1978

The Effect of a Calcium Hydroxide Paste on Resorption of Replanted Teeth in Dogs

Dale Marshall Anderson
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_theses

Part of the Oral Biology and Oral Pathology Commons

Recommended Citation
https://ecommons.luc.edu/luc_theses/2978

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.
Copyright © 1978 Dale Marshall Anderson
THE EFFECT OF A CALCIUM HYDROXIDE PASTE ON RESORPTION OF REPLANTED TEETH IN DOGS

by

Dale M. Anderson, B.S., D.D.S.

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science April 1978
DEDICATION

To my parents, Marshall and Virginia Anderson, whose love and guidance have meant so much to me, to my wife, Kathy, for her love and understanding, and to her parents, David and Lotta Borgardt, for their love and constant support.
ACKNOWLEDGMENTS

To Dr. Bruce Felder and Dr. John Gillan, whose hard work and moral support allowed this research to be completed.

To Dr. Norman Wood, whose abilities, knowledge, and integrity hopefully have rubbed off on me during the years I have known him.

To Dr. John Madonia, teacher, administrator, and friend to all his students.

To Dr. Marshall Smulson, whose guidance and friendship has gone far beyond the field of endodontics.

To Dr. Franklin Weine for his friendship, excellence in teaching and personal counsel.
The author, Dale Marshall Anderson, is the son of Nels Marshall and Virginia Ruth Anderson. He was born in Chicago, Illinois, on May 18, 1949.

He received his elementary education at West Northfield School in Northbrook, Illinois, and his secondary education at Glenbrook North High School, where he was a member of the National Honor Society, and graduated in June of 1966. In September of the same year he entered Wheaton College, Wheaton, Illinois, where he received a Bachelor of Science degree in June of 1970 with a major in Biology.

In September of 1970 he entered the Loyola University School of Dentistry (Chicago College of Dental Surgery,) where he graduated in June of 1974 with the degree of Doctor of Dental Surgery. Honors included Omicron Kappa Upsilon national dental honor society, and the American Academy of Oral Medicine award.

After receiving his D.D.S. degree, he was in private dental practice in Palatine, Illinois from August, 1974 to August, 1976. He held the position of part-time Clinical Instructor in the Department of Oral Diagnosis at Loyola University School of Dentistry from December, 1975 until August, 1976, when he began the
graduate program at the same institution, pursuing the degree of Master of Science in Oral Biology and a specialty certificate in the Department of Endodontics.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>VITA</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
</tbody>
</table>

Chapter

1. INTRODUCTION                                1
2. REVIEW OF THE LITERATURE                    3
3. MATERIALS AND METHODS                       47
4. RESULTS                                     54
5. DISCUSSION                                  71

SUMMARY AND CONCLUSIONS                       80
REFERENCES                                    82
FIGURES                                       97
LIST OF TABLES

Table | Page
-----|-----
1. Results, Dog Number One       | 62
2. Results, Dog Number Two       | 63
3. Results, Dog Number Three     | 64
4. Results, Dog Number Four      | 65
5. Results, Dog Number Five      | 66
6. Results, Dog Number Six       | 67
7. Summarized Histologic Scores  | 68
8. Statistical Analysis          | 69
9. Comparison of Contralateral Gutta-Percha and Ca(OH)₂ Filled Teeth | 70
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>99</td>
</tr>
<tr>
<td>2.</td>
<td>99</td>
</tr>
<tr>
<td>3.</td>
<td>101</td>
</tr>
<tr>
<td>4.</td>
<td>101</td>
</tr>
<tr>
<td>5.</td>
<td>103</td>
</tr>
<tr>
<td>6.</td>
<td>103</td>
</tr>
<tr>
<td>7.</td>
<td>105</td>
</tr>
<tr>
<td>8.</td>
<td>105</td>
</tr>
<tr>
<td>9.</td>
<td>107</td>
</tr>
<tr>
<td>10.</td>
<td>107</td>
</tr>
<tr>
<td>11.</td>
<td>109</td>
</tr>
<tr>
<td>12.</td>
<td>109</td>
</tr>
<tr>
<td>13.</td>
<td>111</td>
</tr>
<tr>
<td>14.</td>
<td>111</td>
</tr>
<tr>
<td>15.</td>
<td>113</td>
</tr>
<tr>
<td>16.</td>
<td>113</td>
</tr>
<tr>
<td>17.</td>
<td>115</td>
</tr>
<tr>
<td>18.</td>
<td>115</td>
</tr>
<tr>
<td>19.</td>
<td>117</td>
</tr>
</tbody>
</table>
Proper treatment of traumatic injuries has always been a particular problem in dentistry as well as medicine, because of the need for quick, decisive, and correct treatment. Emergency treatment, often rendered in the middle of an already hectic day, may have long lasting negative effects on the unfortunate patient presenting to his dentist with his teeth in his hand after an accident, especially if the treatment rendered is inadequate or incorrect. Although replantation of avulsed teeth has been practiced for centuries, only in the last twenty years has proper research on the effect of the many contradictory methods of treatment begun to change tooth replantation from a pessimistic temporary measure to a more predictable procedure.

Although adults may suffer tooth avulsion, often as the result of automobile accidents or physical altercations, children may suffer severe psychological trauma in addition to the physical injury. Children most frequently lose teeth due to sports accidents or overexuberant roughhousing. Because anatomical considerations usually prevent the construction of fixed bridge-work in children and young teenagers, these youngsters
are forced to wear bulky and often unaesthetic removable acrylic appliances for years. Therefore, it is fortunate that the replantation procedure is being refined by isolating the pertinent variables and determining which methods lead to increased success. Healthy retention of these teeth through the formative years, when the emotional impact of the loss of anterior teeth may mean severe impairment of the psychosocial development of a child, may soon become a predictable procedure.

Root resorption is a likely sequela of tooth replantation, and is probably the most common reason for failure in these cases(1). Endodontic therapy using gutta-percha and sealer as a filling technique is commonly performed on replanted teeth, but several recent studies have reported either prevention or arrest of external resorption by using calcium hydroxide as an intermediate root canal filling material. The purpose of this study is to determine histologically and radiographically, using dogs as an experimental model, whether teeth replanted with a calcium hydroxide paste in the root canals show any difference in incidence or severity of external resorption from teeth conventionally filled with gutta-percha and sealer.
CHAPTER II
REVIEW OF THE LITERATURE

Replantation of teeth has been defined as "the return of a tooth avulsed by acute trauma, into its alveolus." (2) Grossman (3) defined intentional replantation as "the intentional removal of a tooth and its reinsertion into its socket after endodontic manipulation or obturation of the canals or both," and transplantation as "the removal of a tooth from one socket and insertion into another socket of the same or a different person." The literature pertaining to these three different modes of reinsertion of teeth into empty sockets shows much overlapping of research methods, applicable results, and healing processes, so although there are many important differences between replantation of avulsed teeth, intentional replantation, and transplantation, any review of the literature in the first area would be incomplete without including selected articles from the other two areas.

Albucasis made the first reference in the literature to replantation in 1050, so it has been attempted for hundreds of years. The first research on the subject was reported in 1775 by Hunter (4), who reached the conclusion that a vital periodontal ligament was a prerequisite for successful replantation. In order to
prolong the life of recently extracted teeth, he planted them in the comb of a rooster, until such time as he would have occasion to use them. Barbikow reported that in Elizabethan times, it was fashionable for socialites to have the extracted teeth of paupers transplanted into their mouths. An increase in the incidence of tuberculosis and syphilis and other infectious diseases, however, brought this practice to an end. (5)

Even against resistance from those who supported the focal infection theory and advocated extraction of all "pulpless" teeth, preliminary research into tooth replantation began to appear in the literature in the third decade of this century. Wilkinson (6), in 1926, extracted two monkey lateral incisors and replanted them five days later. Postoperative examination after 53 days showed root resorption. Skillen and Lundquist (7), in 1929, found more promising results. They replanted teeth in dogs and found normal reattachment through formation of cementum. Bödecker and Lefkowitz (8), in 1935, however, extracted anterior teeth in six dogs, placed retrograde amalgam fillings, while keeping teeth moist with sterile saline, and replanted the teeth within thirty minutes. Microscopic examination revealed areas of resorption which they equated with "areas of infection," so they concluded that "this procedure would
probably not come into vogue again because of the real dangers of focal infection in human teeth." The impact of this study may be one of the reasons why very little research was done on replantation until the 1950's, when the focal infection theory rapidly lost acceptance, at least pertaining to teeth with proper endodontic therapy.

Intentional extraction and replantation of teeth was described by Grossman (3), 1966, as being performed when problems encountered in conventional endodontic therapy preclude successful therapy. Such problems as broken instruments lodged in the canal, root perforation, excessive canal curvature, or blockage of the canal were depicted. It is used as an alternative to extraction and prosthetic replacement. When teeth were kept moist with a saline antibiotic solution during extraoral treatment, he reported that 36 out of 45 replantations done intentionally were successful, with no root resorption, at an average of 5.6 years recall time. Emmertsen, et al (9) the same year reported on 100 molars with inaccessible canals and periapical pathosis that were extracted, kept moist with saline, and treated from the apical end and filled with either amalgam or gutta-percha, and replanted after curettage of the periapical area. The teeth were splinted for eight days and radiographed for up to thirteen years. Only 34% showed
periapical healing and absence of root resorption at one year, but they reported that teeth with periapical radiolucencies at time of replantation showed significantly less root resorption than those without. Also, patients less than 30 years of age had inflammatory root resorption and periapical inflammation more frequently than older age groups. Kaplan and Ward (10) in 1971 suggested that repositioning of malposed or rotated teeth, and replantation after extraction of the wrong tooth, were two additional indications for intentional replantation, and listed as contraindications systemic healing problems, loss of crestal bone, root fractures, destruction of labial plate of bone, and hypercementosis. Simon and Kimura (11) in 1974 extracted teeth, treated the roots endodontically, and replanted them in their sockets, the crowns being cut off below the level of the crestal bone, in an attempt to maintain the height and width of alveolar bone for the support of prosthetic appliances. Eighteen month postoperative clinical and radiographic findings indicated that although no bone formed coronal to the roots, and many of the roots were resorbing, clinically some cases appeared to be maintaining bone to different degrees. Simon, et al, (12) the next year, in a histologic study of the same or similar teeth, showed normal periodontal ligament,
resorption, ankylosis, and repaired tooth structure, without predictability as to location or severity. They also described an eosinophilic cuticle-like band found between the epithelium covering replanted dentin surfaces and the resorbed dentin. Even though the success rates reported for most of the intentional replantation studies are not particularly high, a report by Kirsten (13) of an intentional replant done in 1938, successful for 25 years, makes it seem a reasonable alternative to extraction and replacement in selected cases.

Transplantation of teeth from one socket to another is classified by Natiella, et al (14) as being either autogenous (in the same individual), homogenous (within the same species), or heterogenous (one species to another). The same authors give the overall rate of success for autogenous transplants as greater than fifty per cent. Schulman, et al (15) studied autogenous transplants (maxillary left central incisor to right central's socket) and allogeneic (the same as homogenous) transplants, and found that while the autogenous grafts rapidly reattached by normal periodontal ligament, the allografts became firm as a result of ankylosis, with exaggerated periapical inflammation, progressive vertical bone loss, and extensive inflammatory and replacement root resorption. Grewe and Felts (16) found, by studying tritiated thymidine incorporation, that mouse teeth
replanted into their sockets exhibited further growth and maturation. Teeth transplanted from one animal to another, however, were all non-viable, and showed no growth or maturation, many becoming exfoliated. Various techniques have been used in an attempt to improve transplantation success. Weinreb, et al (17) found that if a portion of the accompanying alveolar bone in autogenous transplants was implanted along with the grafted tooth with the periodontal ligament intact, root resorption would not occur. Contrary to these findings, however, Luke and Boyne (18) have shown that the transplantation of teeth with the surrounding periodontal ligament and bone resulted in an extensive root resorptive process. Costich, et al (19) replanted and transplanted 120 hamster molars, finding that freezing teeth at -70°C and storing them at -8°C for one to 66 days before replantation or transplantation led to an overall 60% rate of successful retention for up to 89 days. Storage of teeth in culture medium at 37°C for one to two days led to 77% success.

Transplantation of teeth from one animal to another is complicated by immune reactions to foreign tissue. Boyne (20) reported that the rejection phenomenon after tooth transplantation, although not of the same magnitude as that elicited by other types of tissues, may be evidenced by a chronic inflammatory infiltration of cells
surrounding the transplant and extending into the pulpal tissue. Failure of the pulp to function as a dentin-forming agent and to assist in the completion of the structure of the tooth root, with fibrous encapsulation and root resorption with replacement by osseous tissue are also seen. The results of a study by Robinson and Rowlands (21) in 1972 also show that teeth used in transplantation are antigenic. Prior sensitization of the recipient by skin grafting resulted in more frequent loss of the tooth allografts and more intense lymphocytic infiltration around the graft beds. In a later study (22), 1974, the same authors evaluated tooth allografts in Syrian hamsters, and found that while isolated allogenic mineralized tooth surface, with the periodontal ligament enzymatically removed, was not immunogenic (did not serve as a primary response to secondary skin grafts), isolated periodontal ligament was shown to evoke an accelerated rejection of skin grafts, proving its antigenicity. How the rejection phenomenon and the inflammatory reaction usually associated with transplanted teeth relate to the inflammatory resorption, ankylosis, and too frequent failure of replanted avulsed teeth, is a matter that deserves much more study. As LeCavalier (23) stated, "many authors believe that the body treats replanted teeth as a foreign protein and thus eventually rejects them."
Several studies have been done in the last twenty years to determine the longevity of replanted avulsed teeth, although many failed to isolate the individual factors affecting success.

In 1959, Lenstrup and Skeiller (24) followed the progress for several years of forty-six patients who had traumatically avulsed teeth replanted using various methods, and concluded that "the long-term prospects of preserving a replanted tooth are unfavorable, since root resorption results in most cases." Garber (25), in 1965, stated that replanted teeth had an average retention of five years, as reported by most clinicians. Ingle (26) feels that the average life span of such teeth is between five and ten years. Grossman (27), in 1970, complained that papers on replantation claiming success after only six to twelve months are not valid, and followed 54 cases from various sources for up to three years. Two thirds of the teeth remained in function for two or more years. Kemp, et al (28), in a continuation of Grossman's study including 71 cases, found that the longer the teeth were replanted, the more likely that root resorption would occur, while the teeth replanted for the longest period had the fewest periapical rarefactions. They concluded that replantation of avulsed teeth is justified despite the fact that it is not usually a permanent procedure. "Success" of a replanted tooth is
a relative term, and the success rates reported depend greatly on the criteria on which the judgement is based. Messing (29) used as his definition of success "fixation of the tooth in its socket without residual inflammation, ...either by ankylosis or by regeneration and reattachment of the periodontal ligament, the latter mode obviously preferred." Emmertsen, et al (9) regarded as successful any implant that was firm in normal function and had normal gingival attachments and no root radiolucencies. Talim and Antia (30) followed up fourteen intentional replants for one year and concluded that nine were successful using the following criteria: The teeth must perform masticatory function without discomfort; gingival color, contour, texture, and sulcus depth must be normal, and there must be no radiographic evidence of resorption of tooth or bone. Knight, et al (31) pointed out the great difference between clinical and histologic criteria for success, with the results of his work replanting teeth in dogs. Seven of sixteen teeth were clinically successful, showing stability, function, and absence of inflammation in soft tissue. None of the sixteen teeth, however, met their histologic criteria of success, which was defined as the absence of root resorption, abscess, and cyst formation, reattachment of the epithelium at the proper level, reformation of the periodontal ligament
without ankylosis, and reestablishment of the vitality of the tooth. In practice, one certainly must be satisfied with meeting clinical success criteria, but it should be noted that it is not usually a normal histologic result.

The histologic mechanism of healing of the periodontium surrounding replanted teeth has been studied extensively. Andreasen and Hjorting-Hansen (32) found that the first six weeks are the most critical to the survival of the implant, since this is the period of major progress in the healing of the periodontal membrane. Woehrle (33) found that although there may be a "primary reattachment" or a true healing of the severed periodontal tissue, an extended or adverse extraoral course led to a "secondary reattachment." This involved replacement of degenerating periodontal tissues attached to the replanted tooth by new ligament fibers provided by viable alveolar fragments. The alveolar fragments also provided cellular elements for the secretion of a new cementum matrix. Then, entrapment of the regenerating fibers in the newly deposited calcifying cementum provides a functional periodontium. This secondary reattachment, according to Woehrle, is dependent on the status of the supporting alveolar tissues, the replant being passive, a substrate for cementum deposition dictated by the extraoral history and the periodontal tissues. Johansen, (34) after replanting
teeth immediately, found that true reattachment was an unpredictable occurrence, and postulated that reattachment took place in the vascular area of the periodontal membrane without new formation of bone and cementum as a prerequisite. This healing by first intention was the initial phase of reattachment of the extracted tooth, and it took place without new formation of the entire fiber apparatus across the periodontal membrane. By the eleventh day the fibers and the bone along the original alveolar wall were replaced by new fibers embedded in a layer of new bone. Caffesse, et al (35) replanted teeth in monkeys twenty minutes after extraction, and found that a highly cellular periodontal membrane developed, and although fibers reattached to reparative bone and cementum, they seldom regained functional orientation. Cervical and apical root resorption was a universal complication, but arrested areas of resorption often showed repair by deposition of cementum. Massler (36), in a clinical assessment of healing following tooth replantation, stated that epithelial cuff reattachment occurred at one week, periodontal ligament reattachment in two to four weeks, and root resorption was evident radiographically by six weeks. Nasjleti, et al (37) agreed that a new junctional epithelium was established (in monkeys) at seven days, while at the same time connective tissue proliferation was at its peak,
starting mainly from the supro-crestal connective tissue and the bone marrow spaces. The interface in the periodontal ligament was undetectable histologically by seven days. Large numbers of epithelial rests were seen in the periodontal ligaments of the replanted teeth. Loe and Waerhaug (38) hypothesized that the epithelial rests of Malassez play a role in the maintenance of the periodontal space in teeth replanted with a "vital" periodontal membrane. Kaplan and Ward (10) stressed the importance of the organizing blood clot in the repair of the periodontal ligament when kept vital, the repair being complete in one month. Hamner, et al (39) studying replanted teeth in baboons, described a massive infiltration of lymphocytes and plasma cells in the healing periodontal membrane. Rockert and Ohman (40) found that within one month after replantation, demineralized areas were seen in cementum and dentin using microradiography. These seemed to be areas of degenerative change and they thought that the demineralization was caused by lack of "membrane covering cementum."

The location of the epithelial attachment was unpredictable after replantation, according to Groper and Bernick (41), but Deeb (42) feels that elimination of periodontal disease by curettage of crevicular epithelium and careful repositioning of gingival tissues is
important in replantation procedures in order to improve a predictable reattachment.

Root resorption is such a common occurrence after replantation that many authors have considered it normal and unavoidable. Caffesse, et al (35) after following up replanted teeth in monkeys for three to four years, concluded that cervical and apical root resorption is a universal complication after tooth reimplantation, and that arrested areas of resorption will show repair by deposition of cementum. Andreasen and Hjörting-Hansen (43), however, described three separate and distinct types of root resorption. The first and most desirable was minor "surface resorption" that occurs in what they described as normal healing, which is quickly repaired by cementum deposition. The second was "replacement resorption," or progressive ankylosis with tooth structure replaced by bone. The third type was referred to as "inflammatory resorption," and was the most destructive type, tooth structure rapidly becoming replaced by granulation-type tissue. Within each type, Andreasen (44) later distinguished between active, arrested, or repaired areas of resorption when studied microscopically. He believed that since it was observed that resorption, when present, always started within one year after replantation, only teeth with an observation period greater than one year would be regarded as successful. (43)
Surface resorption was described in a histologic study of replanted human teeth by Andreasen and Hjørting-Hansen (32) as showing small superficial resorption cavities in the cementum and outermost layers of the dentin. No inflammatory reaction in the periodontal membrane was seen, and most cavities showed repair with cementoid. Barbikow, et al (45) working with monkeys, described surface resorption as becoming evident within two weeks after replantation. Andreasen (46) postulated that these superficial resorption lacunae may represent localized areas of traumatic damage to the periodontal ligament or cementum, and that it was self limiting, usually with spontaneous repair. Normally, only the most severe of these are seen radiographically, and clinically a normal percussion sound is heard.

In a histometric study of periodontal healing in rats by Andreasen, (47) an experimental injury with a bur through the vestibular bone, periodontal ligament, and superficial root surface of a mandibular molar was seen to heal in three phases. Bone repair with formation of an ankylosis occurred initially, followed by repair of the periodontal ligament including removal of the established ankylosis, with repair of the cementum occurring last. In another study by the same author (48) assessing periodontal healing after replantation of avulsed
human teeth, he described two distinct types of ankylosis. The first he called a "transient replacement resorption," with radiographic signs of ankylosis and clinical lowering of tooth mobility, both disappearing within several weeks. This may be the same type of healing that he described after the injury to the rats. The second type of ankylosis, however, was a "permanent replacement resorption," with lowered mobility values occurring by five weeks, radiographic signs of resorption without radiolucency of tooth replaced by bone not evident until eight weeks. Andreasen and Hjörting-Hansen (32) described this histologically as showing cementum and bone being replaced by, and in direct contact with, lamellar bone, with osteoblastic activity often evident. Andreasen (46) hypothesized that a blood clot in the periodontal ligament organized into granulation tissue, and was replaced rapidly by bony trabeculae uniting the alveolar socket with the tooth. Root resorption may or may not precede ankylosis. The tooth may be involved in the normal remodeling cycle of the bone, gradually being replaced by bone. The same author also stated that the replacement resorption was usually first seen radiographically by three to four months after replantation, usually starting in the apical one third of the root. Often eruption was halted, and the percussion sound was high-pitched. Barbikow (45) believed that while replacement resorption was caused by devitalized
periodontal ligament tissue, inflammatory resorption was caused by necrotic pulpal products.

Inflammatory resorption, as described by Andreasen and Hjørting-Hansen (43), was often seen as early as three to four weeks after replantation, and was radiographically characterized by loss of root substance with adjacent radiolucency in the bone. Histologically, the same authors (32) described bowl-shaped areas of resorption of cementum and dentin, associated with inflammatory changes of the adjacent periodontal tissue. The resorptive areas were characterized by Howship's lacunae containing multinucleated cells, and in the inflamed periodontal tissue were seen intense concentrations of polymorphonuclear leukocytes, lymphocytes, and plasma cells, with proliferation of capillaries. Andreasen (46) believed that small resorptive cavities in the root surface may expose or open dentinal tubules. This may allow necrotic tissue, toxic autolyzed pulp components, or bacteria from a necrotic pulp or an inadequate root canal filling to penetrate into the periodontal tissues, causing an inflammatory reaction. Andreasen and Hjørting-Hansen (32) carried this further, saying that inflammation of the periodontal ligament from necrotic tissue in the canal provoked a response which could result in complete resorption of the root with no attempt at repair, as long as the necrotic pulp was present.
One of the multitude of variables affecting the successful healing after tooth replantation is the extent of damage to the teeth and supporting structures. Andreasen (49) considered root fracture a contraindication to replantation. According to Humphrey (50), the maxillary anterior teeth are the teeth most often avulsed, and the alveolar bone in this area is often displaced, or even fractured. Woehrle (33) believed that the viable alveolar fragments were the source of the new ligament fibers and cells for new cementum secretion, so it was not surprising that Andreasen and Hjörting-Hansen (43) found that of nineteen cases of replantation with accompanying alveolar bone fracture, all showed extensive resorption and a poor long-term prognosis. McCagie (51), however, reported a case where three teeth were replanted after being out of the mouth for five days, with loss of most of the buccal plate. Two and one-half years later, very little resorption was evident. Lu (52) recommended replanting teeth even adjacent to jaw fractures after soaking them in benzalkonium chloride. He thought that root canal therapy was not necessary as long as the teeth had a "normal appearance clinically," but that healing of a replanted tooth normally occurred by ankylosis.

The stage of root development at the time of avulsion has been shown to affect the course of healing after
replantation. Kaqueler and Massler (53), in a study using dogs, found that replantation of mature teeth was uniformly less successful than that of immature teeth, when endodontic therapy was not done. Although the extraoral time period was more important to the immature teeth with open apices, the temperature and storage medium variables were more important than the extraoral period to mature teeth. Lenstrup and Skeiller (24) found in studying forty-six patients with replanted teeth that the younger the tooth, the more pronounced and progressive was the resorption. Andreasen (46) found that inflammatory resorption was especially frequent in permanent incisors with incomplete root formation, possibly because of thin dentinal walls and wide dentinal tubules, through which toxic pulpal products could more easily pass. The pulps of the immature teeth, however, revascularized more often than those of mature teeth. Massler (36) said that mature teeth healed more slowly than immature teeth after immediate replantation, but that the difference between the two was small if replantation was delayed for more than 24 hours. Andreasen and Hjörting-Hansen (32) found that a functional arrangement of the periodontal fibers after replantation occurred in almost all of the young teeth, except in those areas of the root surface where active resorption and ankylosis were present.
In the mature teeth, the fibers were usually randomly arranged or ran parallel to the root surface.

Probably the two most important factors affecting the survival of replanted teeth are the amount of time that an avulsed tooth is outside the socket and the environment that it is kept in during that time. McElroy (54) reported on a case where a thirteen year old boy knocked out four maxillary anterior teeth, immediately replanted them himself, and they functioned for 44 years afterward. Kemp, et al (28) studying 71 avulsed teeth replanted by many different clinicians, found that only 13.7% of the teeth were replanted within one hour after avulsion, so usually time out of the socket is a major factor. Andreasen and Hjörting-Hansen(43), after studying 110 replanted avulsed human teeth for from two months to thirteen years, concluded that the degree of replacement resorption was proportional to the extraoral time. Ninety per cent of teeth replanted within 30 minutes showed no resorption, while the vast majority of teeth replanted 90 minutes or more after loss showed extensive resorption. A variable frequency of resorption was seen in those with extraoral periods of from 31 to 90 minutes. Flanagan and Myers (55) extracted and replanted hamster teeth after variable time periods, with the teeth kept in sterile saline, and found that teeth replanted immediately or
within 30 minutes showed a minimum of tissue inflammation, while those kept out longer, especially up to six hours, showed very poor results, osteomyelitis, pulp necrosis, and root resorption occurring. Kaplan and Ward (10) felt that the success rate was directly proportional to the amount of viable periodontal ligament present. They concluded that an excessive extraoral period produced irreversible degenerative changes in the periodontal ligament prior to replantation. The presence of this layer of necrotic tissue, which requires removal by phagocytosis, retarded or prevented reattachment. There are several studies, however, that minimized time and environmental factors. Lenstrup and Skeiller (24) studied 46 patients who had had avulsed teeth replanted and found no correlation between the extraoral period or method of preservation, and the degree or rate of resorption. They found that root resorption resulted in most cases. Since this study was reported in 1959, it is possible that replantation methods found preferable today were not used in these patients. Anderson, et al (56) felt that the periodontal tissues can become reattached even after six hours out of the mouth. This was, however, not attachment by a normal periodontal ligament. Groper and Bernick (41) replanted teeth in dogs after extraoral times from zero to twenty-four hours, with the teeth
kept in sterile gauze. They concluded that no evidence appeared to show that dry storage alone for a period of two hours or more had any effect on the presence of a functional periodontal ligament, at least during the period of their study. Repair of resorbed areas along the entire root surface took place regardless of extra-oral time. The thirty day length of their study may not have been long enough to make any broad generalizations. Čvek (57) studied 38 human luxated and replanted teeth for 22 to 78 months, and found that all teeth stored dry for one hour or more became ankylosic. If the dry interval was short, less than fifteen minutes, the further course was good. If the dry interval was moderate, ankylosis was less if the tooth was stored in isotonic saline for about 30 minutes before replantation. Barbikow (45) found that there was no difference in healing after replanting monkey teeth immediately than after storage for fifteen minutes in saline. Loe and Waerhaug (38), after replanting 58 teeth in monkeys and dogs, found that teeth replanted with a vital periodontal ligament always formed a normal periodontal ligament without ankylosis. Teeth allowed to dry for a short time ankylosed, with minor areas of normal periodontal ligament attachment, while teeth with periodontal ligaments dried for longer times never formed normal periodontal ligaments.
Kristerson, et al (58) maintained the viability of periodontal ligament cells of 21 teeth by the use of a transport medium and tissue culture for four weeks. Four teeth replanted after eleven weeks in this medium showed good healing after five months. Nasjlete, et al (59) found that ten monkey teeth stored in a culture medium at 4° C for seven days before replantation showed 100% success at one year, while ten other teeth stored at -10° C for the same time showed only 50% success. Jonck (60), however, stored human teeth in isotonic saline at 4° C, and found active resorption six weeks after replantation. Other teeth which he stored in the patient's own blood (treated with dextrose citrate to prevent coagulation) for three weeks before replantation showed no signs of resorption after two years.

The effects of various methods of direct manipulation of the periodontal ligament on healing after replantation has been discussed by many authors. Hammer, (61) in 1955, believed that the life-span of a replanted tooth was directly proportional to the area of the periodontal ligament remaining attached to the tooth. Loe and Waerhaug (38) found that teeth replanted in dogs and monkeys after removing the periodontal ligament by curettage never regained their normal reattachment apparatus, but had the periodontal space occupied by soft tissue, soon replaced
by bone, with ankylosis complete by 30 days. Bennett (62) found that teeth replanted after scraping the periodontal ligament formed a new periodontal membrane within two or three months, but it was followed by complete bony ankylosis and osseous rebuilding of the root. When the ligament was not removed there was complete restitution of a normal periodontal ligament within four to six weeks. Hammer, et al (39) replanted teeth in baboons, discing the distal root surface of the teeth, while leaving the rest of the root surface alone. The mesial side maintained a good periodontal union twelve months after re plantation, with only focal microscopic resorption, while the disced distal surface showed severe root resorption, with a massive infiltration of lymphocytes and plasma cells in the periodontal ligament. Sherman (63) replanted incisors in dogs after performing root canal therapy. More extensive root resorption and ankylosis were observed on the teeth replanted after scraping the root surface and wrapping with resorbable surgical sponge as a periodontal ligament substitute than those replanted with the periodontal ligament unmanipulated. Ogus (64) described a technique which involved casting and cementing a vitallium thimble implant with a rough external surface over the prepared root surface before replanting teeth, in an effort to prevent root resorption. No actual clinical cases were reported. Keller, et al (65) in an effort to
stimulate fibrotic attachment of replanted monkey teeth to the socket, adapted venous segments over the roots of teeth prior to replantation. Increased sulcus depth, root resorption, and ankylosis were seen in the experimental teeth compared to control teeth. Natkin (66), discussing the value of scraping the periodontal ligament before replantation, stressed the importance of whether or not the ligament alone is removed, or whether the cementum is also removed, exposing dentin. Morris (14), found that after surgical detachment of periodontal tissues, healing was normal against untouched or lightly curetted cementum, but healing did not occur against the bare dentin of teeth with non-vital pulps, including those with previous root canal therapy. Baurer, et al (67) was able to remove the periodontal ligament enzymatically from extracted teeth in as little as fifteen minutes, without observable damage to the cementum. Huard, et al (68) using rhesus monkeys, cultured periodontal ligament fibroblasts on enzymatically debrided root surfaces, establishing a network of viable fibers, and replanted one of these teeth in a monkey. The tooth was reextracted seven months later, and a normal looking periodontal ligament was seen with no visible areas of resorption.

The advisability of apicoectomy before replantation has been debated. Harris (69) advised cutting off the root
apex, cleaning out the root canal, and leaving the lingual access open to avoid fluid pressure in the periapical area. If root end resorption occurred later, he recommended treating it by surgical exposure and "shaving" the root end down to "fresh" tooth structure. Anderson, et al (56) thought that cutting off the root end in older teeth promoted repair by secondary cementum and reattachment of the periodontal ligament in this area, and Massler (36) advocated "snipping off the root tip" if the tooth resists replacement in its former locus. Bennett (62) disagreed with these procedures. He believed that since it has been suggested that resorption begins where small pieces of cementum are torn from the root during extraction, it would follow that apicoectomy should not be performed.

Although there is almost complete agreement in the literature that replanted teeth should be splinted, there is little agreement as to the type and duration of splinting. A most unusual splint was one reported by Serrano (70), where a horizontal hole was drilled through the alveolar bone and root of the replanted tooth, through which was driven a "retention rod" of calf's bone. Others were more conventional. Pearson and Nicolazzo (71) described an arch bar wiring technique, while Barbikow (5) recommended splinting by full arch coverage with an appliance to open the bite from two to four millimeters.
Biven, et al (72) suggested processed acrylic buccal and lingual splints, which were wired together through the interproximals. Jenner (73) recommended a ligature wire splint covered with cold-cure acrylic, while Rand (74) described an unusual technique of suturing through the buccal and lingual periosteum, with the suture running over the incisal edge between the mammelons. Humphrey (50) splinted anterior teeth by soldering together interproximally either stainless steel crowns or orthodontic bands. McEvoy and Mink (75) advocated acid etching the labial surfaces of the teeth, then applying a 3 mm. strip of acrylic resin across the labial surfaces and interproximals. Heiman, et al (76) described a splint attaching an arch wire to the labial surfaces of the teeth by means of an adhesive composite system. Hovland and Gutmann (77) cemented direct-bonded, mesh-backed orthodontic brackets to the teeth, and attached an arch wire with elastic ligatures, and Berman and Buch (78) covered similar direct-bonded brackets with a band of acrylic.

Although Johansen (34) found when replanting guinea pig teeth that splinting or not splinting showed no difference in healing, splinting of avulsed human teeth after replantation has been almost universally accepted. Barbikow (5) thought that 30 day immobilization was needed,
Pearson and Nicolazzo (71) recommended splinting for six to eight weeks, Hargreaves and Craig (79) for eight to twelve weeks, and Henning (80) advised "at least three months." Andreasen (44) has published the only objective research into this area, replanting teeth in 21 monkeys, splinting for six weeks, two weeks, or no splint at all. The monkeys were sacrificed at eight weeks, and although teeth that were out for 120 minutes showed extensive ankylosis irrespective of splinting method, teeth that were out for eighteen minutes showed significantly less frequency and extent of replacement resorption when not splinted compared to the splinted teeth. Andreasen (81) at the present time recommends splinting replanted teeth for one week only.

Antibiotic coverage has been advised (71), and in cases of severe tissue injury is probably warranted but Massler (82) and Andreasen and Hjörting-Hansen (43) have both shown experimentally that systemic antibiotic administration did not significantly affect the rate of healing of the periodontal tissues.

Disinfection of root surfaces before replantation has been advised by some authors. Loe and Waerhaug (38) washed roots in 1% phenyl mercuric acetate, Messing (29) soaked teeth in chlorhexidine diacetate solution for five minutes, and Lu (52) soaked them in benzalkonium chloride. Massler (82), however, found that applying
caustic drugs such as phenol or silver nitrate in an attempt to disinfect the tooth increased the rate and extent of root resorption. Andreasen (49) concurred, saying that attempts to sterilize the tooth surface may damage or destroy vital periodontal tissue and cementum.

Other chemical treatments of avulsed teeth beside germicides have been used in an attempt to improve healing. Fluorides have been the most extensively tested. Schulman, et al (83) soaked teeth from monkeys in sodium fluoride solution before replantation and found mixed results, with some apparent lessening of resorption of the fluoride-treated teeth. Bjorvatn and Massler (84) soaked rat teeth in 10% stannous fluoride solution for five minutes before replantation, and found increased resorption. Dilution to a 1% solution showed an improvement of root resorption over control teeth, although not totally preventing it. Bjorvatn and Weiss (85) found that a 2% solution of sodium fluoride clearly decreased root resorption in replanted rat molars.

Colletti (86) described a "root incision technique," where grooves were cut in the root exposing the canals which were then filled with amalgam, and these teeth were soaked in a 10% stannous fluoride solution for two minutes. He claimed a 94% success rate in human teeth, although he accepted ankylosis as an acceptable result.
In another study Schulman, et al (83) found that after tooth immersion in fluoride and replantation, the fluoride was taken up significantly into the bone of the socket wall. White (87), in 1975, related a case where teeth traumatically avulsed were replanted after lying at the bottom of a swimming pool for 24 hours. Healing was good, and the author thought that since both were halogens, the effect of chlorine may have been similar to that reported by fluoride. Robinson and Shapiro (88) soaked hamster molars in a diphosphonate solution before transplantation, but found no difference in healing compared to control teeth. Huebsch (89) treated root surfaces of dog teeth with methyl-2-cyanoacrylate adhesive before replantation, but the teeth were all exfoliated within fourteen to sixty days. Nordenram, et al (90) superficially demineralized roots of monkey teeth in hydrochloric acid, replanted teeth, and found that the demineralizing treatment caused an accelerated resorption and ankylosis of the teeth. Bjorvatn and Weiss (85) found that soaking roots in tetracyclines before replantation produced a marked increase in alveolar bone growth, so that roots so treated became quickly ankylosed. Fong and Berger (91), attempting to reduce the antigenicity of tooth transplants, irradiated the teeth with high doses of x-radiation, but observation of the transplants showed that the radiation destroyed the viable tissues of the
autograft, so that poor healing and no further root development resulted.

Many studies have been undertaken to determine the effect of endodontic therapy on replanted teeth. Some authors felt it preferable to replant without pulp therapy. In 1929, Skillen and Lundquist (7) replanted teeth in dogs, and found that "the (endodontically) treated tooth suffered much more extensively through resorption than the untreated, fusion of bone and cementum also occurring in the former." The exact method of endodontic treatment was not mentioned, nor was it clear whether or not the extraoral times were equal. Various studies have shown that sometimes pulp vitality can be maintained to some extent. Costich, et al (92) immediately replanted 29 hamster molars immediately after extraction. At three months, most had vital tissue in the canals, some with osteodentin formation, but almost one half also showed ankylosis. Sorg (93) replanted twenty hamster second molars with developed apices within five minutes after extraction. Sixteen of twenty teeth were retained until the 34 to 63 day sacrifice time. Nine of these sixteen showed regenerated nerves within the pulp, three of these pulps being histologically normal, six pulps showing normal tissue with varying amounts of osteoid material. Morrison (94) reported a case
where a central incisor with an undeveloped apex was replanted within 30 minutes of traumatic avulsion. Three weeks later the tooth responded to thermal and electric vitality tests, and two years later a normal completely developed root apex was seen on x-ray examination. Ten years later the pulp chamber was completely filled in radiographically, but the periapex and periodontal ligament appeared normal. Henning (80) felt that it was probable that the time taken in performing root filling before replantation led to damage to the root surface because of increased extraoral time, which may outweigh the benefits of pulp removal. Rothchild, et al (95) replanted teeth in dogs, and concluded that there appeared to be little difference, if any, between endodontic and non-endodontic replants with respect to ankylosis and bone deposition (at 30 days.) This was not in agreement with the earlier study by Knight, et al (31), also using dogs. Knight found that although all teeth showed mild to severe diffuse resorption, localized cervical resorption was much worse in the untreated teeth. Ankylosis occurred with equal frequency in treated and untreated teeth, but general tissue response was better in the treated teeth. They recommended that replanted teeth have root canal therapy done as soon as possible. Barbikow (45) replanted central incisors in monkeys with and without root canal therapy, with an average time extraoral of
fifteen minutes. They found no difference in healing for up to eight weeks, other than the presence of periapical abscesses in the non-treated teeth. Andreasen and Hjörting Hansen (43) followed up 110 replanted human teeth. They concluded that if a replanted tooth was not endodontically treated and the pulp became necrotic, toxic products from the pulp could destroy the periodontal membrane and cementum via the dentinal tubules. This would cause poor healing and inflammatory resorption, usually leading to loss of these teeth in from two to ten months.

Often the stage of root development has been used as a factor in deciding whether to replant and hope for pulp revitalization, or to perform endodontic therapy. Anderson, et al (56) found that the size of the opening of the apical foramen was important to pulpal survival. Andreasen and Hjörting-Hansen (43) reported that seven of thirteen human teeth with open apices replanted without endodontic therapy showed pulp survival, with gradual obliteration of the pulp chamber. Six teeth showed pulp necrosis, five of these with extensive rapid inflammatory resorption. Andreasen (46) found that teeth with open apices may revascularize if out of the socket for less than 30 minutes, with some chance of revascularization up to two hours. Most of these teeth showed arrested root development and pulp canal obliteration with osteoid or bone.
Monsour (96) replanted anterior teeth with open apices in eight dogs. Most teeth showed the entire pulp chamber filled with granulation tissue leading to "osteodentin" deposition by cells differentiated from the fibrous replacement tissues. He felt that vital replantation was superior to root canal therapy if done immediately and with open apices. Öhman (97 & 98) studied teeth that were replanted immediately and reextracted at various later times, finding that regenerating nerve fibers were evident at one month. This was consistent with vital pulp test responses that were seen two months after replantation in 33 of 37 teeth tested. He said that a better indication of vitality was the reduction in the size of the pulp chamber radiographically. Smith (99) reported a case where a 26 year old man replanted his own tooth immediately after it was totally avulsed into his mouth. Although the apex was fully developed, it remained in function for 25 years with calcific degeneration of the pulp, but no root resorption or apical rarefaction was evident radiographically. In spite of an isolated case like this, Whiteacre and Edwards (100) said that pulp necrosis occurred in over 80% of replanted teeth with closed apices, and root canal therapy should be instituted as soon as this occurs.
The length of time that an avulsed tooth is out of the socket, and thus the degree of pulp tissue breakdown, should be of prime importance in deciding whether or not to institute immediate endodontic therapy. Heithersay (101) normally recommended replanting the tooth immediately, then commencing root canal therapy within ten days, unless the tooth was out longer than two hours, in which case endodontic therapy can be carried out first. Deeb (42) said to perform endodontics prior to replantation, unless the tooth was out less than one hour, in which case endodontics should be delayed three to four weeks to evaluate pulpal healing. Andreasen and Hjörting-Hansen (43) believed that pulp removal and endodontic therapy should be performed as soon as possible to prevent inflammatory resorption from pulp breakdown products, but not at the expense of increasing the extraoral time. The same authors (32) in another study found that pulp necrosis usually occurred between two and seven weeks after replantation, if at all, so they recommended immediate replantation with endodontic therapy done within two weeks afterward.

Many materials and methods have been recommended for performing root canal therapy on avulsed teeth. Bennett (62) believed that the material used for the root filling does not appear to influence the reattachment, provided there is an adequate apical seal. Pulp extiration alone
is not advised, as Woehrle (33) found that this caused abscess formation in replanted teeth, while complete endodontic therapy showed minimum periapical pathosis. Andreasen and Hjörting-Hansen (32) recommended gutta-percha and sealer as a root canal filling, while Deeb (42) and Messing (29) advised performing an apicoectomy and reverse filling with amalgam. Harndt and Hoefig (102) described the use of a chromium-vanadium steel endodontic implant to aid in the retention of a replanted avulsed tooth. Marosky (103) recommended a vitallium post as a filling material, as it could be easily removed if severe resorption occurred necessitating extraction, with calcium hydroxide as a sealer, as it is resorbable and may retard resorption. Recently calcium hydroxide has itself been shown to have an effect on root resorption. In 1973 McKinley (104) described a case where external root resorption following avulsion and replantation of a central incisor was arrested by placement of a calcium hydroxide paste in the root canal. The tooth was later filled with gutta-percha and sealer.

Calcium hydroxide has had a multitude of uses in dentistry, beginning in 1930 when Hermann (105) introduced it as calxyl for pulp capping, showing the ability of the pulp to protect itself through formation of a dentin bridge.
Zander (106), in 1939, described pulp healing after treatment of pulp exposures with pure calcium hydroxide. Later, in 1949, Glass and Zander (107) found that calcium hydroxide acts as a stimulus for the conversion of undifferentiated mesenchymal cells of the pulp to form a new odontoblastic layer after pulp injury. When calcium hydroxide was used as a base, Mjör (108), in 1967, found that sound dentin showed increased density through intratubular secondary mineralization. Avery (109) found that calcium hydroxide would induce reparative dentin underlying a cavity much more readily than under an exposure. Heys et al (110) showed that calcium hydroxide, when placed in cavities of moderate depth, produced at three days a moderate pulpal inflammatory response. By five weeks a decrease in inflammation and the formation of repairative dentin was seen, with sclerosis of dentinal tubules. Macchetti and Toledo (111) used tetracycline to study secondary mineralization of healthy dentin under calcium hydroxide bases, and found increased mineralization in the dentin below the base, and at the dentinopulpal boundary. The time during which calcium hydroxide acted on the dentin was relatively short, reaching its maximum on the second day after treatment, with the effects disappearing completely by the fifth day. Eidelman, et al (112) placed calcium hydroxide over the deepest one millimeter of remaining carious dentin
(indirect pulp cap) after deep excavations. They found a significant increase in phosphorous content when the remaining dentin was checked after two to twelve weeks, indicating that a remineralization of the carious dentin occurred. Spedding, et al (113) found that 60% of pulp caps in monkeys were successful when calcium hydroxide was used, while formocresol showed 70% success and was judged superior as a pulp capping agent. Kozlov and Massler (114), however, found that pulp caps in rat molars with calcium hydroxide produced a good repairative reaction, with a small zone of inflammation, well encapsulated. Under this a wide bridge of repairative dentin was formed centrally and laterally to bridge the site of amputation, with normal, relatively uninflamed, pulp beneath.

Krakow, et al (115) and Moodnik (116) recommended vital calcium hydroxide pulpotomy for immature teeth with damaged pulps, in order to allow for continued apical development. While Moodnik advised that root canal therapy be performed after apical closure, Krakow said that it was not necessary unless calcification of the canal was seen on recall examination. Via (117) studied 800 cases of calcium hydroxide pulpotomies, and found only 31.1% success, most failures because of internal resorption. Schröder and Granath (118) stressed the importance of the calcium hydroxide being in intimate
contact with vital pulp tissue, because otherwise an eosinophilic calcium-hydroxide-fixed extra-pulpal blood clot was formed between the two. This could give rise to chronic granulation tissue and internal dentin resorption. Schröder (119), and Schröder and Granath (120) found, after experimental pulpotomy, a layer of necrosis immediately beneath the calcium hydroxide which irritated the underlying pulp to respond with hard tissue formation. They believed that the high pH of the calcium hydroxide was responsible for the tissue changes.

In 1961, Nygaard-Ostby (121) described apical closure by hard tissue deposition of teeth with open immature apices and necrotic pulps. He accomplished this by placing a short root canal filling after purposely lacerating the periapical tissues to produce a blood clot, which organized into cementum-like tissue. Kaiser (122) in 1964 introduced the idea of treating these teeth with a paste made by mixing calcium hydroxide with camphorated parachlorophenol, which produced a bridging over the apex allowing proper obliteration of the canal and eliminating the need for surgery. Frank (123) in 1966 expanded on Kaiser's treatment, outlining a step-by-step procedure for calcium hydroxide and camphorated parachlorophenol treatment of open-apex permanent teeth with necrotic pulps. Michanowicz and Michanowicz (124) in 1967 recommended
placing a layer of calcium hydroxide at the end of a canal with an open apex, and condensing gutta-percha and sealer into the canal. Frank (125) in 1967 explained that calcium hydroxide was used for the apexification technique because it was simple and inexpensive, but that it was the removal of infection, not the calcium hydroxide, that allowed Hertwig's sheath to resume its function of apical closure. Steiner, et al (126) thought that introduction of the calcium hydroxide paste after stimulating apical bleeding (Nygaard-Ostby) may be the most favorable condition for stimulating closure of the apex. Van Hassell and Natkin (127) in 1970 reported calcium hydroxide-induced root end closure of immature molar teeth with necrotic pulps. In 1971 Dylewski (128) studied calcium hydroxide apexification of immature monkey incisors, and found that instead of a continuation of normal root development, the calcium hydroxide induced a repair process in the connective tissue of the apical area. That same year Steiner and Van Hassell (129) found that the calcific root end closure that formed when calcium hydroxide was used satisfied the usual criteria for identification as cementum. Torneck and Smith (130) and Torneck, et al (131, 132, 133) studied the effects of various procedures on the apical development of immature monkey teeth. Some degree of root growth and foraminal closure were seen after pulpectomy with and without coronal seal,
and with and without canal disinfection and medication. The use of a calcium hydroxide treatment paste, however, increased the predictability and amount of hard tissue formation over any of the other treatments.

Other authors have questioned the value and effect of the calcium hydroxide in apexification. Ham, et al (134) found that periapical healing and calcified tissue formation could occur in monkeys with either short gutta-percha or calcium hydroxide paste fillings. Roberts and Brilliant (135) in 1975 found that tricalcium phosphate was as effective as calcium hydroxide in inducing apical closure in human immature pulpless teeth. They concluded that it was not the highly alkaline pH of calcium hydroxide (11.8) that induced closure, because the pH of the tricalcium phosphate was only 8.6. In 1977 England and Best (136) extripated the pulps in 40 permanent premolars with immature apices in seven young dogs, leaving half of the teeth open to the oral environment and sealing the other half with a temporary cement. Without the use of any drugs in the canals, by seven to eleven weeks 85.5% of the open teeth and 50% of those that were closed showed complete apical closure. It was concluded that drugs such as calcium hydroxide were not necessary to stimulate apical closure in dogs.
Calcium hydroxide pastes also have been evaluated as a root canal filling material in teeth with mature apices. Matsumiya and Kitamura (137) found that filling the root canals with a paste made of calcium hydroxide and sterile water had the effect of accelerating the natural healing functions in the periapical tissues of dogs. They felt that this was due in part to an antimicrobial effect of the calcium hydroxide in the apical tissues. Holland (138) and Martin and Crabb (139) reported that calcium hydroxide was an excellent root canal filling material, showing periapical healing by hard tissue formation, through a process similar to the pulp healing when the same material is used there. Stromberg (140), however, showed no statistically significant differences in healing between teeth filled with calcium hydroxide, dibasic calcium phosphate (pH7) and gutta-percha with chloroform-resin. Weinstein and Goldman (141) found that no apical bridging was observed after mature monkey teeth were debrided and filled with calcium hydroxide paste. They concluded that the metabolism of teeth with mature apices was quite different from teeth with immature apices.

Calcium hydroxide has been shown by Frank and Weine (142) to effect the periodontal healing of a perforative defect of internal resorption when placed in the debrided canal. A later permanent root canal filling may then be placed against a matrix of normal periodontal tissues.
Frank (143) further applied this technique to aid in the repair of mechanical perforations as the result of a misdirected bur. Stewart (144) also demonstrated the healing of internal or external root resorptive defects after placement of a calcium hydroxide paste into the canal. He suggested that metacresylacetate was less irritating and gave more rapid healing with calcium hydroxide than did the camphorated parachlorophenol used by the previous two authors quoted. Sinai (145) in 1977 suggested that the induction of calcification external to the tooth by calcium hydroxide is basically the same process whether resorptive perforations or open apices are the sites of calcific repair.

Andreasen (46) in 1971 suggested that endodontic therapy may arrest inflammatory resorption in replanted teeth by eliminating toxic degenerating pulpal products. He recommended calcium hydroxide as the root canal filling material in immature teeth. Nine out of ten cases treated with calcium hydroxide showed new periodontal ligament formation and arrest of resorption. In five cases the apical foramen sealed with hard tissue or completed root formation. The same author (49) recommended conventional root canal therapy with gutta-percha and sealer for mature replanted teeth showing inflammatory root resorption. In some cases he thought that it would lead to an arrest of the resorptive process. Cvek (146, 147)
found that treatment of replanted avulsed human incisors showing external root resorption with calcium hydroxide or gutta-percha and sealer showed the same results. 98% of the foramen and 94% of the latter showed arrest of pre-existing external root resorption after treatment. He felt that the high frequency of success in both groups suggested that the removal of necrotic pulp tissue was the decisive factor in the healing of external root resorption of the inflammatory or non-ankylosing type. In 1976 Burke (148) described a clinical case where external inflammatory root resorption was arrested in a replanted tooth after filling of the canal with a calcium hydroxide paste, with the development of a radiographically normal periodontal ligament. A thorough histologic study has not been reported investigating the value of the placement of calcium hydroxide paste into the root canal at the time of replantation, or the subsequent effect on root resorption.

Vital stains or dyes have been used in various studies as markers to study the apposition and resorption of bone, cementum, and dentin. Hoyte (149) described the use of alizarin red for this purpose, and Frost (150) the use of tetracycline, which can be seen under fluorescent microscopic examination. Goland and Grand (151) found that Procion dyes, when given intravenously, permanently
marked incremental lines and zones of growth in teeth and bone. The markers persisted during processing and decalcification of the specimens. Prescott, et al (152) found that Procion Brilliant Red H-8BS was the most efficient marker of the Procion dyes, and could be seen under both ultraviolet light and light microscopy, even in decalcified paraffin sections cut at 7μ. This same dye was used by Sherman (63) in 1968 to study resorption of teeth after replantation, and by Ham, et al (134) in 1972 to study apexification. Binnie and Mitchell (153) used both tetracycline and Procion H-8BS to study induced calcification in rat connective tissues, and Reames, et al (154) used the Procion dye to label new bone overlying submerged retained roots.
CHAPTER III
MATERIALS AND METHODS

Six healthy young adult beagle dogs, of inter­minate age, randomly selected, three male and three female, each weighing between 9.9 and twelve kilograms were used in this study. The animals were individually caged and maintained on a diet of standard laboratory meal and water ad libitum. All were radiographed preoperatively to ensure normal anterior tooth anatomy with complete apical development, and were examined to determine that periodontal disease was either not present or was limited to marginal gingivitis. All radiographs in this study were taken using standard bisecting angle radiographic technique, with a portable hand held x-ray generator giving 60 K.V.P. at 20 mili­amperes at 0.4 second exposure time. Kodak (a) peri­apical ultra-speed film, size two, was held by a spring type mouth-prop, with the x-ray source-to-film distance kept close to twenty centimeters. Radiographs were developed in a portable light-tight developing box in Insta-neg (b) rapid developing solution for twenty seconds,

a Eastman Kodak Company, Rochester, New York.
b Microcopy, Culver City, California.
fixed in Insta-fix (b) concentrated fixer for forty seconds, and washed in running water for five minutes.

Each suitable animal was premedicated with an intramuscular injection of 1 cc. of Innovar (c) per 10 kilograms of body weight, the active ingredients being fentanyl citrate 0.4 mg./ml. and droperidol 20 mg./ml., to sedate the animals and make them more manageable, and also with a subcutaneous injection of 1.5 cc. of Atrosed (d) per 10 kilograms body weight, active ingredient atropine sulfate 0.5 mg./ml., to limit salivary flow. When the desired sedative effect was reached, a foreleg was shaved, swabbed with alcohol, the large anterior vein located, and an intravenous injection of sodium pentabarbital (e) 65 mg./ml., an intermediate acting barbiturate, was given to effect (approximately 4 cc./10kg.,) as determined by the loss of pedal reflex and the passage through the stage of excitement into one of slow, steady resperation. When the proper level of anesthesia was reached, the left and right maxillary second incisors were extracted, using sterile elevators and forceps, as atraumatically and aseptically as possible, and held in sterile, [c] Pitman-Moore, Inc. Washington Crossing, New Jersey. [d] Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California. [e] W.A. Butler Company, Columbus, Ohio.
dry, gauze during endodontic therapy, to allow the periodontal ligament to dry, and serve as a controlled stimulus for root resorption in experimental and control teeth equally. In both left and right incisors lingual access cavities were prepared to the pulp with a sterile bur, and a number fifteen K-type endodontic file was inserted until the tip could be seen through the apex, sometimes easily accomplished, and sometimes requiring some degree of force due to the multiple small foramina common to dog root apices. The length of the files were adjusted, and the canals were filed, using frequent irrigation with 5.25% sodium hypochlorite solution, to approximately a number forty file, one millimeter short of the apex, and subsequently step-flared (1) several sizes larger. Care was taken that the canal irrigant not touch the external root surface. One of the incisors, alternating left and right side on every other dog, was immediately filled with laterally condensed gutta-percha (f) and Procosol (g) non-staining root canal sealer, while the contralateral tooth was filled with calcium hydroxide powder and barium sulfate powder (for radio-opacity) in a proportion of 6:1, mixed to a thick paste with camphorated monoparachlorophenol, and vertically condensed to the apex with hand pluggers. The access

f Premier Dental Products Co., Philadelphia, Pennsylvania
g Proco-sol Chemical Co., Inc., W. Conshohocken, PA
cavities were sealed with amalgam, and each tooth was slowly but firmly replanted into its socket after being held in dry sterile gauze for exactly sixty minutes extraoral time. The periodontal ligament tissues on the root surfaces were disturbed minimally during treatment (other than allowing them to dry out,) and the sockets were not curetted, but were flushed with sterile saline, so as to loosen the blood clot but not to remove periodontal ligament tissue on the socket wall. Then the mandibular second incisors were extracted, treated identically to the maxillary teeth, but filed to one instrument size smaller apically due to the smaller size of the canals, and each tooth was replanted also in sixty minutes after its extraction. The labial enamel of the six incisors in each arch was etched with a phosphoric acid solution for one minute, great care being taken not to let the etching solution touch the gingival tissues, washed with a gauze pad soaked with water, and air dried. Each replanted tooth, and the tooth on either side of it, were notched on the labial surface with a bur, wrapped with orthodontic ligature wire in a tightly twisted figure-eight fashion, and the labial surfaces of the teeth and wire were covered with an orthodontic adhesive resin (h) for stabilization.

h Ortho International Services, Inc., Wilmington, Delaware
Postoperative radiographs were taken, and the animal was placed back in its cage and given its normal diet and water *ad libitum*. Each animal appeared normal and healthy by the first postoperative day.

Thirty days after the replantation procedure each dog again was sedated with the same intramuscular dosage of Innovar, and anesthetized lightly with intravenous sodium pentobarbital. The splints were removed with a scaler if still present, and the teeth were checked for mobility and periodontal condition, specifically pocket depth and gingival inflammation. Radiographs were taken, and each dog received an intraperitoneal injection of the vital dye Procion Red H-8B (i), 200 mg./kg. body weight, in sterile saline to make 10 cc. of solution.

Sixty days postoperative each dog was sedated, anesthetized, radiographed, and the teeth examined for mobility and periodontal condition.

At ninety days postoperative time, each dog was sedated with Innovar, anesthetized with pentobarbital, radiographed, and examined for tooth mobility and periodontal condition. It was then sacrificed by giving an intravenous injection of 10 cc. of Bueuthenasia-D (j),

---

*i* Polysciences, Inc., Warrington, Pennsylvania.

*j* Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc., Oakland, California.
a highly toxic parenteral solution of sodium pentobarbital 195 mg./ml., and phenytoin sodium 25 mg./ml., specifically designed for rapid and painless euthanasia of animals. Cessation of vital signs occurred usually within ten seconds after injection of the drug. The soft tissue surrounding the anterior maxilla and mandible was quickly and carefully dissected free, and the anterior teeth with the surrounding bone was cut free with an electric bone saw, and immediately placed in 10% neutral buffered formalin for fixation. The labial and lingual plates of cortical bone were thinned and opened in areas adjacent to the teeth to be studied to allow for better fixation, and the formalin solutions were changed every 24 hours for the first few days. The specimens remained in the formalin for at least two weeks.

Subsequent to fixation, the jaws were decalcified for four to five weeks in a solution made up of equal parts of solutions of 50% formic acid and 20% sodium citrate, until radiolucent radiographically and of a rubbery consistancy, when they were cut with a razor blade into blocks of bone containing the individual replanted teeth. A tooth which was not replanted was included from each animal as a control. The individual blocks were decalcified for several days to allow better penetration. Each individual tooth-bone block was then cut into three segments, apical, middle, and cervical,
which were dehydrated in increasing concentrations of alcohol and embedded in paraffin. Seven micron sections were cut from the apical end of each segment, seven slides with from ten to sixteen sections per slide from each tooth segment. Of these seven slides, the first, fourth, and seventh slides were stained with hematoxylin and eosin, while the other four slides were left unstained and mounted for examination by fluorescent microscopy or for possible future special staining.

The radiographs of each pair of teeth were mounted in plastic mounts, in the sequence of preoperative, immediate postoperative, thirty day, sixty day, and ninety day, so that the radiographic progression of healing and/or pathosis could easily be determined.
CHAPTER IV

RESULTS

The operative procedures and postoperative course of healing were accomplished quite smoothly. The only problem encountered during the experimental replantation was the difficulty of extraction of the mandibular incisors of the dog. Because of the combination of very resilient periodontal ligaments and thin roots of these teeth, roots were fractured in dogs one and five, in spite of extremely careful extraction technique. This necessitated surgical extraction of the apical segments, and resulted in four less teeth being used in the study than originally planned, giving a total of twenty teeth extracted and successfully replanted. The dogs seemed to suffer no ill effects from the procedure after the first day, and all animals appeared healthy throughout the study, with no serious weight loss seen. The splints were well tolerated and functioned well, most lasting until the 30 day evaluation when they were removed. All replanted teeth remained in the socket throughout the study, with the exception of three teeth in dog number four, which were exfoliated due to severe inflammatory root resorption, two before 30 days and one between 30 and 60 days.

The vital dye which was injected intraperitoneally at 30 days seemed to cause no ill effects on the health of the animals, but one particular side effect was noted.
The sclera of the eye, the gingiva and lips, and the abdominal skin were all stained a bright red color, which persisted throughout the study. Apparently collagen was stained by the dye as well as bone.

After sacrifice and histologic preparation of the specimens, a systematic microscopic examination was made of the histologic sections, which had been assigned a coded numbering system. This allowed evaluation of histologic criteria without knowledge of or bias towards the filling material used. It was difficult to visually differentiate between filling materials microscopically, because much of it was lost during processing and both the calcium hydroxide and gutta-percha appeared as an amorphous, granular mass when present. Thirty microscopic cross-sections were examined from each tooth segment (apical, middle, and cervical thirds.) Each section was examined and graded in each of five categories: normal periodontal ligament, surface resorption with a regenerated periodontal ligament, replacement resorption (ankylosis), slight inflammatory resorption, and severe inflammatory resorption. Each of the 30 sections from each tooth segment was given a numerical score from zero to four in each of the five previously described categories. This score was determined by mentally dividing each section into four quadrants, then noting how many quadrants in which the process
described in each category was seen. Thus for a given segment of a tooth a score of between zero and 120 was given in each histologic category. Twenty teeth studied, divided into three segments each, with five histologic categories, resulted in 300 histologic scores. The results of this examination were charted for each dog in tables one through six. The radiographs were examined without knowledge of the corresponding histologic results, and were also included in these tables, along with the clinical periodontal observations.

The histologic categories were based on Andreasen's (43) three types of external root resorption, with the added classifications of normal (unresorbed) cementum. It was found during examination of the sections that inflammatory resorption was seen in two extremes. One was called slight inflammatory resorption, and showed shallow punched out areas through the cementum, extending into the dentin not more than one quarter of its thickness. The periodontal ligament in these areas showed mild inflammatory cell infiltration, with occasional osteoclasts present along the dentinal walls of the resorbed defects. Repair of resorption by cementum was sometimes seen adjacent to these areas. The second type was called severe inflammatory resorption, and was seen as large areas of granulomatous tissue extending from the periodontal ligament deep into the dentin,
often encompassing one-quarter to one-half of the tooth in
cross section, and extending into the root canal space. At
the interface with the dentin, under high-power examina-
tion, was seen scooped-out areas of resorption, most
filled with a multinucleated osteoclastic cell (figure 12,13.)
The granulomatous tissue was composed of a loose connective
tissue framework with many capillaries, densely infiltrated
with many polymorphonuclear leukocytes and lymphocytes,
with occasional plasma cells, macrophages, and giant cells.
These two types of inflammatory resorption were probably
differing degrees of the same pathologic process, or ar-
rested and active forms. Nevertheless, they appeared in
separate and distinct areas, with little gradation in
between. For the purposes of comparing the two filling
materials, the presence of each type of inflammatory res-
orption was computed separately. Inflammatory resorption
was seen radiographically as radiolucent areas within the
tooth or bone (figure 14, 17.)

The maxillary left incisor of dog number two, filled
with calcium hydroxide, was cut in two by severe inflammatory
resorption, with loss of the cervical two thirds of the
tooth. Histologic sections through the cervical area showed
a massive infiltration of plasma cells, indicating a severe
inflammatory response (figure 18,19) It is possible that this
might suggest the presence of an autoimmune response.
The third histologic category was replacement resorption, or ankylosis. Histologically, this was seen as areas of resorbed dentin fused intimately with alveolar bone (figure 3,8.) This was seen sometimes as isolated trabeculae of bone occasionally in contact with dentin, or as large areas of contact, sometimes involving almost the entire periphery of the root. Osteoblasts were often seen lined up along the periphery of the dentin and bone, indicating active bone formation, even at the 90 day sacrifice time. Radiographic findings in ankylosis included disappearance of the normal periodontal ligament space, and continuous replacement of resorbed root surface with bone (figure 9.)

The most prevalent histologic category seen in this study was surface resorption repaired by cementum. Small resorptive lacunae were seen along the dentin and cementum surface, repaired by cellular secondary cementum (figure 5.) A normal periodontal ligament was present. This type of resorption was also seen in areas of the roots of unoperated control teeth, and is evidently an area of spontaneous repair of small areas of damaged cementum.

The fifth histologic category was the presence of normal periodontal ligament and unresorbed cementum (figure 3.) There were relatively few areas showing this lack of root resorption, although it was more prevalent in cervical
areas than in apical or middle. Both repaired surface resorption and normal ligament and cementum were seen radiographically as having a normal periodontal space around the replanted tooth.

It must be stressed that never was an entire root or root-third seen histologically as fitting into one category of healing or resorption. Usually even one histologic cross-section showed two or three categories (figure 3.) For example, repaired surface resorption was often interspersed with areas of ankylosis, and even severe inflammatory resorption was seen in the same cross-section with normal cementum and periodontal ligament. From this it is obvious that accurate radiographic interpretation of the root resorptive processes is impossible, unless a large area is grossly affected.

The three teeth in dog number four, which were exfoliated because of extensive inflammatory root resorption, were histologically scored as showing total inflammatory root resorption, given 120 points at each level, even through by the 90 day sacrifice time the sockets were in an advanced state of healing. The inflammatory resorption was clearly evident in the 30 day radiographs, with large radiolucent areas surrounding the roots, leading to exfoliation. By 90 days radiographic evidence of bony healing of the sockets could be seen.
The Procion vital dye was not seen to fluoresce as was expected when unstained sections, or those stained with hematoxylin and eosin, were examined with the fluorescent microscope. Unresorbed cementum on unreplanted control teeth and on a very few sections of replanted teeth demonstrated a darker-staining red band in the hematoxylin and eosin stained sections. Without the confirmation of the fluorescence, it was not determined whether this was a layer of secondary cementum laid down and stained by the vital dye, or merely a zone of darker staining by the hematoxylin and eosin.

Table 7 shows a summary of the histologic scores for all twenty teeth, grouped according to the five histologic criteria studied, the three segments of the teeth studied, and the two different canal filling materials used. Since the purpose of this study was to determine whether a difference in root resorption would be seen between teeth filled with gutta-percha and sealer or calcium hydroxide, the histologic results were subjected to statistical analysis. Within each of the five resorption categories studied, and for each third of the root surface studied, a two-sample t-test was applied, comparing the scores of the ten teeth filled with each material. The formulas used, the limits at 5% and 1% probability, and the t-test results
can be seen in Table 8. The results showed no statistically significant difference in histologic scores between the two filling materials, at either the 5% or 1% level of probability, at any level of the root examined. Since no differences were seen within any of the groups as divided according to root third, statistical analysis of the total-tooth scores was thought to be unnecessary and was not done.

In Table 9, the teeth were grouped in the same categories, but the histologic results were evaluated to specifically compare the paired contralateral teeth, one filled with each material. This may also be a valid analysis, because variations in healing and tissue response between animals are eliminated. For each category including ten pairs of teeth, the number of teeth filled with each filling material showing the higher score of the pair in each category of evaluation is listed. The number of times the two teeth showed equal scores in a given category is also shown. Careful examination of this table may give additional information beyond the point totals and statistical analysis in tables seven and eight.
<table>
<thead>
<tr>
<th>Dog #1</th>
<th>Normal Periodontal Ligament &amp; Cementum</th>
<th>Surface Resorption, Regenerated P. D. L.</th>
<th>Replacement Resorption (Ankylosis)</th>
<th>Slight Inflammation, Resorption</th>
<th>Severe Inflammation, Resorption</th>
<th>Radiograph, Clinical Periodontal Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>8</td>
<td>84</td>
<td>82</td>
<td>5</td>
<td>0</td>
<td>Good, mild Ankylosis, Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Middle</td>
<td>4</td>
<td>56</td>
<td>117</td>
<td>7</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cervical</td>
<td>43</td>
<td>49</td>
<td>55</td>
<td>34</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap.</td>
<td>0</td>
<td>53</td>
<td>76</td>
<td>17</td>
<td>11</td>
<td>Good, mild Ankylosis, Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Mid.</td>
<td>12</td>
<td>106</td>
<td>81</td>
<td>20</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cerv.</td>
<td>34</td>
<td>63</td>
<td>14</td>
<td>38</td>
<td>30</td>
<td>&quot;</td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td></td>
<td>Tooth fractured during extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)₂</td>
<td></td>
<td>Tooth not extracted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog #2</td>
<td>Normal Periodontal Ligament &amp; Cementum</td>
<td>Surface Resorption Regenerated P. D. L.</td>
<td>Replacement Resorption (Ankylosis)</td>
<td>Slight Inflammation Resorption</td>
<td>Severe Inflammation Resorption</td>
<td>Radiograph Appearance</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td>Histologic Scores, 30 sections each 1/3, scored 0-120</td>
<td>Apical 0</td>
<td>68</td>
<td>75</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle 6</td>
<td>49</td>
<td>113</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cervical 2</td>
<td>44</td>
<td>29</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)_2</td>
<td></td>
<td>Ap. 0</td>
<td>49</td>
<td>68</td>
<td>32</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid. 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerv. 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td></td>
<td>Ap. 0</td>
<td>96</td>
<td>0</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid. 15</td>
<td>105</td>
<td>0</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerv. 0</td>
<td>66</td>
<td>0</td>
<td>37</td>
<td>86</td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)_2</td>
<td></td>
<td>Ap. 0</td>
<td>65</td>
<td>10</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid. 27</td>
<td>82</td>
<td>30</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cerv. 0</td>
<td>120</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 3

<table>
<thead>
<tr>
<th>Dog #3</th>
<th>Normal Periodontal Ligament &amp; Cementum</th>
<th>Surface Resorption, Regenerated P. D. L.</th>
<th>Replacement Resorption (Ankylosis)</th>
<th>Slight Inflammation Resorption</th>
<th>Severe Inflammation Resorption</th>
<th>Radiograph Appearance</th>
<th>Clinical Periodontal Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td>Histologic Scores, 30 sections each 1/3, scored 0-120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>38</td>
<td>31</td>
<td>45</td>
<td>25</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)₂</td>
<td>Ap.</td>
<td>0</td>
<td>26</td>
<td>99</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mid.</td>
<td>24</td>
<td>35</td>
<td>57</td>
<td>52</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerv.</td>
<td>30</td>
<td>20</td>
<td>38</td>
<td>21</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td>Ap.</td>
<td>0</td>
<td>57</td>
<td>0</td>
<td>45</td>
<td>17</td>
<td>Severe inflammation, resorption</td>
</tr>
<tr>
<td>Mid.</td>
<td>23</td>
<td>53</td>
<td>49</td>
<td>38</td>
<td>0</td>
<td>Good</td>
<td>Tooth slightly mobile, gingiva inflamed</td>
</tr>
<tr>
<td>Cerv.</td>
<td>45</td>
<td>90</td>
<td>0</td>
<td>63</td>
<td>0</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)₂</td>
<td>Ap.</td>
<td>0</td>
<td>42</td>
<td>58</td>
<td>44</td>
<td>0</td>
<td>Mild inflammation, resorption</td>
</tr>
<tr>
<td>Mid.</td>
<td>30</td>
<td>49</td>
<td>88</td>
<td>37</td>
<td>0</td>
<td>Good</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Cerv.</td>
<td>64</td>
<td>52</td>
<td>10</td>
<td>50</td>
<td>0</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Dog #4</td>
<td>Normal Periodontal Ligament &amp; Cementum</td>
<td>Surface Resorption, Regenerated P. D. L.</td>
<td>Replacement Resorption (Ankylosis)</td>
<td>Slight Inflammation Resorption</td>
<td>Severe Inflammation Resorption</td>
<td>Radiograph Appearance</td>
<td>Clinical Periodontal Appearance</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------------</td>
<td>-----------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td>Apical</td>
<td>Histologic Scores, 30 sections each 1/3, scored 0-120</td>
<td>Severe inflammatory resorption, tooth exfoliated by 30 days.</td>
<td>120</td>
<td>Healing socket by 90 days</td>
<td>Gingiva healed by 90 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cervical</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)₂</td>
<td>Ap.</td>
<td>Severe inflammatory resorption, tooth exfoliated between 30 and 60 days.</td>
<td>120</td>
<td>Healing socket by 90 days</td>
<td>Gingiva healed by 90 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid.</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerv.</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td>Ap.</td>
<td>Severe inflammatory resorption, tooth exfoliated before 30 days.</td>
<td>120</td>
<td>Healing socket by 90 days</td>
<td>Gingiva healed by 90 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid.</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerv.</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)₂</td>
<td>Ap.</td>
<td>0</td>
<td>55</td>
<td>70</td>
<td>26</td>
<td>0</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Mid.</td>
<td>10</td>
<td>63</td>
<td>73</td>
<td>21</td>
<td>0</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Cerv.</td>
<td>38</td>
<td>71</td>
<td>33</td>
<td>30</td>
<td>0</td>
<td>Good</td>
</tr>
<tr>
<td>Dog #5</td>
<td>Normal Periodontal Ligament &amp; Cementum</td>
<td>Surface Resorption, Regenerated P. D. L.</td>
<td>Replacement Resorption (Ankylosis)</td>
<td>Slight Inflammation Resorption</td>
<td>Severe Inflammation Resorption</td>
<td>Radiograph Appearance</td>
<td>Clinical Periodontal Appearance</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
<td>------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild ankylosis</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Apical 21</td>
<td>48</td>
<td>53</td>
<td>19</td>
<td>0</td>
<td></td>
<td>Mild ankylosis</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Middle 25</td>
<td>83</td>
<td>88</td>
<td>20</td>
<td>0</td>
<td></td>
<td>Severe Inflammation Resorption</td>
<td></td>
</tr>
<tr>
<td>Cervical 0</td>
<td>15</td>
<td>61</td>
<td>33</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate infl. or replac. res.</td>
<td></td>
</tr>
<tr>
<td>Ap. 1</td>
<td>45</td>
<td>80</td>
<td>40</td>
<td>0</td>
<td></td>
<td>Severe Inflammation Resorption</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Mid. 1</td>
<td>71</td>
<td>84</td>
<td>41</td>
<td>0</td>
<td></td>
<td>Severe Inflammation Resorption</td>
<td></td>
</tr>
<tr>
<td>Cerv. 29</td>
<td>50</td>
<td>46</td>
<td>33</td>
<td>27</td>
<td></td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td>Tooth fractured during extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)₂</td>
<td>Tooth not extracted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog #6</td>
<td>Normal Periodontal Ligament &amp; Cementum</td>
<td>Surface Resorption, Regenerated P. D. L.</td>
<td>Replacement Resorption (Ankylosis)</td>
<td>Slight Inflammation Resorption</td>
<td>Severe Inflammation Resorption</td>
<td>Radiograph. Appearance</td>
<td>Clinical Periodontal Appearance</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Maxillary Incisor Gutta-Percha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>2</td>
<td>57</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>Mild ankylosis</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
<td>45</td>
<td>120</td>
<td>10</td>
<td>0</td>
<td>Severe ankylosis</td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>7</td>
<td>33</td>
<td>66</td>
<td>35</td>
<td>0</td>
<td>Severe ankylosis</td>
<td>Tooth mobile, gingiva uninflamed</td>
</tr>
<tr>
<td>Maxillary Incisor Ca(OH)_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap.</td>
<td>0</td>
<td>43</td>
<td>120</td>
<td>58</td>
<td>0</td>
<td>Severe ankylosis</td>
<td></td>
</tr>
<tr>
<td>Mid.</td>
<td>12</td>
<td>70</td>
<td>120</td>
<td>63</td>
<td>0</td>
<td>Very severe inflammat. resorption</td>
<td></td>
</tr>
<tr>
<td>Cerv.</td>
<td>24</td>
<td>32</td>
<td>0</td>
<td>34</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Gutta-Percha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap.</td>
<td>0</td>
<td>68</td>
<td>120</td>
<td>73</td>
<td>0</td>
<td>Mild infl. resorption</td>
<td>Crown lost, gingiva inflamed</td>
</tr>
<tr>
<td>Mid.</td>
<td>0</td>
<td>65</td>
<td>101</td>
<td>80</td>
<td>16</td>
<td>Severe infl. resorption</td>
<td></td>
</tr>
<tr>
<td>Cerv.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td>Crown lost</td>
<td></td>
</tr>
<tr>
<td>Mandibular Incisor Ca(OH)_2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ap.</td>
<td>0</td>
<td>82</td>
<td>88</td>
<td>42</td>
<td>0</td>
<td>Good</td>
<td>Tooth firm, gingiva uninflamed</td>
</tr>
<tr>
<td>Mid.</td>
<td>37</td>
<td>81</td>
<td>88</td>
<td>38</td>
<td>0</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Cerv.</td>
<td>33</td>
<td>91</td>
<td>0</td>
<td>68</td>
<td>0</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal Periodontal Ligament &amp; Cementum</td>
<td>Surface Resorption, Regenerated P. D. L.</td>
<td>Replacement Resorption (Ankylosis)</td>
<td>Slight Inflammatory Resorption</td>
<td>Severe Inflammatory Resorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gutta-percha</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical 1/3</td>
<td>25</td>
<td>478</td>
<td>450</td>
<td>227</td>
<td>377</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>1</td>
<td>460</td>
<td>669</td>
<td>324</td>
<td>220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle 1/3</td>
<td>73</td>
<td>456</td>
<td>708</td>
<td>234</td>
<td>356</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>153</td>
<td>557</td>
<td>621</td>
<td>305</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical 1/3</td>
<td>135</td>
<td>328</td>
<td>256</td>
<td>274</td>
<td>541</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>252</td>
<td>499</td>
<td>141</td>
<td>294</td>
<td>351</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>233</td>
<td>1262</td>
<td>1414</td>
<td>735</td>
<td>1274</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>406</td>
<td>1516</td>
<td>1431</td>
<td>923</td>
<td>811</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 8
Statistical Analysis

Two-sample t-test applied to histologic results of Table 7, comparing the two filling materials tested.

\[
t = \frac{(\bar{x}_1 - \bar{x}_2) - \varnothing}{\sqrt{s^2_p \left( \frac{1}{N_1} + \frac{1}{N_2} \right)}}
\]

where

\[
s^2_p = \frac{(x_1 - \bar{x}_1)^2 + (x_2 - \bar{x}_2)^2}{(N_1 - 1) + (N_2 - 1)}
\]

Both \(N_1\) and \(N_2\) are constant at 10, therefore degrees of freedom is 18.

At \(\alpha = .05\), limits are \(\pm 2.10\)

At \(\alpha = .01\), limits are \(\pm 2.88\)

<table>
<thead>
<tr>
<th></th>
<th>Normal Surface Replacement</th>
<th>Periodontal Resorption, Regenerated P. D. L.</th>
<th>Replacement Resorption (Ankylosis)</th>
<th>Slight Inflammatory Resorption</th>
<th>Severe Inflammatory Resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical</td>
<td>&quot;t&quot; value comparing gutta-percha vs. Ca(OH)(_2)</td>
<td>1.16</td>
<td>0.135</td>
<td>-1.09</td>
<td>-0.976</td>
</tr>
<tr>
<td>Middle</td>
<td>0.151</td>
<td>-0.634</td>
<td>0.413</td>
<td>-0.657</td>
<td>0.480</td>
</tr>
<tr>
<td>Cervical</td>
<td>-1.30</td>
<td>-1.10</td>
<td>1.07</td>
<td>-0.212</td>
<td>0.815</td>
</tr>
</tbody>
</table>
### TABLE 9

Comparison of Contralateral Gutta-Percha and Ca(OH)$_2$ filled Teeth

<table>
<thead>
<tr>
<th>Normal Periodontal Ligament &amp; Cementum</th>
<th>Surface Resorption &amp; Regenerated P. D. L.</th>
<th>Replacement Resorption (Ankylosis)</th>
<th>Slight Inflammatory Resorption</th>
<th>Severe Inflammatory Resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NUMBER OF TEETH SHOWING THE HIGHER HISTOLOGIC SCORE IN EACH CATAGORY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>APICAL 1/3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P Higher 3</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CH Higher 0</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Equal 7</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>MIDDLE 1/3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P Higher 2</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>CH Higher 7</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Equal 1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>CERVICAL 1/3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P Higher 3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>CH Higher 5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Equal 2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-P Higher 8</td>
<td>G-P 14</td>
<td>G-P 13</td>
<td>G-P 12</td>
<td>G-P 9</td>
</tr>
<tr>
<td>CH Higher 12</td>
<td>CH 13</td>
<td>CH 10</td>
<td>CH 14</td>
<td>CH 9</td>
</tr>
<tr>
<td>Equal 10</td>
<td>Equal 3</td>
<td>Equal 7</td>
<td>Equal 4</td>
<td>Equal 12</td>
</tr>
</tbody>
</table>

*Note: The table shows the number of teeth showing the higher histologic score in each category for apical, middle, and cervical thirds, as well as the total.*
CHAPTER V
DISCUSSION

The results of this study indicate that it was impossible to demonstrate a difference in any type of root resorption at any level of the root when calcium hydroxide was used as an immediate root canal filling material, when compared with gutta-percha and sealer. No extensive conclusions, however, should be made from the data presented here. Due to the low sample size (twenty teeth) and because only one set of experimental replantation conditions were used, further work in this area would seem to be warranted. The effects of a different extraoral time and environment are unknown as to their interaction with the role of the canal filling material. The time of placement of the calcium hydroxide canal dressing also may be very important. The studies that showed promising results with calcium hydroxide in the arrest of root resorption all had the paste being placed into the canals at sometime after replantation, after resorption had begun, and after some periodontal ligament healing had taken place. Andreasen (81) has suggested that calcium hydroxide placed into the root canal at the time of replantation may act as a necrotizing agent to an unregenerated periodontal ligament, preventing initial healing. Therefore, he suggested canal debridment one week after replantation, with delay of calcium
hydroxide placement for two more weeks after that, to allow for primary healing of the periodontal ligament. This may act as a natural barrier to prevent a gross flooding of the socket with the caustic, highly alkaline calcium hydroxide. Nevertheless, in this study the calcium hydroxide treated teeth showed no worse resorption than the gutta-percha and sealer treated teeth. Gutta-percha has been shown by many studies, including Feldman and Nyberg (155), to be very well tolerated by the tissues, while Procosol sealer has been shown by Kapsimalis and Evans (156) to have excellent sealing ability, and by Rappaport (157) to evoke a very mild inflammatory response in rat connective tissue. Therefore, this combination should serve as a good control with which to compare the effects of the calcium hydroxide. Further study into the precise effect of calcium hydroxide, in the root canal, on the periodontal tissues after replantation is warranted. The material has such proven effects on pulpal and periapical tissues when used as a capping or apexification agent that it would be surprising if it had no effect when used in replanted teeth. Other experiment conditions other than those in the present study should be evaluated.

Calcium hydroxide has been shown to definitely alter dentin, which may transmit changes through the tubules to the cementum surface and periodontal ligament. Linden (158)
demonstrated, with an in vitro study, the presence of molecular level pathways of communication between the pulp chamber and the root surface. Calcium hydroxide is a small molecule, so it may be able to penetrate these tubules.

It is not clear which part of the calcium hydroxide molecule exerts the active effect on pulp, dentin, and periapical tissues. Sciaki and Pisanti (159) and Pisanti and Siaki (160) demonstrated, by the use of labeled calcium ions, that the calcium used in the formation of the dentin bridge over a healing pulp cap is not contributed by the calcium hydroxide, but comes from the circulating serum calcium. Raisz (161), studying the osteoclastic resorption of fetal rat bone in tissue culture, stimulated by parathormone, found that raising the calcium ion concentration from five to twelve mg./100ml. had no effect on the rate of active bone resorption. An increased calcium ion content, however, did cause a decrease in mineral loss, through a decrease in dissolution of bone mineral. In a similar study, Raisz and Niemann (162) found that while a calcium ion increase did not affect active bone resorption, an increased phosphate concentration significantly decreased parathormone-induced osteoclastic resorption of bone in tissue culture. Possibly a high-phosphate root canal filling should be studied as a root canal filling material for treatment of root resorption after replantation. Whether
the fact that changes in ion concentration have been found to alter bone resorption, may be extended to include external root resorption is unknown. Orban (163) stated, however, that osteoclasts are the active cells in both bone resorption and resorption of the roots of teeth, so research findings on bone resorption, although a separate process, may be at least partially applicable to root resorption.

The extreme alkalinity of calcium hydroxide, usually reported to be around pH 12, has been suggested as being responsible for its actions on tissue. Laws (164) found that the pH of calcium hydroxide from a treated pulpotomy was 7.4, the decrease attributed to dilution by the surrounding tissue fluids, whose pH may have been at the same time raised somewhat. This pH effect has been suggested by Schröder and Granath (120) as being the cause of the dentin bridging under a pulp cap by irritation of remaining pulp tissue. Raisz (161) found in his tissue culture study that parathormone could stimulate osteoclastic bone resorption from pH 6.78 to 7.45. Raising the pH to 7.6 with bicarbonate inhibited the bone resorption significantly. This is near the pH of the spent calcium hydroxide after equilibration with tissue fluid reported by Laws. Although it has not been proven directly, it would seem to follow that perhaps calcium hydroxide, when placed in the root
canal, may, by penetration through the dentinal tubules or apical foramena, have a direct effect on osteoclastic resorption after replantation.

Menkin (165) created a severe inflammatory response in the pleural cavity of dogs by injecting irritants. He discovered that as the inflammation progressed, the exudate became more acidic. When the pH increased, however, the outlook was more favorable and the animal tended to improve. It is possible that calcium hydroxide may change the environment of the periodontal ligament from one of inflammation and resorption to a more alkaline pH which is more conducive to healing and calcification of resorbed areas. Even though calcium hydroxide has been shown by Spangberg (166, 167) to be highly toxic to HeLa cells and human skin fibroblasts in tissue culture, it has also been demonstrated that it can induce calcification in connective tissue of rats by both Mitchell and Shankwalker (168) and Binnie and Mitchell (153). This calcification was described as being an "osteoid" material, which is morphologically very similar to secondary cementum, the method by which root resorption is healed.

Binnie and Rowe (169) found that calcium hydroxide materials seemed to be particularly useful in an infected environment, when used as a root canal filling material. Cvek (170), in studying apexification of teeth with
pulp necrosis and periapical pathosis, "assumed that calcium hydroxide per se may act as a long-acting antibacterial agent." Fisher (171) found that viable microorganisms were not recoverable from previously infected carious dentine in vital permanent teeth exposed to a lining paste of pure calcium hydroxide and water, while simple isolation did not kill the organisms. Matsumiya and Kitamura (137) filled experimentally infected root canals in dogs with a calcium hydroxide paste. They found that bacteria living in the periapical tissues were clearly observed to diminish and disappear as healing progressed. They concluded that "calcium hydroxide has an antibacterial effect in the dental tissue." Since Andreasen (46) attributes inflammatory root resorption after replantation largely to infected necrotic tissue within the root canal escaping through dentinal tubules, especially in young teeth, perhaps the antibacterial effects of calcium hydroxide would be beneficial to the periodontal healing of this type of resorption. To what extent this is true has not yet been proven.

All of these theories on the possible modes of action of calcium hydroxide on replanted teeth must remain theories until proven. They are not consistent with the findings of the present study. Within its very limited confines, their results showed no discernable differences in several types of root resorption between calcium hydroxide-filled
teeth, and teeth filled with gutta-percha and sealer, which are control materials well accepted as being relatively innocuous in their effect on tissue.

Splinting for 30 days may have had an effect on the results of this study, possibly causing higher incidence of ankylosis than if they had been removed at one week as recommended by Andreasen (44). Whatever effect that this had, however, would have been shared equally by teeth filled by both methods. It was effective in preventing exfoliation of teeth through eating and chewing, before the regenerating periodontal ligament had enough strength of its own.

As in any animal study, great care must be taken when extrapolating results to humans. Although Nygaard-Ostby (121) and Matsumiya and Kitamura (137) found similar healing in humans and dogs, Gad (172) found that periodontal disease progressed five times as fast in dogs than in humans. Whether or not there is a similar difference in healing after replantation is unknown, as no controlled studies have been done in this area.

Systemic conditions and individual healing variations may have great effect on the healing and subsequent resorption of replanted teeth. All replanted teeth in five dogs in this study remained in the socket, with varying degrees of resorption, by the termination of the study. Dog number four, however, had three teeth exfoliate
because of gross inflammatory resorption, even though all six animals were treated identically throughout the study. Why this dog reacted so differently to the treatment is unknown, but the fact that there was such a variation between animals leads to the conclusion that systemic healing differences may account for some of the clinical unpredictability of replantation.

Most clinical replantation studies are largely evaluated radiographically, unless the teeth are extracted because of failure of treatment. In general, in this study, the correlation between radiographic and histologic results was good only when an advanced state of pathosis was present, such as severe inflammatory root resorption. More moderate resorption was often difficult to diagnose properly by the use of the radiograph. Often the loss of the integrity of the lamina dura and lack of sharpness of detail of the root surface was evident radiographically. Microscopic examination, however, revealed anything from repaired surface resorption to moderate ankylosis or mild inflammatory resorption, or a combination of histologic entities. Care should be taken in evaluating replanted teeth radiographically, especially when testing various methods of treatment.

It is not known why the Procion vital dye was not seen in the histologic sections. Procion red H-8BS has
been reported in the literature to stain bone and cementum that was being laid down while the dye was present in the circulation. When an attempt was made to purchase this dye from the manufacturer, their representative stated that Procion H-8B reactive red 31 had replaced, and was identical to, Procion red H-8BS, so the former is what was used in this study. They may have been in error, however, because the dye was not seen in unstained sections, and no fluorescence was seen. The dye may have stained collagen but not calcified tissues, it may have been removed from bone and cementum during fixation and decalcification, or it may show no fluorescence even if present. Its importance to the study was not critical, however, and its use was intended merely as a tool to see whether resorption occurred before or after the 30 day time that it was injected, by the presence or absence of a fluorescent ring of cementum.
SUMMARY AND CONCLUSIONS

Twenty maxillary and mandibular incisors in six beagle dogs were extracted and replanted after drying in sterile gauze for one hour. During that hour, the root canals were debrided, prepared, and filled, half with gutta-percha and sealer, and half with a paste made of calcium hydroxide and camphorated parachlorophenol. The animals were radiographed and observed for ninety days, at which time they were sacrificed. Histologic sections were examined and graded in five categories ranging from normal cementum to severe inflammatory resorption. The histologic results were statistically evaluated to determine whether or not there was a difference in root resorption when the teeth were filled with calcium hydroxide paste or gutta-percha and sealer. The experiment showed the following:

a) All the replanted teeth showed extensive resorption when examined histologically, ranging from surface resorption repaired by cementum deposition to severe anklosis or inflammatory resorption.

b) The reaction at the surface of an individual tooth was not uniform. Usually one type of resorption was seen in close relationship to another, in almost any combination. This was
true within the same histologic cross-section, and also between different levels of the root from apex to cervix.

c) This study was unable to demonstrate, at any level of the root, any statistically significant differences in any type of resorption between teeth filled with calcium hydroxide or gutta-percha and sealer.

d) Radiographic examination proved inadequate in critically evaluating the results of replantation. Only large areas of resorption were correctly interpreted radiographically, when compared with histologic sections.

e) No broad conclusions as to the value of calcium hydroxide in treating or preventing root resorption in replanted teeth should be made from these results alone. Due to the limited number of teeth and the single set of replantation conditions, this study should be regarded as a pilot study. Further work is necessary using varied extraoral times and environments, as well as with delayed placement of the calcium hydroxide paste for several weeks after replantation.
REFERENCES


FIGURES
Figure 1: Dog #4, maxillary pre-operative radiograph.

Figure 2: Dog #4, maxillary immediate post-operative radiograph. Tooth on left of photograph filled with Ca(OH)₂, contralateral tooth filled with gutta-percha and sealer.
Figure 3: Dog #3, mandibular incisor, Ca(OH)\textsubscript{2} filling. Unreplanted control tooth (CT), replanted tooth showing normal cementum (NC), severe ankylosis at right (A). (Hematoxylin and eosin stain, original magnification X40).

Figure 4: Surface resorption, repaired with cementum. Schematic drawing from Andreasen (49).
Figure 5: Dog #2, mandibular incisor, Ca(OH)$_2$ filling. Normal periodontal ligament and repairing surface resorption. (Hematoxylin and eosin stain, original magnification X100).

Figure 6: Dog #2, mandibular 90 day radiograph. Tooth at left, filled with Ca(OH)$_2$, showing surface resorption, repaired by cementum. Tooth at right, filled with gutta-percha and sealer, showing cervical severe inflammatory resorption.
Figure 7: Progressive replacement resorption (ankylosis). Schematic drawing from Andreasen (49).

Figure 8: Dog #3, maxillary incisor, gutta-percha and sealer filling. Ankylosis. Dentin(D), bone (B), osteoblast (OB), osteoclast (OC). (Hemat­oxylin and eosin stain, original magnification X100).
Figure 9: Dog #3, maxillary 60 day radiograph. Tooth at left, filled with gutta-percha and sealer, showing ankylosis with spotty inflammatory resorption. Tooth at right, filled with Ca(OH)$_2$, showing severe ankylosis.

Figure 10: Severe progressive inflammatory resorption. Schematic drawing from Andreasen (49).
Figure 11: Dog #4, mandibular incisor, Ca(OH)$_2$ filling. Slight inflammatory resorption (SIR) next to normal cementum (NC). (Hematoxylin and eosin stain, original magnification X40).

Figure 12: Dog #1, maxillary incisor, Ca(OH)$_2$ filling. Cervical severe inflammatory resorption. Granulomatous tissue (GT), active osteoclastic resorption of dentin (OR). (Hematoxylin and eosin stain, original magnification X40).
Figure 13: Same histologic section as Figure 12. Higher power view of osteoclasts (OC), or dentinoclasts, in resorptive lacunae in dentin. (Hematoxylin and eosin stain, original magnification X100).

Figure 14: Dog #4, maxillary 30 day radiograph. Tooth at left, filled with Ca(OH)$_2$, showing extreme inflammatory root and bone resorption. Tooth at right already lost, residual socket showing diffuse radiolucency with loss of lamina dura.
Figure 15: Dog #4, maxillary incisor, Ca(OH)$_2$ filling. Tooth exfoliated because of inflammatory resorption. Osteoblasts forming bone around residual filling material. (Hematoxylin and eosin stain, original magnification X40).

Figure 16: Same histologic section as Figure 15. Higher power view of osteoblasts (OB) forming bone around mass of connective tissue containing islands of calcified tissue (CT) and granular Ca(OH)$_2$ particles (CH). (Hematoxylin and eosin stain, original magnification X100).
Figure 17: Dog #2, maxillary 60 day radiograph. Tooth at left, filled with Ca(OH)$_2$, showing severe cervical inflammatory root resorption. Tooth at right, filled with gutta-percha and sealer, showing mild ankylosis.

Figure 18: Dog #2, maxillary incisor, Ca(OH)$_2$ filling. Crown lost because of cervical inflammatory resorption. Epithelium (E) with dense infiltration of inflammatory cells (IC) in connective tissue. (Hematoxylin and eosin stain, original magnification X40).
Figure 19: Same histologic section as Figure 18. Higher power view of inflammatory cell infiltration, showing predominance of plasma cells (arrows). (Hematoxylin and eosin stain, original magnification X400).
APPROVAL SHEET

The thesis submitted by Dale M. Anderson, B.S., D.D.S., has been read and approved by the following committee:

Dr. John V. Madonia, Director
Associate Dean,
Professor of Microbiology,
Loyola University School of Dentistry

Dr. Marshall H. Smulson
Professor and Chairman,
Department of Endodontics,
Loyola University School of Dentistry

Dr. Norman K. Wood
Professor and Chairman,
Department of Oral Diagnosis,
Loyola University School of Dentistry

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

[Signature]
April 18, 1978
Director's Signature