatory infiltrate in the subepithelial tissues which consisted mainly of lymphocytes, but plasma cells and macrophages were also present. Furcation and interproximal alveolar bone were absent while the periapical alveolar bone showed signs of osteoblasts laying down new bone.

No histological evidence of apical closure or calcification was seen at any time in the acidic-treated during this study.

**INFECTED AND BASIC-TREATED TEETH**

One maxillary and one mandibular premolar in each animal, which served as the infected and basic-treated teeth, were infected with *S. faecalis* for one week following pulp extirpation before an alkaline paste (pH 11) was inserted into the root canal for the duration of the study.

Table III lists the average periapical tissue pH values at various intervals during this study. The average initial periapical tissue pH of the basic-treated teeth in each animal ranged from 7.03 to 7.15 with an overall average of 7.11. The average periapical tissue pH of these teeth in each animal after one week of infection ranged from 6.30 to 6.68 with an overall average of 6.48. The average periapical tissue pH at the time of sacrifice ranged from 6.93 to 7.10 in the individual animals with an overall average of 7.02.

Clinical findings (Table IV) revealed that all of the basic-treated teeth had a mobility of two or greater after the first week of infection to the end of the experiment. One out of two basic-treated
teeth in the one month animal and three out of four basic-treated teeth in the six month animals were exfoliated while the rest of the animals retained these teeth at the time of sacrifice (Table V). Draining sinus tracts were present in ten out of sixteen basic-treated teeth after one week of infection, and in four out of eight so treated teeth at the time of sacrifice (Table IV).

Radiographic results (Table I) showed that all basic-treated teeth had periapical and bifurcation radiolucent lesions present after one week of infection to the end of the study. These lesions did not noticeably increase in size after one more week as seen in the one week animal at sacrifice (Table VII). However, all other radiolucent lesions in the remaining animals did increase in size as the postoperative sacrifice interval increased. There was no evidence of apical closure in any of the basic-treated teeth (Table I).

Histological evaluation of the basic-treated teeth in the one week animal revealed severe degenerative changes (Figure 26). No evidence of vital or necrotic pulp tissue was seen in the root canal, nor was Hertwig's epithelial root sheath or root resorption demonstrable at the root apex. Viable periodontal ligament, alveolar bone and epithelial attachment were present on the mesial interproximal root surface, but a chronic draining sinus tract was seen along the distal interproximal root surface. Furcation alveolar bone was nearly absent and a proliferating sling of epithelium surrounded the root surfaces that lacked periodontal membrane (Figure 27). Periapical alveolar bone showed signs of osteoblastic activity but this deposition was on the nonlesion side. The subepithelial tissues
contained a moderate acute inflammatory infiltrate consisting mainly of polymorphonuclear leukocytes, but some lymphocytes, plasma cells, macrophages, and fibroblasts were present.

In the one month animals, one of the four basic-treated teeth had exfoliated. Complete healing of the socket areas was evident but an unusual finding of an embedded piece of dentin was seen in the lamina propria (Figure 28). There was no evidence of inflammation around this dentin which indicated it was well tolerated by the animal. The rest of the basic-treated teeth exhibited severe loss of the periodontal attachment apparatus. The epithelial attachment was absent with only some of the interproximal areas of periodontal ligament remaining (Figure 29). A well differentiated epithelial sling surrounded most of the root surfaces and a chronic round cell infiltrate was present in the subepithelial tissues. In one root canal, there was an evagination of the well differentiated epithelium into the apical foramen while small pieces of temporary filling material could be seen in the root canal and surrounding tissues (Figure 30). Another root apex showed evidence of cementum and dentin resorption but no evidence of Hertwig's sheath was seen (Figure 31).

In the three month animals, severe degenerative changes were seen in histological sections of the basic-treated teeth (Figure 32). The epithelial attachment and most of the periodontal ligament were absent and replaced by a stratified squamous epithelial sling with rete pegs, basal and prickle cell layers, and parakeratin (Figure 33). Furcation and periradicular alveolar bone were nearly absent and there was a moderate inflammatory infiltrate and hyperemia in the subepithelial tissues.
There was some amorphous basic paste remnants in the canals, but Hertwig's sheath and dentin resorption were not present.

Only one of the four basic-treated teeth remained in the six month animals. Although this tooth was lost during histological preparation, the tissue sections clearly showed the severe degenerative tissue changes present in the tooth socket (Figure 34). No evidence of any connective tissue attachment could be seen which indicated this tooth would have exfoliated soon. The socket was surrounded by a well differentiated stratified squamous epithelium. A severe chronic inflammatory cellular infiltrate was seen in the subepithelial tissues and furcation and interproximal alveolar bone were absent.

No histological evidence of apical closure or calcification was seen at any time in the basic-treated teeth during this study.

**SUMMARY OF pH RESULTS**

Table VI lists the average periapical tissue pH values at various intervals during the experiment. Initial normal periapical tissue pH ranged from 7.09 to 7.13 with an average pH=7.11. Following one week of infection with *S. faecalis*, the periapical tissue pH dropped to a range of 6.33 to 6.62 with an average pH=6.49. At the time of sacrifice, the periapical tissue pH had increased to a range of 6.89 to 7.16 with an average pH=7.03.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F Value</th>
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<tbody>
<tr>
<td>Variance in pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>between dogs</td>
<td>5</td>
<td>0.02</td>
<td>0.004</td>
<td>0.03</td>
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<tr>
<td>Variance in pH</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>within dogs</td>
<td>12</td>
<td>1.49</td>
<td>0.124</td>
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</table>
This analysis of variance table shows that there was no statistically significant variation in response between or within animals at a specific time interval during the experiment (P>.5). That is, each animal responded in the same manner during a specific operative session in this study.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sums of Squares</th>
<th>Mean Squares</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance in pH between time intervals</td>
<td>2</td>
<td>1.40</td>
<td>0.7</td>
<td>95.9</td>
</tr>
<tr>
<td>Variance in pH within time intervals</td>
<td>15</td>
<td>0.11</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

This analysis of variance table shows that there was a statistically significant difference in periapical tissue pH at the various operative sessions during this study. Using the K distribution, these significant differences were determined. The periapical tissue pH after one week of infection is significantly lower than the initial normal periapical tissue pH (P<.01). That is, the pH of acutely inflamed periapical tissue was significantly lower than the pH of normal periapical tissue in dogs. Furthermore, the periapical tissue pH at sacrifice was significantly higher than the periapical tissue pH after one week of infection (P<.01). Hence, the pH of chronically inflamed periapical tissue was significantly higher than the pH of acutely inflamed periapical tissue in dogs. However, there was not a statistically significant difference in tissue pH between chronically inflamed and normal periapical tissue in dogs (P>.05).
Root end induction procedures have become a valuable addition to the modern endodontic practice. In the literature review, numerous clinical and animal studies were cited that have attempted to clarify our understanding of the apexification process and to increase the success rate of this treatment regimen. Although calcium hydroxide pastes have become the most widely used agents to induce apexification, the use of a blood clot, polyantibiotic and iodoform pastes, resorbable tricalcium phosphate ceramic, and collagen-calcium phosphate gel have yielded similarly successful results.

In order to further clarify the underlying reparative mechanism of apexification, this study was undertaken in an attempt to delineate the effects of the pH of the intracanal paste on the hard tissue repair which occurs at the apices of pulpless immature teeth with periapical involvement. The results, at first glance, were very discouraging since hard tissue repair did not occur in any of the experimental teeth, most probably as a result of overwhelming infection and tissue damage. However, one must reflect upon Spallanzani's dictum as cited by Naidorf (149):

"If I set out to prove something, I am no real scientist. I have to learn to follow where the facts lead me; I have to learn to whip my prejudices."

62
The following analysis of the experimental design will delineate the significant findings of this study as well as to suggest improvements for future investigations.

**SUMMARY OF THE OVERALL CLINICAL AND HISTOLOGICAL RESULTS**

The results of this study indicated that the presence or absence of a treatment paste did not induce root end closure. However, no conclusions could be drawn concerning the effects of the pH of the intracanal treatment paste on the apexification process due to the overwhelming infection and tissue damage seen in the experimental teeth.

Tables IV and V summarize the clinical findings at various intervals during this experiment. There were approximately equal numbers of exfoliated teeth, draining sinus tracts, and mobile teeth in each of the acidic, basic, and infected groups throughout this study. Hence, the presence or absence of a paste in the root canal did not in itself affect the number of exfoliated teeth, draining sinus tracts, or the mobility of the experimental teeth in this study. However, the number of exfoliated teeth and severity of tooth mobility did increase as the postoperative interval increased.

Tables I and VII summarize the radiographic findings at various intervals during this experiment. There was no radiographic evidence of apical closure in any of the experimental teeth in this study. Periapical and bifurcation radiolucent lesions were seen in all experimental teeth and the size of these lesions increased as the postoperative sacrifice interval increased, regardless of the type of paste used in the canal.
Hence, the presence or absence of a paste neither induced apical closure nor affected the growth of periapical or bifurcation radiolucent lesions in this study.

Clinical results revealed that all experimental teeth suffered severe degenerative changes regardless of the presence or absence of a treatment paste in the root canal. The one week animal had lost considerable periodontal ligament support for these teeth and an epithelial sling was beginning to form around the roots. Although no definitive conclusions could be made, the origin of this epithelium was from cellular proliferation of either the epithelial attachment at the gingival opening of the sinus tract or the epithelial rests of Malassez in the periodontal ligament. After the loss of the periodontal ligament attachment to the root surface, the epithelium grew into this area as a protective mechanism to prevent further injury to the underlying periodontal tissues. Neither vital nor necrotic tissue was seen in the root canals and neither Hertwig's sheath nor evidence of root resorption was evident at the root apex.

An acute inflammatory cellular infiltrate of polymorphonuclear leukocytes was present in the subepithelial tissues and severe loss of furcation bone was seen.

As the postoperative interval increased, the degenerative changes became more severe with increasing loss of the periodontal attachment apparatus. The epithelial sling surrounding the root surfaces became more organized and displayed rete pegs, basal and prickle cell layers, and a surface of parakeratin. The subepithelial cellular inflammatory
infiltrate became chronic in nature and consisted of lymphocytes, plasma cells, and macrophages scattered among the dense fibrous connective tissue of the developing lamina propria.

One could deduce from these results that as the postoperative interval increased, all experimental teeth would eventually be exfoliated due to the infection and tissue damage. The observed degenerative changes were similar in all experimental teeth and hence did not depend on the presence or absence of a treatment paste in the root canal.

**INTRACANAL pH CONCEPT**

Table VI discloses the average periapical tissue pH at various intervals in the experiment. Initial normal periapical tissue pH ranged from 7.09 to 7.13 in the individual animals with an overall average of 7.11. Following one week of infection with *S. faecalis*, the periapical tissue pH dropped to a range of 6.33 to 6.62 with an overall average of 6.49. This result verifies Menkin's (104) hypothesis that the normal tissue pH will become acidic during the acute inflammatory process. At the time of sacrifice, the periapical tissue pH had risen to a range of 6.89 to 7.16 with an average of 7.03. This result verifies Menkin's (104) hypothesis that the pH of chronic inflammation will rise and may become slightly alkaline. The pH of periapical tissues at the time of sacrifice was independent of the presence of a paste in this study since the overall average pH was 7.03 while the average pH of the infected-only teeth was 7.04, the acidic-treated teeth was 7.01 and the basic-treated teeth was 7.02. Hence the insertion of an acidic or basic paste had no effect on
the periapical tissue pH at sacrifice.

This study did show that healthy periapical tissue pH in dogs averaged 7.1 which is in agreement with the results of Wolpert, et al., (141), on mongrel dogs. Following one week of pulpal infection with *S. faecalis* (human origin), the average periapical tissue pH dropped to 6.5. An analysis of variance of the experimental results showed that each animal responded in the same manner and that this decrease in periapical tissue pH was statistically significant at the one per cent level of probability. From one week to six months later, the average periapical tissue pH increased to 7.03. An analysis of variance of the experimental results showed that each animal responded in the same manner and that this increase in periapical tissue pH was statistically significant at the one percent level of probability.

Menkin (140) created a severe inflammatory response in the pleural cavity of dogs by injecting irritants. He reported that the exudate became more acidic as inflammation progressed. However, when the pH increased, the animal tended to improve and the outlook was more favorable.

It is not clear which part of the calcium hydroxide molecule exerts the active effect on pulp, dentin, and periapical tissues. Sciaky and Pisanti (92) and Pisanti and Sciaky (93) have shown, using labelled calcium ions, that the calcium used in the formation of the dentin bridge over a healing pulp capping is not contributed by the calcium hydroxide medicament placed over the pulp, but rather comes from the circulating serum calcium. Laws (150) found that the pH of calcium hydroxide from a treated pulpotomy was 7.4 and he attributed this decrease in the normal
pH of 12.5 of the calcium hydroxide material to be due to dilution by the surrounding tissue fluids. However, Tronstad, et al., (148), found that the pH of calcium hydroxide in the root canals of immature monkey's teeth maintained a high pH which was greater than 12.2 during observation periods up to thirteen months. Their results on the pH changes in immature teeth are shown in diagramatic form on page 68.

It is conceivable that the mode of action of calcium hydroxide may be related to the alkaline pH of the treatment paste. It is possible that calcium hydroxide may change the environment of the periapical tissues to a more alkaline pH which is more conductive to healing. This increase in pH by the hydroxide ions could inhibit the action of acid hydrolases released by polymorphonuclear leukocytes and osteoclasts which function best in a slightly acidic pH. Raisz (151), studying fetal rat bone resorption stimulated by parathyroid hormone in tissue culture, found that increasing the pH to 7.6 inhibited osteoclastic bone resorption significantly. In an acidic pH, acid hydrolases cause a demineralization of the mineral components of tissues. However, a rise in tissue pH would be unfavorable for osteoclastic acid hydrolase activity. Furthermore, an alkaline pH and the presence of calcium ions may activate alkaline phosphatase which has been proposed to play an important role in hard tissue formation as stated by Tronstad, et al., (148).

Binnie and Rowe (134) found that calcium hydroxide, when used as a root canal filling material, was particularly useful in an infected environment. Cvec (18), studying apexification of teeth with periapical
pH CHANGES IN DENTAL TISSUES*

* from Tronstad, et al.,: pH changes in dental tissues following root canal filling with calcium hydroxide after induced pulp necrosis in replanted and non-replanted teeth. An experimental study in monkeys. In publication.
pathosis, presumed that calcium hydroxide *per se* was acting as a long-lasting antimicrobial agent. Matsumiya and Kitamura (94) filled experimentally infected root canals in dogs with a calcium hydroxide paste and found that bacteria living in the periapical tissues disappeared as healing progressed. They concluded that calcium hydroxide has antibacterial action in dental tissues. The result is not surprising since most microorganisms will not survive in a highly alkaline environment.

Calcium hydroxide has been shown to induce ectopic calcification in rat connective tissue in studies by Mitchell and Shankwalker (90), Yoshiki and Mori (145), and Binnie and Mitchell (152). Yoshiki and Mori (145) carried out a histochemical survey of enzymes present in the tissues surrounding the implant of calcium hydroxide and found they were similar to enzymes associated with normal calcification. They suggested the high hydroxide ion concentration may be the factor that induces calcification.

When one considers that all pastes used in successful apexification procedures have an alkaline pH, one may conclude that the pH of the intracanal treatment paste may conceivably play a role in the apexification process and will warrant further investigation in future studies.

**THE DOG AS AN EXPERIMENTAL ANIMAL**

The choice of an animal model for apexification research has been a subject of controversy. It is neither possible to histologically examine the response of human tissue beyond the apex of treated teeth nor feasible to obtain adequate pre- and postoperative experimental controls. These difficulties preclude the use of humans for basic research.
The monkey has been chosen as the experimental animal in a number of studies because of its evolutionary and anatomical resemblance to man. The use of an animal species closely related to man would permit a more convincing extrapolation of the experimental results to actual clinical practice as stated by Steiner and Van Hassel (120). However, the recuperative power of the monkey is greater than man. Citrome (116) pointed out that primate pulpal tissue is very resistant to the damaging effects of oral contamination and that despite the existence of severe inflammatory disease, the periapical tissues of the monkey still permit hard tissue repair in the form of dentin, cementum, and bone. He stated that all materials tested in the monkey have been successful, but not so in the dog. The high cost of obtaining and boarding monkeys may be a further deterrent to their use as an animal model (153).

On the other hand, the canine species has been found to bear a closer similarity in healing processes when compared to man. In 1932 Orban (154) stated that canine dental tissues are more sensitive to any kind of injury than human teeth due to the greater permeability of dentin and cementum. He concluded that if treatment was successful in the far more sensitive dog, it could be expected to be satisfactory in humans.

Hill (155) in 1932 reported that dental granulomas could be produced more quickly and easily in dogs than humans. He felt this was due to the difficulty in removing all the organic debris in the apical arborization of canals and large multiple apical foramina. He noted that dental granulomas produced in dogs were histologically similar to those found in humans.
Ostby (64), Vojinovic (82), Matsumiya and Kitamura (94), and Gottlieb, et al., (156) found close similarities in healing processes between dogs and humans. Torneck and Tulananda (132) in 1969 stated that although the basic periapical tissue response to injury was similar in dogs and human beings, the greater degree of hard tissue resorption seen in dogs could be due to accumulation of pus and inflammatory exudate which occurred about the orifices of the numerous apical accessory canals. They reported that no evidence of repair was seen where pus accumulated or the inflammatory exudate was intense.

In 1971 Barker and Lockett (157), evaluating the mandibular premolars of dogs for endodontic experimentation, concluded that canine tissue is more sensitive to injury and experiences greater difficulty in healing than humans due to the protected sites for microorganisms in the apical canal arborizations. They stated that dogs' premolar teeth are an ideal model for endodontic research.

In 1975 Mazukelli (158) commented that the periapical tissue response after endodontic treatment of dogs' teeth would be similar to that which would occur in humans after similar therapy.

In 1977 England and Best (71) reported that dogs appear to be much more resistant to injury than humans since apical closure was seen in teeth whose pulpal tissue was exposed to saliva. However, examination of the published photomicrographs revealed that the pulpal tissue in the root canals appeared normal which may indicate that pulpal trauma and infection was not very severe. In light of the unusual results of this study, another study of similar design would lend credence to their
conclusions.

In 1977 Citrome (116) concluded that the dog is a more sensitive animal than the monkey for apexification studies and hence the appropriate animal model for such experiments.

Based on the conclusions of these investigators, the beagle dog was selected as the experimental animal for this study. As a result of the overwhelming tissue damage seen in the experimental teeth, one must agree that indeed the dog is very sensitive to tissue injury and infection as reported by most investigators.

The age of the animal and the degree of apical closure of the experimental teeth when the project begins must be carefully considered in future studies. The eruption of the experimental teeth was closely observed and the operative procedures began three weeks after eruption. Experimental hindsight revealed that there was still a substantial number of control teeth with widely divergent apices five weeks later as seen in the animals sacrificed one month after operative procedures were completed. If this study had been started at this later stage of tooth development, the more fully developed teeth and further maturity of the animals may have shown more resistance to the devastating tissue injury which occurred in this experiment. Perhaps the immaturity and underdevelopment of these dogs may have been a major reason for the failure of any hard tissue deposition to occur at the apices of the experimental teeth.

THE USE OF STREPTOCOCCUS FAECALIS

In 1943 Hayes (159) cultured the root canals of 340 teeth and
microscopically studied the bacteria. Of the 211 teeth which exhibited positive cultures, ninety-five contained a pure culture of some species of streptococcus.

Teeth with root canals that were unexposed to the oral environment were studied for the presence of bacteria by Brown and Rudolph (160) in 1957. They found that streptococci, diptheroids and micrococci were the most frequently isolated organisms, and that mixed infections were prevalent. They discovered that the bacterial flora of unexposed canals varied from that of exposed canals.

Blechman (161) in 1957 reported that alpha and gamma hemolytic streptococci and *Staphylococcus albus* were the organisms most frequently isolated from root canals. Approximately eighty per cent of isolated cultures contained streptococci while forty-eight per cent of these were pure cultures.

In 1958 Leavitt, Naidorf and Shugaevsky (162), using Trypticase Soy broth and agar, studied the bacterial flora of root canals. They indicated that streptococci were the largest single group of organisms present.

In 1960 Shovelton and Sidaway (163) cultured and subcultured the root canals of 147 teeth and reported that alpha hemolytic streptococci were the most prevalent microorganisms. Engstrom and Frostell (164) noted that streptococci were present in the majority of teeth with positive cultures that had non-vital pulps and intact pulp cavities.

The *Streptococcus faecalis* organism, which is not an overt pathogen and is generally considered to be an opportunistic organism, has been
implicated as a significant pulpal pathogen. In 1959 Cran (165) studied the microflora of root canals and stated that the streptococci were the most difficult bacteria to eradicate. He reported that the enterococci organisms, for example *S. faecalis*, were very insensitive organisms and were resistant to many antibiotics.

In 1959 Winkler and van Amerongen (166) commented on the bacteriologic results from 4,000 root canal cultures taken prior to and after endodontic treatment. From the 1,141 positive cultures, fifty-one percent were streptococci in pure culture and alpha hemolytic *S. faecalis* was the most common isolate. This organism, which was isolated more frequently in subsequent than initial cultures from teeth with necrotic pulps, tended to persist in root canals once established. *S. faecalis* and its variant *S. liquefaciens* caused clinical infections which were very difficult to eliminate and hence they should be considered potentially pathogenic for the dental pulp.

In 1961 Crawford and Shankle (167), comparing the bacterial flora of root canals which were open to those closed to oral contamination, found that non-beta hemolytic streptococci predominated in both environments. They reported that enterobacteria were among the most common microorganisms retrieved from the initial culture of a tooth left open to the oral environment.

In 1964 Engstrom (168) studied the significance of enterococci in endodontic therapy. He found that the first antiseptic treatment failed to eradicate the organisms from the root canal of sixty-five per cent of
the teeth infected with enterococci, *S. faecalis* being the usual isolate. This compared to the figure of 39.5 per cent for teeth infected with other varieties of microorganisms. He concluded that pulpal infections with enterococci were a treatment problem since the infections were harder to eliminate and would require a longer treatment period.

In 1969 Torneck (169) isolated a high proportion of enterococci and staphylococci from acutely infected dental pulps. He noted the presence of enterococci in cultures taken from the canals of teeth with a history of previous endodontic therapy.

In 1971 Barker and Lockett (157) concluded that *S. viridans* was an ideal microorganism for the infection of mandibular premolars in dogs. They reported that it will incite a chronic to subacute periapical response without danger of acute exacerbation, while apical granulomas will be visible radiographically within two or three months. They stated that a nutrient broth culture, which had been incubated for twenty-four hours following transfer of a standard inoculum from a stock culture, would be suitable for animal experimentation.

Mazukelli (158), in dogs, and Felder (170), in monkeys, inoculated dental pulps with pure cultures of *S. faecalis* which induced radiographically visible periapical pathosis within three to four months.

Based on these studies, *S. faecalis* was chosen for infection of the dogs' root canals in this study. This organism, having verified its virulence as a significant pulpal pathogen in dogs by Mazukelli (158), was obtained in pure culture from the Microbiology Department of Loyola
University. Although the specific source of this organism could not be determined, it was likely a hospital isolate of a non-oral human infection. The significance of this ill-suited choice will be discussed after one considers that the periapical lesions of bacterial etiology are the result of the number of invading organisms, the virulence of the organism, and the intrinsic ability of the host to combat infection. Zeldow and Ingle (171) stated that the relationship of this triad of factors could be expressed by the equation:

$$\text{Severity of the disease state} = \frac{\text{Number} \times \text{Virulence}}{\text{Host Resistance}}$$

Steiner and Van Hassel (120) explained that the experimental procedure and animal model being examined should be tested under the most unfavorable situations likely to be encountered clinically. In this manner, the validity of the effectiveness of a treatment regimen as determined in an animal model would be reinforced for use in a human clinical situation. A major reason for the extreme tissue damage seen in this study may have been related to the use of a human non-oral strain of *S. faecalis*. The extensive destruction of periapical tissues seen in these animals after one week of infection is rarely seen in clinical situations. As this strain of bacteria was too virulent for the resistance of the dog to overcome, future research projects should consider the use of oral strains of *S. faecalis*. These organisms could be obtained from the root canals of human teeth with positive cultures. However, one must consider that human strains of this microorganism may be more virulent than the bacterial flora normally found in the canine species. Perhaps a more
logical choice would be the isolation of an indigenous strain of \textit{S. faecalis} from the dog's saliva for use in future studies.

The use of a standard inoculum of microorganisms in this study ensured that a constant number of organisms was introduced into each experimental tooth. However, no attempt was made to analyze the bacteriologic status of the root canals. Ham, \textit{et al.}, (73), reported that when complete pulpal necrosis and extensive periapical destruction were present, the likelihood of successful apexification results were lessened if a negative culture could not be obtained. Future experimentation should delay insertion of the treatment pastes until a negative culture has been obtained. The use of a parenteral antibiotic to reduce systemic disturbances should also be considered. As discussed previously, the host resistance of the experimental animals may have been increased if the study had begun at a later date when the animals were more mature. One should also consider using non-infected experimental teeth, in addition to infected experimental teeth, to evaluate the effectiveness of the treatment pastes. This method would simulate the clinical situation of apexification of non-infected pulpless immature teeth.

\textbf{USE OF VITAL DYE MARKERS}

Histological evidence of hard tissue deposition at the apex of a root canal can be visualized in a hematoxylin and eosin stained tissue section. Unfortunately, one cannot be sure whether this deposition occurred before, during, or after experimental procedures. This realization necessitates the use of a biological marker to label the tissue
so that one can determine the sequence of hard tissue deposition.

Tomich (172) in 1968 reported that Procion Brilliant Red H-8BS was an effective in vivo hard tissue marking agent which was used safely at a dosage of 100 mg./kg. of body weight in rats. Goland, et al., (173, 174), noted that the reactive Procion dyes formed very stable covalent bonds and crosslinkages under physiological conditions in rats. These dyes were retained in incremental lines and zones of growth in teeth and bones, and these dyes were preserved in decalcification and preparation of tissue sections. Prescott, et al., (175), in 1968 showed that a single dose of Procion Brilliant Red H-8BS given intraperitoneally to a dog would selectively stain bone, dentin and cementum being formed at the time of administration. Sherman (176) used this dye to observe deposition of cementum and alveolar bone apposition following intentional replantation of teeth in dogs and monkeys. Ham, et al., (73), used a single injection of this dye to observe hard tissue deposition in an apexification study using monkeys while Citrome (116) used multiple injections of this dye to monitor hard tissue repair in an apexification study using dogs.

Due to the extensive tissue damage which occurred in this study, the use of a vital dye did not aid in the evaluation of the histological results. However, the use of a biological marker to monitor calcific repair after experimental procedures should not be neglected in future studies. In this manner, one can objectively state that the calcific repair occurred after the treatment was performed.
CHAPTER VI

SUMMARY AND CONCLUSIONS

In an effort to delineate the role of pH in healing of developing periapical lesions in immature teeth of dogs, the pulps of twenty-four premolar teeth were extirpated in six young purebred beagle littermates. Then a standardized innoculum of a pure culture of \textit{S. faecalis} was placed in the root canal and the access cavities were sealed. Untreated premolar teeth adjacent to the operated teeth served as controls. One week later, the root canals of two-thirds of the experimental teeth received endodontic canal enlargement, were irrigated and dried before the insertion of either an acidic paste (pH=3) or a basic paste (pH=11). These teeth served as the acidic-treated and basic-treated teeth while the remaining experimental teeth served as infected-only teeth. The periapical tissue pH in the region of the root apex was measured at various intervals during the experiment using pH microelectrodes. Regular radiographic and clinical observations of the experimental and control teeth were conducted throughout the entire experiment. The animals were sacrificed at four different postoperative intervals: one dog at one week, two dogs at one month, one dog at three months, and two dogs at six months. The jaws were removed at necropsy and following fixation, decalcification and histologic preparation, the teeth and surrounding structures were evaluated microscopically.

Under the conditions of this experiment, the following conclusions
could be drawn:

a) *S. faecalis* is capable of producing pathologic periapical lesions in dogs.
b) Hertwig's epithelial root sheath is destroyed following pulpal extirpation, infection and severe periapical inflammation.
c) In the presence of severe periapical tissue inflammation, further root development will not occur in immature teeth of dogs in response to change in pH of periapical tissue.

Under the conditions of this experiment, the following impressions could be drawn:

a) Young beagle dogs appear to be sensitive to severe periapical tissue injury and infection.
b) The normal periapical tissue pH in dogs is 7.1. In the presence of acute inflammation, the periapical tissue pH of the beagle dogs in this study dropped to approximately 6.5. As the inflammation became chronic, the periapical tissue pH rose to about pH=7.
c) The use of a human isolate of *S. faecalis* for periapical infection of young dogs' teeth may be too virulent to allow healing of the periapical tissues. The use of a strain of *S. faecalis* indiginous to the canine species may be preferable in future studies of this nature.
CHAPTER VII

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81


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CHAPTER VIII

TABLES AND FIGURES
TABLE I

COMPARISON OF RADIOGRAPHIC FINDINGS AFTER ONE WEEK OF INFECTION AND AT SACRIFICE

<table>
<thead>
<tr>
<th>Control Teeth</th>
<th>Experimental Teeth</th>
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<td></td>
<td>Apical Closure</td>
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<tr>
<td></td>
<td>N.E. Partial Complete</td>
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</tr>
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<td>animal A.S.</td>
<td>4</td>
</tr>
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<td>6 month I.</td>
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<td>4</td>
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<td>animal A.S.</td>
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LEGEND

I. - After one week infection with S. faecalis
A.S. - At sacrifice
N.E. - Not evident on radiograph
E. - Evident on radiograph
* - Includes exfoliated teeth
<table>
<thead>
<tr>
<th>Animal</th>
<th>Tooth</th>
<th>Initial Tissue pH</th>
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<th>pH At Sacrifice of Infection</th>
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**Table II**

**INDIVIDUAL PERIAPICAL TISSUE pH VALUES AT VARIOUS INTERVALS**

**DURING THE EXPERIMENT**
TABLE II (continued)

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<th>Animal</th>
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LEGEND

E - Exfoliated
UR3 - Upper right third premolar
UL2 - Upper left second premolar
LR3 - Lower right third premolar
LR2 - Lower right second premolar
LL2 - Lower left second premolar
LL3 - Lower left third premolar
TABLE III

AVERAGE PERiapICAL TISSUE pH VALUES AT VARIOUS TIMES

INTERVALS DURING THE EXPERIMENT COMPARING THE

EFFECTS OF PASTE INSERTED

<table>
<thead>
<tr>
<th></th>
<th>Acidic Paste</th>
<th>Basic Paste</th>
<th>Infected Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.10</td>
<td>7.08</td>
<td>7.14</td>
</tr>
<tr>
<td>Animal</td>
<td>6.53</td>
<td>6.30</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
<td>7.04</td>
<td>7.01</td>
<td>7.00</td>
</tr>
<tr>
<td>One Month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.13</td>
<td>7.13</td>
<td>7.15</td>
</tr>
<tr>
<td>Animal</td>
<td>6.76</td>
<td>6.37</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
<td>6.92</td>
<td>7.03</td>
<td>6.98</td>
</tr>
<tr>
<td>One Month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.13</td>
<td>7.14</td>
<td>7.17</td>
</tr>
<tr>
<td>Animal</td>
<td>6.55</td>
<td>6.68</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
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<td>6.93</td>
<td>6.88</td>
</tr>
<tr>
<td>Three Month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.20</td>
<td>7.03</td>
<td>7.05</td>
</tr>
<tr>
<td>Animal</td>
<td>6.45</td>
<td>6.61</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
<td>7.12</td>
<td>7.10</td>
<td>7.21</td>
</tr>
<tr>
<td>Six Month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.10</td>
<td>7.13</td>
<td>7.10</td>
</tr>
<tr>
<td>Animal</td>
<td>6.40</td>
<td>6.58</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Six Month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.03</td>
<td>7.15</td>
<td>7.10</td>
</tr>
<tr>
<td>Animal</td>
<td>6.33</td>
<td>6.35</td>
<td></td>
</tr>
<tr>
<td>A.S.</td>
<td>7.05</td>
<td></td>
<td>7.15</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inf.</td>
<td>7.12</td>
<td>7.11</td>
<td>7.12</td>
</tr>
<tr>
<td>A.S.</td>
<td>6.50</td>
<td>6.48</td>
<td></td>
</tr>
<tr>
<td>Averages</td>
<td>7.01</td>
<td>7.02</td>
<td>7.04</td>
</tr>
</tbody>
</table>

LEGEND

I. - At beginning of the experiment
Inf. - After one week infection with S. faecalis
A.S. - At sacrifice
### TABLE IV

COMPARISON OF CLINICAL FINDINGS AFTER ONE WEEK OF INFECTION AND AT SACRIFICE

<table>
<thead>
<tr>
<th>Number of Exfoliated Teeth</th>
<th>Number of Draining Sinus Tracts</th>
<th>Tooth Mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. B. I. Total</td>
<td>Normal Two Three</td>
</tr>
<tr>
<td>One Week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Animal A.S.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>One Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Animal A.S.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>One Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Animal A.S.</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Three Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Animal A.S.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Six Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
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<td>2</td>
</tr>
<tr>
<td>Animal A.S.</td>
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<td>0</td>
</tr>
<tr>
<td>Six Month</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal I.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Animal A.S.</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

**LEGEND**

I. - After one week infection with *S. faecalis*
A.S. - At sacrifice
A. - Acidic-treated teeth
B. - Basic-treated teeth
I. - Infected-only teeth
* - Exfoliated teeth included
TABLE V

CLINICAL FINDINGS AT TIME OF SACRIFICE

COMPARING EFFECTS OF PASTE INSERTED

<table>
<thead>
<tr>
<th>Number of Exfoliated Teeth</th>
<th>Number of Teeth With Draining Sinus Tracts</th>
<th>Number of Teeth With Mobility Two or Greater</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>B.</td>
<td>I.</td>
</tr>
<tr>
<td>One Week Animal</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>One Month Animal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>One Month Animal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Three Month Animal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Six Month Animal</td>
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<td>2</td>
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<tr>
<td>Six Month Animal</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

LEGEND

A. - Acidic-treated teeth
B. - Basic-treated teeth
I. - Infected-only teeth
* - Exfoliated teeth included
### TABLE VI

**AVERAGE PERIAPICAL TISSUE pH VALUES OF THE EXPERIMENTAL TEETH AT VARIOUS TIME INTERVALS DURING THE EXPERIMENT**

<table>
<thead>
<tr>
<th>Initial Normal Tissue pH</th>
<th>pH After One Week of Infection</th>
<th>pH at Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Week Animal</td>
<td>7.10</td>
<td>6.41</td>
</tr>
<tr>
<td>One Month Animal</td>
<td>7.13</td>
<td>6.58</td>
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<tr>
<td>One Month Animal</td>
<td>7.15</td>
<td>6.62</td>
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<tr>
<td>Three Month Animal</td>
<td>7.09</td>
<td>6.53</td>
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<tr>
<td>Six Month Animal</td>
<td>7.11</td>
<td>6.48</td>
</tr>
<tr>
<td>Average</td>
<td>7.11</td>
<td>6.49</td>
</tr>
</tbody>
</table>
TABLE VII

RADIOGRAPHIC FINDINGS AT TIME OF SACRIFICE

COMPARING EFFECTS OF PASTE INSERTED

<table>
<thead>
<tr>
<th>Lesion Size</th>
<th>Acidic Paste</th>
<th>Basic Paste</th>
<th>Infected Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smaller</td>
<td>Same</td>
<td>Larger</td>
</tr>
</tbody>
</table>

| One Week Animal | 2 | 2 | 2 |
| One Month Animal | 2 | 2 | 2 |
| Three Month Animal | 2 | 2 | 2 |
| Six Month Animal | 2* | 2* | 2* |

| Total | 0 | 2 | 10* | 0 | 2 | 10* | 0 | 2 | 10* |
Figure 1: Typical preoperative radiographic survey. Note the wide open apices of the premolar teeth. Second premolar (2), third premolar (3).

Figure 2: Typical radiographic survey after one week of infection with *Streptococcus faecalis*. Acidic-treated tooth (A), basic-treated tooth (B), infected-only tooth (I), control tooth (C). Note the small periapical radiolucent lesions and larger bifurcation radiolucent lesions.
Figure 3: Radiographic survey of one week animal at sacrifice. Acidic-treated tooth (A), basic-treated tooth (B), infected-only tooth (I), control tooth (C). Periapical radiolucent lesions are continuous with bifurcation radiolucent lesions.

Figure 4: Radiographic survey of one month animal at sacrifice. Acidic-treated tooth (A), basic-treated tooth (B), infected-only tooth (I), control tooth (C). Note severe bifurcation and periapical radiolucent lesions.
Figure 5: Radiographic survey of three month animal at sacrifice. Acidic-treated tooth (A), basic-treated tooth (B), infected-only tooth (I), control tooth (C). Note severe radiolucent lesions present in upper left and lower right quadrants.

Figure 6: Radiographic survey of six month animal at sacrifice. Note that only one acidic-treated tooth (A) and one basic-treated tooth (B) remain and very little supporting bone is evident.
Figure 7: Apex of control tooth in one week animal. Dental pulp (P), odontoblastic layer (O), dentin (D), Hertwig's epithelial root sheath (H), periodontal ligament (L), nerve (N), alveolar bone (B). (Hematoxylin and eosin stain, original magnification X25).

Figure 8: Furcation area of control tooth in one week animal. Dental pulp (P), dentin (D), periodontal ligament (L), alveolar bone (B). (Hematoxylin and eosin stain, original magnification X25).
Figure 9: Interproximal gingival sulcus area in one week animal. Note the normal gingival attachment (G) adjacent to the control tooth (C) while the adjacent acidic-treated tooth (A) appears to have a deepened gingival sulcus due to the plane of section. (Hematoxylin and eosin stain, original magnification X25).

Figure 10: Apex of control tooth in one month animal. Note some blood vessel congestion (C) in the dental pulp and nerve (N) in periapical tissue. (Hematoxylin and eosin stain, original magnification X40).
Figure 11: Apex of control tooth in three month animal. Note the completed root development and the apical arborization of the root canal in cementum. Dentin (D), cementum (C). (Hematoxylin and eosin stain, original magnification X40).

Figure 12: Apex of control tooth in six month animal. Note that apical development is complete and the apical arborization of the root canal in cementum is clearly seen. Dental pulp (P), dentin (D), cementum (C) maxillary sinus (S), periodontal ligament (L). Pulp fixation is poor and separation artifacts (A) can be seen. (Hematoxylin and eosin stain, original magnification X25).
Figure 13: Apex of infected-only tooth in one week animal. Note loss of alveolar bone, epithelial sling at apex (E), and temporary filling material in root canal (F). (Hematoxylin and eosin stain, original magnification X25).

Figure 14: Higher magnification of periapical tissue in Figure 13 showing acute inflammatory cellular infiltrate consisting mainly of polymorphonuclear leukocytes (arrows). (Hematoxylin and eosin stain, original magnification X250).
Figure 15: Periapical alveolar bone around infected-only tooth in one week animal. Note bone deposition and osteoblasts (arrows) on the non-lesion side. (Hematoxylin and eosin stain, original magnification X40).

Figure 16: Apex of infected-only tooth in one month animal. Note well differentiated epithelial sling (E) around apex and probable puncture site (M) of microelectrode. (Hematoxylin and eosin stain, original magnification X40).
Figure 17: Higher magnification of periapical tissue in Figure 16 showing chronic inflammatory cellular infiltrate of lymphocytes, plasma cells, and macrophages. (Hematoxylin and eosin stain, original magnification X250).

Figure 18: Furcation area of infected-only tooth in one month animal. Note complete loss of furcation alveolar bone and periodontal ligament and their replacement by epithelium (E) with a severe subepithelial chronic inflammatory response. Pulp chamber (P), bifurcation area (B), dentin (D). (Hematoxylin and eosin stain, original magnification X40).
Figure 19: Apex of infected-only tooth in 3 month animal. Note the absence of periodontal ligament support, epithelial sling (E), and focus of pus (P) in the sinus tract. (Hematoxylin and eosin stain, original magnification X40).

Figure 20: Apex of acidic-treated tooth in one week animal. Note the epithelial sling (E), sinus tract (S), and remnants of the acidic paste (P). (Hematoxylin and eosin stain, original magnification X40).
Figure 21: Apex of acidic-treated tooth in one month animal. Note the well differentiated epithelium (E) in the apical region and remnants of the acidic-paste (A) in the root canal. (Hematoxylin and eosin stain, original magnification X40).

Figure 22: Isolated periodontal ligament attachment (L) of an acidic-treated tooth in one month animal. (Hematoxylin and eosin stain, original magnification X40).
Figure 23: Apex of acidic-treated tooth in three month animal. Note the epithelial proliferation in the periapical region. (Hematoxylin and eosin stain, original magnification X25).

Figure 24: Periodontal cyst-like area adjacent to root apex seen in Figure 23. Note the well demarcated cyst-like area (C) lined by epithelium and a chronic inflammatory infiltrate. (Hematoxylin and eosin stain, original magnification X25).
Figure 25: Apex of acidic-treated tooth in six month animal. Note the lack of any periodontal ligament support and the severe subepithelial chronic inflammatory infiltrate. (Hematoxylin and eosin stain, original magnification X40).

Figure 26: Apex of basic-treated tooth in one week animal. Note the epithelial sling (E), sinus tract (S), and acute inflammatory response (A) in the periapical tissues. (Hematoxylin and eosin stain, original magnification X40).
Figure 27: Proliferating epithelium (E) is adjacent to an acute focus of pus (P) in a sinus tract of a basic-treated tooth in one week animal. Note the epithelial rests of Malassez (M). (Hematoxylin and eosin stain, original magnification X40).

Figure 28: Embedded piece of dentin in lamina propria of 1 month animal. Note the absence of inflammation around the embedded piece of dentin (D) and temporary filling material (F). (Hematoxylin and eosin stain, original magnification X25).
Figure 29: Isolated periodontal ligament attachment of a basic-treated tooth in one month animal. Note the degenerating epithelial attachment (A) and proliferating epithelium (E). (Hematoxylin and eosin stain, original magnification X40).

Figure 30: Apex of basic-treated tooth in one month animal. Note epithelial sling evagination (E) into the apical region of the root canal. (Hematoxylin and eosin stain, original magnification X40).
Figure 31: Apex of basic-treated tooth in one month animal. Note dentin and cementum resorption (R) in the apical region. (Hematoxylin and eosin stain, original magnification X40).

Figure 32: Apex of basic-treated tooth in three month animal. Note extensive epithelial proliferation (E), probable puncture site of micro-electrode (M), and basic paste (B), blood clot (C), and temporary filling remnants (F) in the root canal. (Hematoxylin and eosin stain, original magnification X40).
Figure 33: Well differentiated epithelial sling of a basic-treated tooth in three month animal. The rete pegs (R) and basal and prickle cell (P) layers are evident. (Hematoxylin and eosin stain, original magnification X40).

Figure 34: Remaining socket of basic-treated tooth in six month animal. Note the remaining tooth structure was lost in preparation and a severe subepithelial chronic inflammatory response is present. (Hematoxylin and eosin stain, original magnification X40).
This thesis submitted by James E. McCormick, D.M.D., has been read and approved by the following committee:

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2/13/79
Date

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Signature of Advisor