

THE EFFECTS OF APICAL MODIFICATION ON THE VITALITY OF
REPLANTED PERMANENT MONKEY TEETH

by

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INTRODUCTION

Replantation has been a part of dental therapy for centuries, with its popularity varying from one historical period to another. Research has generally focused on the role of the periodontal membrane in reattachment and prevention of the resorption which usually occurs. The reparative ability of the pulp after replantation has received little attention. Consequently, when advocated, vital replantation is primarily for immature permanent teeth which have been recently avulsed.

The purpose of this study was to determine the effects of increasing the surface area of the pulp in the apical region before replantation of mature teeth. It was thought that the results of this study might provide an alternative to present forms of treatment for the mature avulsed tooth.

The hypotheses for the study are as follows:

- 1) Mature permanent monkey teeth can be extracted, receive an enlargement of the apical pulpal tissue, be replanted, stabilized and sustain a healthy periodontium clinically for a period of ninety days.
- 2) A significant amount of apical revascularisation and nerve regeneration will occur as compared to control teeth which have not received any apical modification prior to immediate replantation.

REVIEW OF LITERATURE

Tooth replantation has been defined as the reinsertion of a tooth into the alveolar socket from which it has been displaced.¹ One of the first persons known to attempt replantation of teeth was Albucasis de Condue,² an Arabian surgeon, who in the eleventh century replanted teeth using fine gold wires as lifetime splints.

Ambroise Pare,³ a French barber who later became a surgeon, was said to have practiced replantation successfully in the sixteenth century. He recommended immediate replantation of the tooth followed by binding to its neighbors. His criterion for success was the ability of the patient to masticate properly.

In the seventeenth century, Christopher Schelhammer,⁴ a German otologist and anatomist, recommended extraction of carious teeth, removal of decay, restoration and subsequent replantation. He thought the tooth would take root again and not be the source of pain.

Pierre Fauchard,⁵ the founder of modern scientific dentistry, was also a firm advocate and practitioner of replantation in the eighteenth century. He gave as an example a case report of a young girl from whom he extracted a first molar. Since he achieved this without any gingival laceration or bone destruction, he proceeded to replant the tooth immediately. Thread was then used to anchor it to the adjacent teeth and postoperatively a filling was placed. The tooth decreased in mobility and caused pain for only two days. He also recommended immediate replantation in instances when the wrong tooth was extracted. Fauchard later found that notching the tooth in

three or four places produced greater stabilization. Thirty days after this treatment the mobility decreased due to formation of alveolar bone in the notches.

In the eighteenth century, Hunter⁶ was a strenuous partisan of replantation. In one of his animal experiments, he transplanted teeth into roosters' combs and observed the condition of the periodontal membrane after varying periods. He concluded that the vitality of the dental periosteum was essential for reattachment.

Bourdet⁷ in 1775 proposed that grossly carious teeth should be treated by extraction, removal of both decay and pulp, root filling, and finally replantation.

Heinreich Callisen,⁸ in a treatise on surgery published in 1788, declared himself in favor of replantation. He maintained that after the tooth had been replanted and stabilized there was no possibility of further pain since the nerve had been severed.

The value of transplantation was also recognized by Hirsch⁹ in the nineteenth century. From a child who had died a violent death he transplanted teeth into the mouth of an adult. In 1866 Younger¹⁰ reported the successful transplantation of teeth into artificially created alveoli in humans.

In 1914 Morrison¹¹ extracted a lower second molar to facilitate removal of an impacted third molar. He then sealed the foraminae of the second molar with alloy and replanted it. Immobilisation was achieved with a silver splint which was removed after seven weeks. He reported that 14 weeks after the operation the replanted tooth was quite firm in its socket.

In 1917 Wilkinson¹² stated that the amount of periodontal membrane remaining on the tooth had no effect on the prognosis of a replanted tooth. In an effort to prove this, he stripped the periodontal membrane from the root of one extracted monkey incisor. He extracted the adjacent incisor for use as a control. Both teeth were kept in a test tube for five days before replantation. The roots of both teeth were seen to be resorbing two months after replantation but more resorption was taking place on the incisor from which the periodontal ligament had not been removed.

Again in 1926, Wilkinson¹³ stated that the presence or absence of a periodontal membrane had no effect on the mode of reattachment. Sections of teeth replanted with or without periodontal membrane showed similar amounts of resorption.

Kells¹⁴ in 1918 contrasted the techniques of replantation and apicoectomy. He recognized that ankylosis occurred due to the destruction of the periodontal membrane. He also foresaw a time when this would be eliminated. He recommended the use of proper forceps, wrapping the tooth in gauze during the extraoral period, and possibly reducing the root prior to replantation.

In 1928 Zemsky¹⁵ stated that single-rooted teeth that had been avulsed or extracted in error could be replanted. However, even with his recommended technique of sterilization, root filling, and curettage of the socket, the procedure was not always a success. As soon as resorption became apparent, he advised extraction of the offending tooth.

Three years later Payne¹⁶ described how he had replanted two teeth in a young woman in 1912. The teeth had been extracted due to the presence of a fibrous epulis. The root canals were filled with a material of creamy consistency and the teeth were splinted in place for four weeks. The teeth were asymptomatic until nine years later when deep periodontal pockets were noted. Radiographs revealed root rarefaction. Extraction was advised but due to patient reluctance this was not done until three years later. Thus, in effect the teeth had remained functional for 12 years.

In 1931 Hankey¹⁷ described the replantation of two incisors in a 13 year-old. Two years later, although clinically the teeth appeared normal, radiographic examination revealed almost complete resorption of both roots.

Maley¹⁸ described his personal method of replantation in 1932. He explained that many of the previous failures had been due to inadequate cleansing of the root canal. His technique differed in that the root filling was supplemented by inserting a gold point through the apex to complete the seal.

In 1935 Bodecker and Lefkowitz,¹⁹ reporting a study on dogs, stated that following extraction, islands of periodontal membrane remained on the tooth surface. Upon replantation, these islands of tissue proliferated to effect reattachment. Replanted teeth were studied from periods varying from 28 to 730 days. The authors showed that complete reattachment of the membrane was possible under favorable conditions.

The opposite view was expressed by Pleasant²⁰ in 1942. He described several cases in which he deliberately removed all the remaining periodontal membrane before replantation. His criteria for success were a firm tooth with no symptoms and the absence of radiographic involvement.

Similar reports of success²¹⁻³⁹ and failure⁴⁰⁻⁴² following replantation have been published by many authors.

Another variation of the accepted technique was proposed by Maxmen⁴³ in 1945. He thought that an air pocket existed at the apex of the alveolar socket when a tooth was replanted. To eliminate this, he raised a semilunar flap over the apex of the tooth. After removal of a window of bone, the clot was curetted until fresh bleeding took place. In this approach no air was trapped on replantation and the root no longer had to be shortened. Suturing of the flap created a negative pressure vacuum which aided in retaining the tooth. The fresh blood surrounding the tooth later became organized and developed into fibrous tissue and new bone.

The biological basis for replantation was explained by Schaffer⁴⁴ in 1946. He said that the prognosis depended on the amount of damage to the periodontal tissue and alveolar bone. The physiological status of the surrounding epithelium and the connective tissue also played an important part. The first step in healing was the organization of the blood clot that existed between the alveolar bone and the root. New periodontal membrane was formed by the proliferation of the small islands of tissue that remained on the root surface. In addition, ex-

tension and proliferation of the connective tissue, vessels and nerve endings occurred. Once granulation tissue formed, fibroblasts from the marrow spaces helped to fill in the defects created by resorption.

Corseo⁴⁵ outlined his indications for replantation in 1949. He considered it the treatment of choice when patients resisted apicoectomy or when a tooth was extracted by mistake. Lateral perforations occurring during root canal therapy could also be treated with it.

In 1951 Perint⁴⁶ stated the contraindications to replantation, including exudative diatheses, blood dyscrasias, hormonal disturbances, gingivitis, pyorrhoea, and decreased tolerance to infection. Other factors such as divergent roots and proximity to anatomical structures were also a consideration.

Tilley⁴⁷ was one of the first to realize that root filling was not always necessary prior to replantation. In a letter to the British Dental Journal in 1933, he expressed such an opinion. However, he also recognized the difficulty of obtaining sufficient stability to allow regeneration of nerves.

Herbert⁴⁸ reported three cases of immediate vital replantation in 1953. He hypothesized that because the patients were young the blood supply was good. In two of the cases there was regeneration of nerves as indicated by positive responses to vitality tests.

In a brief review of replantation in 1953, Healey⁴⁹ noted that three distinct types of reaction were seen, varying from prolonged retention, as reported by Kromer,⁵⁰ to a rapid and complete resorption, as reported by Smith.⁵¹ An intermediate form of gradual and partial root resorption such as reported by Payne⁵² was also possible.

Pindborg⁵³ published a case report in 1951 in which he histologically examined a tooth which he had replanted seven months previously in a 10-year-old. In the distal part of the periodontal membrane complete healing had occurred. In other parts of the root, resorption lacunae were seen with hard tissue repair taking place. An important finding was that, although the pulp was necrotic on histological examination, the vitality tests prior to extraction had been positive.

In 1955 Hammer⁵⁴ transplanted teeth into the greater omentum of dogs. He observed that although resorption occurred, the defects produced were rapidly filled in with new cementum. Since preservation of the periodontal membrane was necessary for this deposition, he considered it sufficient proof of the survival and continued function of the periodontal membrane. He also observed that the amount of periodontal membrane remaining on a root was significantly less on a multi-rooted tooth. The prognosis for an incisor was better than for a molar tooth.

The importance of proper fixation was stressed by Basharer⁵⁵ in 1953. He stated that anything less than rigid splinting stimulated osteoclastic activity.

In 1955 Butcher and Vidair⁵⁶ investigated the factors affecting periodontal fibre reattachment in Rhesus monkeys. Following extraction and root canal therapy, the teeth were subjected to various modifications. In some cases the remaining periodontal membrane was removed, while in other teeth the root was notched. No splints were

applied. Despite apical infections the periodontal relations were normal. Reattachment existed above the alveolar bone, illustrating that the latter was not essential for support.

Costich and Hoek⁵⁷ reported an experiment in 1958 in which they replanted teeth in Syrian hamsters. No pulp therapy was performed and the teeth were not splinted. They found that vital pulp tissue existed in the roots of the replants in most cases. However, they also found material resembling osteodentin in the canals. Ankylosis occurred in most of the replanted teeth.

Replantation of primary teeth has been carried out by Sakellariou,⁵⁸ James⁵⁹ and Eisenberg.⁶⁰ Sakellariou reported five cases of replantation, including teeth with chronic periapical infection, in addition to avulsed teeth. Replantation after root canal filling produced rapid healing, firm retention in the socket and normal resorption patterns. Consequently, the author advocated this treatment in preference to extraction and space maintenance.

In an effort to inhibit resorption in replanted teeth, Sather⁶¹ treated the teeth with formalin before replantation. However, histologic examination later revealed no difference between the treated and untreated teeth.

The effect of fluoride on root resorption was investigated by Shulman, Kalis and Goldhaber.⁶² In 1967 they reported a study in which delayed replantation was carried out on Cebus monkeys to determine the effects of immersing the teeth in concentrated sodium fluoride before replacement. Radiographic evaluation of the effect on resorption

was performed biweekly. The findings indicated that fluoride did inhibit root resorption but the actual mechanism was unknown.

Possible theories on the inhibition of root resorption by fluoride were described by Bjorvatn and Massler⁶³ in 1971. They stated that either the hydroxyapatite of the cementum was converted to fluorapatite or else the fluoride had a specific inhibitory effect on the cells that cause root resorption. In their experiment they found that applying 1% stannous fluoride to the root for five minutes before replantation greatly reduced resorption. At a greater concentration, the fluoride considerably reduced the chance of survival of the pulp, periodontal membrane and alveolar bone.

In 1971 Monsour⁶⁴ stated that vital replantation of teeth was performed for two reasons: traumatic dislocation of teeth and surgical repositioning. In a study performed on eight dogs, he selected maxillary central incisors whose roots were two-thirds formed. Both immediate and delayed replantations were carried out. In the delayed replantation the extracted teeth were placed in sterile normal saline and left for three minutes. The teeth were then firmly replaced in their sockets and splinted in place with wrought wire splints. After 14 days they were removed. Radiographic examination was carried out every seven days and the material was recovered for histological examination between seven and 190 days post-operatively. Although there were two cases of pulpal recovery, the general pulpal behavior followed a pattern of slow degeneration and replacement by the ingrowth of tissues through the wide apical foramen. Later there was progressive obliteration of the pulp chamber with osteodentin.

In 1960 Sorg⁶⁵ replanted second molar teeth of hamsters immediately after extraction. After 36 to 63 days he extracted the teeth and examined them histologically for nerve regeneration. Nerve regeneration was seen in over one-half of the replanted teeth examined, while periodontal membrane reattachment and cementum deposition were seen in all of the specimens.

In 1963 Raby⁶⁶ stated that the time elapsed between avulsion and successful replantation can vary from a few minutes to several hours. He also said that if a young tooth, particularly one with an open apex, can be replanted within minutes of the accident, the pulp and the periodontal membrane have an excellent chance for survival. Raby added that if pulp death occurs at a later date, root canal therapy can be instituted.

Loe and Waerhaug⁶⁷ reported in 1961 on the replanting of 58 teeth in four monkeys and six dogs. Thirteen teeth were replanted without periodontal membrane; 15 were dried in air for varying periods, and 13 were reinserted into the alveolus immediately after extraction. Histologic examination showed that teeth replanted without periodontal membrane never regained a normal attachment apparatus; the dried teeth exhibited minor areas of normal periodontal attachment, while in the teeth which had a vital periodontal membrane the attachment was always reformed after replantation.

One of the first reports of studies in which extracted teeth were replanted without root canal therapy was by Myers, Nassimbene, Alley and Gehrig⁶⁸ in 1954. Eight 36-day-old hamsters had mandibular second

and third molars extracted carefully and replanted immediately. Of the teeth included in the histological study, 75% had a viable pulp. However, the authors felt that the slow rate of reattachment of the periodontal membrane could contribute to periodontal pocket formation.

In one of the largest studies to be undertaken, Deeb, Prietto and McKenna⁶⁹ in 1965 pointed out that post-operative ankylosis and root resorption were not inevitable after replantation. On the basis of the clinical and radiographic evidence in their 270 cases, they concluded that replantation can be successfully carried out if a rigid aseptic technique is used.

In an effort to resolve the controversy surrounding the retention or removal of the pulp of replanted teeth, Kaqueler and Massler⁷⁰ performed a study on dogs in which teeth with incomplete root formation and teeth with complete root formation were replanted. No attempt was made to submit the teeth to endodontic treatment and the periodontal ligament was left undisturbed. Ten minutes was chosen to approximate the extra-oral period for immediate replantation and 120 minutes to approximate the extra-oral period for delayed replantation. Although all the experimentally replanted teeth exhibited some pathologic changes, the results suggested that the more immature the tooth to be replanted, the better the prognosis. In addition, the extra-oral period appeared to be critical, a short extra-oral period being preferable to a longer period.

Andreasen and Hjorting-Hansen⁷¹ in 1966 found that 90% of the teeth replanted within 30 minutes after the accident did not exhibit

root resorption. As the length of time the tooth was out of the alveolus increased, the amount of root resorption increased. The teeth replanted without endodontic treatment showed root resorption opposite an intense inflammatory reaction in the periodontal membrane.

Most of the studies before the report of Ohman⁷² in 1965 were primarily interested in the mechanism by which the root of the replanted tooth is anchored to the jaw; little had been published on what happens after complete interruption of the blood supply of human teeth. The result of Ohman's clinical study on the effects of immediately replanting young immature teeth which were due for orthodontic extraction indicated that in cases with a wide apical foramen the pulp chamber was still filled with vital tissue two or three months after replantation, while in teeth with a narrow apical foramen, partial necrosis was present several months later.

In a comprehensive animal study published in 1971, Massler⁷³ investigated the effects of local factors on the healing of periodontal tissues in both dogs and rats. These studies confirmed previous reports that the time the avulsed tooth is out of the mouth is very important in respect to the healing of the periodontium and the survival of the pulp. Removal of the periodontal membrane, either mechanically or chemically, increased the rate and extent of root resorption. The size of the apical foramen was also a factor in pulpal survival. In addition, the administration of systemic antibiotics did not significantly affect the rate of healing of the periodontal tissues. However, a topical application of fluoride to the tooth root prior to replantation significantly retarded the rate and extent of root resorption.

Topical fluoride application was not recommended by Shulman, Gedalieu and Feingold⁷⁴ for teeth replanted within 30-60 minutes, as the concentrated fluoride might devitalize viable surface periodontal ligament and prevent normal ligament reattachment and thus adversely affect replant survival.

Present-day attitudes regarding the technique of replanting teeth were discussed by Massler⁷⁵ in 1974. From his article the most acceptable technique would appear to be to replant teeth as soon as possible after avulsion, to avoid scraping the periodontal membrane, to use no caustics on the root surface and to treat the root surface with topical fluoride prior to replantation. He suggested replantation without pulp therapy for immature permanent teeth which can be replanted soon after avulsion. Completely developed permanent teeth should receive root canal therapy before replantation. In addition, he recommended a type of splinting which will allow functional arrangement of the periodontal membrane.

Many types of splints have been used to stabilize replanted teeth. Albucasis de Condue² used fine gold wires in the eleventh century. However, the most common method has been to use stainless steel wire in the form of an Essig type splint.

Katz⁷⁶ described how he stabilized a replanted upper left central incisor. A matrix of compound was molded around the teeth for protection. Falvello⁷⁷ used .016 inch stainless steel wire to stabilize a replanted central incisor. The splints remained in place for two months. An acrylic splint was used by Lewis and Dow.⁷⁸ They created

an escapeway for air pressure produced upon replacing the tooth in the socket. They drilled a hole in the alveolar bone opposite the apex. Establishing firm replantation in this way obviated the need for extensive splinting. A simple cold cure acrylic splint was used for seven days.

The use of soft alloy splints was described by Slack and Birch⁷⁹ in 1958. The splint consisted of a soft alloy plate molded to the shape of the teeth by finger pressure. It was then cemented in place with Ames Black Copper Cement. This type of splint was rigid and well tolerated by the soft tissues. The splint was removed after eight to twelve weeks. The limitation of this technique was that it could be applied to one or at the most two displaced teeth.

Maxmen⁸⁰ stated in 1959 that the compound splint was objectionable from the esthetic standpoint. In addition, he believed that a splint which allowed biological functioning of the tissues was important. For this reason he suggested using a Hawley splint which had been modified by the addition of a wire support for the replanted tooth. This he advocated wearing for two months.

Maley¹⁸ used gold bands to splint the teeth. Three gold bands were adapted to the replanted tooth and to the adjacent teeth. An impression of the teeth with the bands in place was then taken. On the model produced a small metal splint was made.

In 1974 Polachek⁸¹ described a case in which he had replanted five maxillary teeth. He used .024 wire to anchor the teeth to the adjacent bicuspid. A quick setting acrylic was applied to enhance the stability. The splints were removed after 12 weeks.

In 1975 Rand⁸² outlined a simple technique for splinting replanted teeth. It had the advantage of not requiring the presence of adjacent teeth for anchorage. A suture, .000, was passed through the labial gingiva over the incisal edge of the replanted tooth. Then it was passed through the lingual tissue, back over the incisal edge, and tied tightly on the labial gingival surface. A permanent splint could then be made at a later stage.

The use of a splint combining bracket type orthodontic bands and cold curing acrylic was described by Berman and Buch⁸³ in 1973. The bands were adapted and cemented. Cold cure acrylic was applied to the labial surface of the bands. The splints were removed after six weeks.

Temporary splinting using an adhesive system was described by Heiman, Biven and Kahn⁸⁴ in 1971. After properly repositioning the teeth, they used pumice to clean the surface. The teeth were then etched for 60 seconds. A rectangular arch wire .0215x .028 inch was contoured to the labial surface of the teeth and held in place with carding wax. Adhesive resin was then applied to the labial middle third of the teeth and in this way the wire was firmly secured. Six weeks later the splint was removed.

Autogenous Tooth Transplantation

Human studies of autogenous tooth transplantation have mainly involved the transplantation of third molars with incompletely formed roots into the extraction sites of cariously involved first molars. Root formation of the teeth was usually not more than 50% complete prior to transplantation.

Miller⁸⁵ reported a figure of 52 percent as the success rate of a two-year clinical study. A variation of the basic technique was the situation in which the socket had to be surgically created for the transplanted tooth.

Fong⁸⁶ outlined his criteria for success in transplantation in 1953. These were: 1) The gingival tissues should be attached at the same level as in the adjacent teeth. 2) The gingiva should have physiologically healthy contours with normal texture, color and appearance. 3) The transplanted tooth should erupt into normal occlusion with complete root development and normal pulp vitality. 4) Radiographically, the transplanted tooth and its environment should appear normal. After analyzing the results of 30 cases, Fong reported a success rate of 80 percent. However, he also observed cases in which root formation was not completed.

Noble⁸⁷ described three cases of allotransplantation of third molars in 1954. He concluded that root completion is more likely to occur when the unerupted third molar has at least reached the bifurcation stage of root development. In only one case did completion of the root occur.

Collings⁸⁸ reported transplanting bilateral mandibular third molars into the sockets of extracted first permanent molars in 1951. Both of the transplanted teeth reacted to the electrical pulp tester after 26 months. They also showed root completion. Similar accounts of normal or slightly reduced vitality status of transplanted teeth have been reported by Noble⁸⁷ and Apfel.⁸⁹

Authors have differed as to whether the pulp should be removed before transplantation. Guralnick and Shulman⁹⁰ thought that a well formed tooth in which the apical portion was not completely developed had the best chance of survival. This was due to the fact that the incompletely formed apex would allow vascularization of the pulp to continue. Hence, they contended that it was illogical to remove the pulpal tissue.

In contrast to the literature on replantation, resorption was not emphasized in the reports on transplantation. Muller,⁹¹ however, reported that endodontic treatment was necessary in 25 percent of cuspid transplants, in an attempt to restrict post-operative resorption.

Heslop⁹² reported a study in which he transplanted cuspids in surgically created sockets. In over 70 percent of the cases ankylosis was present in varying degrees after two years.

METHODS AND MATERIALS

This research was designed as a laboratory study. A rhesus monkey was used since its dentition is similar to that of humans. The value of the monkey as an experimental animal has already been established by several investigators, as typified by the encouraging reports of Walshe.⁹³

The animal was obtained from an animal clearing house^a and upon arrival at the Indiana University School of Dentistry animal quarters it was given a gross physical examination and a routine dusting with D.D.T. powder. The monkey was a female approximately three years old, having a full complement of teeth except for the unerupted third molars.

On each of the occasions that the monkey was anesthetized, 5 mg. of phencyclidine hydrochloride^b was initially administered intramuscularly to tranquilize the animal. After about five minutes the monkey was transferred from the cage to the operating room. An intravenous injection of approximately 75 mg. of sodium pentobarbital^c was given and the syringe was anchored in the vein to facilitate further increments of the drug if necessary.

On the first occasion, an oral examination was conducted and periapical radiographs of all the teeth were taken to identify teeth with pulpal or periapical pathology and to assess the degree of apical closure.

a. Primate Imports, Chicago, Illinois

b. Sernylan. (for veterinary use only), Bio-Ceutic Laboratories, St. Joseph, Mo.

c. Nembutal, Abbott Laboratories, Chicago, Illinois

All of the teeth which were to receive apical modification had closed apices. The apices of the maxillary cuspids, which were to be used as controls, were not completed. The apices of the other two controls, the mandibular cuspids, were completely developed. No pathology was detected upon clinical or radiographic examination. The experimental procedures were carried out two weeks later (Figure 1).

Having induced anesthesia in the manner described above, the tooth to be extracted was first loosened with a number 40 elevator. The tooth was removed with forceps numbered either 32 or 22 and the appropriate experimental procedure was carried out before moving onto the next tooth. In the maxillary arch the extracted teeth were the first permanent molars, the cuspids and the central and lateral incisors. In the mandibular arch it was initially intended to use the corresponding teeth but fracture of the lower right cuspid and first permanent molar resulted in the use of the lower right second permanent molar, the lower left first permanent molar, the first bicuspid, and the central and lateral incisors. Apical development of the mandibular first bicuspid, the controls in the lower arch, was completed.

Each of the four control teeth, namely the maxillary cuspids and the mandibular first bicuspid, was immediately replanted without apical modification. Twelve teeth received the experimental apical modification technique as follows. In an effort to use an aseptic technique, rubber gloves were worn and after extraction the tooth was held in a sterile gauze square which had been previously moistened with sterile normal saline. The purpose of this was to protect the perio-

dontal membrane from trauma and desiccation. With a new sterile number 69L bur in a high speed handpiece with air and non-sterile water used as a coolant spray, a vertical groove was created both on the facial and lingual surfaces of the root at the apex. This groove was approximately 1.5mm long, .5mm in width and its depth was determined by the proximity of the pulpal tissue (Figure 2). The purpose of this groove was to expose an additional area of pulpal tissue available for the revascularization and regeneration of neural tissue. It was thus necessary to minimize the trauma to the pulp. The enlargement of the apical pulp exposure was carefully completed by removing the final thin pieces of dentin overlying the apical facial and lingual pulpal tissue by using a small spoon excavator, number 70 (Figure 3). In the molar region this procedure was performed on each root. The time each tooth was out of the mouth was approximately 1½ minutes. Replantation was accomplished by replacing each tooth in its socket with gentle finger pressure and this procedure was uneventful. Each tooth was operated on separately to ensure the shortest extra-oral period possible.

It had been originally intended to splint the replanted teeth with .026 inch brass ligature wire. However, it was apparent that forces from many directions would be placed on the mobile teeth, because several adjacent teeth required stabilizing. It was decided to use Sevritron^a acrylic splints. The teeth were cleaned, dried, and isolated with gauze squares. Enamel etching was produced with 50 percent phosphoric acid which was applied to the teeth for one minute.

a. Sevritron, Amalgamated Dental Trade Distributors Ltd. London, England

After thorough irrigation with non-sterile water and drying with compressed air, Sevritron was applied with a bead-on technique to the entire facial and lingual surfaces. This extensive coverage was necessary because of the short clinical crowns. The splints extended around the arches connecting both second permanent molars. After the splints were adjusted to eliminate any interference in occlusion, a coating of petroleum jelly was applied to them to protect the soft tissue from mechanical irritation.

A neck collar approximately 3 feet in diameter was applied to prevent the monkey from interfering with the splints or teeth (Figure 4). The monkey was then transferred to its cage and monitored carefully until it regained consciousness. Penicillin in the dosage of 100,000 units was given intramuscularly before the monkey regained consciousness and repeated each day for 10 days to reduce susceptibility to infection.

The monkey was anorexic for four days, but on the fifth day commenced to eat a nutritionally complete soft diet. Each week subsequently, the splints were inspected and the gross oral debris and plaque removed with a toothbrush. No anesthesia or tranquilization was necessary for these procedures. On three occasions, replacement of a portion of the splints was necessary. The weak area appeared to be the space between the cuspids and the first bicuspid (Figure 5). Periapical radiographs were taken at one month, two months post-operatively and at the time of sacrifice. Ninety-three days post-operatively an overdose of sodium pentobarbital^a was given in order to sacrifice

a. Nembutal, Abbott Laboratories, Chicago, Illinois

the monkey. The jaws were removed intact by making cuts at the angles of the mandible and the tuberosities of the maxilla with an electric bone saw (Figure 6). After fixation in 10 percent formalin block sections were made with a band saw by making cuts through the unoperated teeth. Care was taken to reduce vibration during cutting. Sections were fixed for one week in 10 percent formalin and then decalcified for approximately eight weeks in 5 percent formic acid. After processing, the tissue was embedded in paraffin blocks and 7μ sections were prepared and subsequently stained with hematoxylin and eosin. Representative sections were also stained with Brown & Brenn stain. Cell nuclei should be stained dark-reddish brown; cytoplasm yellowish; gram positive bacteria deep violet or almost black; gram negative bacteria bright red.

Criteria for Evaluation of the Pulp

Three categories were used. (1) Vital: A pulp which contained the following structural elements in normal appearance: fibroblasts, collagen fibres, blood vessels and nerves. (2) Vital and Inflamed: A pulp which contained the normal elements described above along with either acute or chronic inflammatory cells. (3) Necrotic: A pulp in which destruction of the normal elements had taken place.

RESULTS

Clinical Observations

At the time of sacrifice all of the replanted teeth were stable. The soft tissues appeared normal although the gingiva showed mild generalized marginal inflammation. This gingivitis was severe in the lower right molar region. The splints were intact but this had not been the case throughout, as portions of them had to be replaced on three separate occasions. The area of the splint susceptible to fracture was between the lower lateral incisors and the first bicuspid. The fractured portions of the splint were noticed during the weekly oral hygiene procedures and were immediately repaired.

Radiographic Results

All of the teeth appeared normal during the pre-experimental radiographic survey (Figure 7). The dentition was complete except for the third molars. Radiographs at monthly intervals postoperatively showed no pathology, except for a periapical radiolucency around the mesial root of the lower right second permanent molar.

Histologic Results

The histological findings are summarized in Table I.

Gingivae

The epithelial attachment was intact and attached at the level of the cemento-enamel junction on 11 of the replanted teeth, but a slight apical migration was observed in five teeth. A mild chronic inflam-

matory infiltrate consisting of lymphocytes and plasma cells was present in the interdental papillae of 12 of the replanted teeth.

Periodontal Ligament

The periodontal membrane reattached in all of the replanted teeth. Normal orientation of the fibers was seen in four teeth, while 12 teeth showed a disorientation and vertical rearrangement of the reattached fibers (Figure 8).

Root Resorption and Ankylosis

Areas of external root resorption were observed in 14 of the replanted teeth (Figure 9), while ankylosis was noticed in nine (Figure 10).

Pulpal Vitality

The pulps of three of the four control teeth were vital, containing intact fibroblasts, blood vessels, and nerves without any inflammatory cells (Figure 11). The two maxillary cuspids which had incomplete apices were both vital. One of the mandibular first bicuspids was vital while the other was necrotic. Both of these teeth had complete apical development. All of the teeth which had received apical modification before replantation showed necrotic pulps. The type of necrosis was coagulative, the cell outlines being still visible, but cellular detail and nuclear staining were lost. The cells assumed a homogeneous eosinophilic appearance (Figures 12 and 13). Ingrowth of granulation tissue, which consisted of blood vessels, fibroblasts, collagen fibres and chronic inflammatory cells was seen in nine of the apically modified teeth and in one control tooth. This ingrowth extended to a varying distance in the root canals (Figure 14). There was a noticeable absence of inflammatory cells in the necrotic pulps.

Periapical Region

Periapical pathosis in the form of granulomas was seen in nine of the experimental teeth. These granulomas consisted of chronic inflammatory cells, chiefly lymphocytes and plasma cells, with new capillaries and proliferating fibroblasts. Proliferation of cell rests of Malassez was seen in some of the granulomas. These lesions were surrounded by connective tissue capsule. Lateral pathosis showing a similar microscopic picture was also seen in five of the experimental teeth (Figure 14).

Internal Resorption and Dentin Deposition

Internal resorption was seen in three of the experimental teeth. New dentin deposition, irregular in nature, was observed in three control teeth. All of the latter had reestablished blood and neural supplies (Figure 15).

Brown and Brenn Stain

The Brown and Brenn stain showed that bacteria were present in small numbers in the pulp of five replanted teeth (Figure 16). Hemosiderin granules and acid hematin deposits were noted in many of the sections and these were found to be associated with a high vascularity, as shown on hematoxylin and eosin sections of the corresponding areas.

TABLES AND FIGURES

TABLE I
Summary of The Microscopic Findings

Tooth	Apical migration of epithelial attachment	Gingivae	Periodontal membrane reattachment	External resorption	Ankylosis	Pulp	Ingrowth of granulation tissue	Apical pathosis	Lateral pathosis	Internal resorption	Irregular dentin deposition
<u>control</u>											
6	-	normal	+	+	-	vital	-	-	-	-	+
11	-	normal	+	+	+	vital	-	-	-	-	+
21	+	inflamed	+	+	-	vital	-	-	-	-	+
28	-	inflamed	+	-	-	necrotic	+	-	-	-	-
<u>modified</u>											
3	+	inflamed	+	+	+	necrotic	+	+	+	+	-
7	+	inflamed	+	+	-	necrotic	+	+	-	-	-
8	+	inflamed	+	+	-	necrotic	+	+	+	-	-
9	-	normal	+	+	-	necrotic	+	+	-	-	-
10	-	normal	+	+	-	necrotic	+	+	+	-	-
14	-	inflamed	+	+	+	necrotic	+	+	-	+	-
19	+	inflamed	+	+	+	necrotic	-	-	-	-	-
23	-	inflamed	+	+	+	necrotic	-	-	-	+	-
24	-	inflamed	+	-	+	necrotic	-	-	-	-	-
25	-	inflamed	+	+	+	necrotic	+	+	+	-	-
26	-	inflamed	+	+	+	necrotic	+	+	-	-	-
31	-	inflamed	+	+	+	necrotic	+	+	+	-	-

Key: + = evident

- = not evident

FIGURE 1 Preoperative appearance of the oral region of
the monkey.

FIGURE 2 Photograph of the completed apical modification
procedure on an extracted monkey molar illustrating
the groove which was created to expose the pulp.



FIGURE 3 Line drawing to illustrate the removal of
the final pieces of dentin to expose the
pulp.

FIGURE 4 Collar used to prevent the monkey from inter-
fering with the splints.

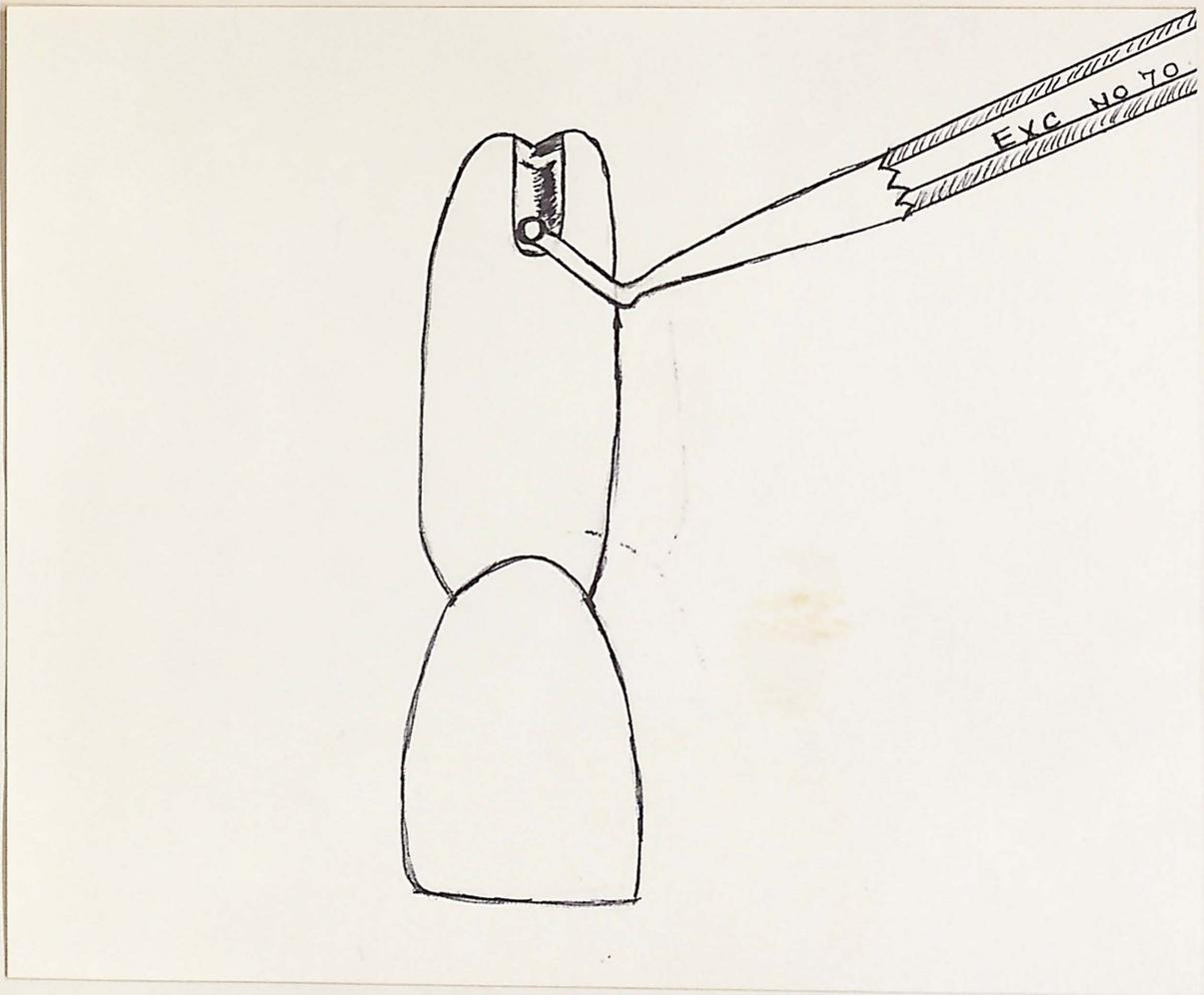
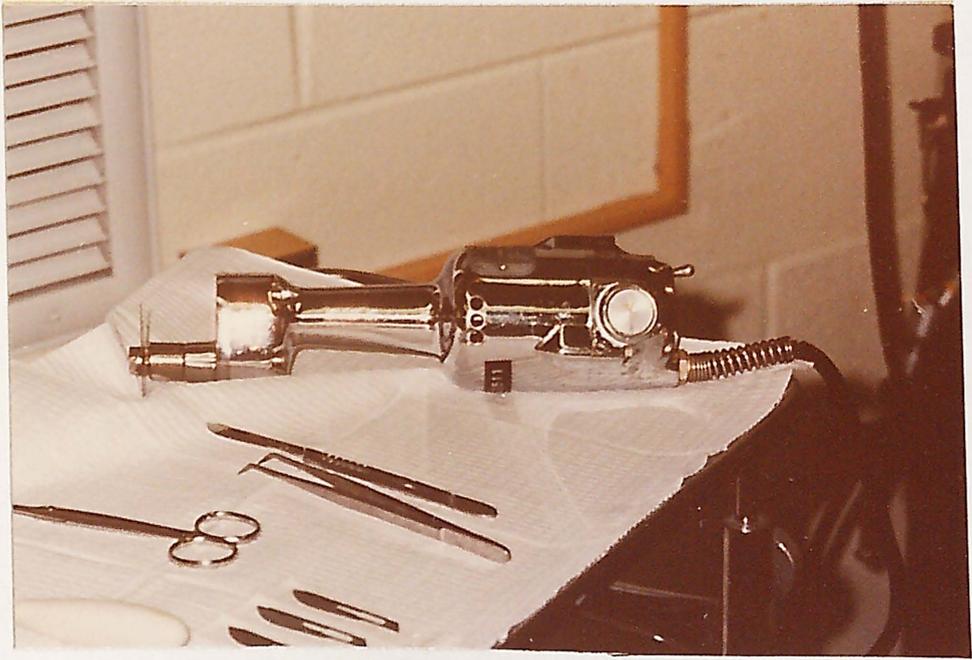


FIGURE 5 The resin splint as seen during one of the
routine weekly examinations.

FIGURE 6 Standardized tray set-up for sectioning the
jaws.



- FIGURE 7 Periapical radiographs of the upper anterior region used to examine root development, pulp size and position and the periapical region.
- (a) Preoperative
 - (b) 1 month postoperative
 - (c) Immediately before sacrifice



a



b



c

FIGURE 8 Photomicrograph of the upper right first permanent molar demonstrating the vertical orientation of the reattached periodontal ligament fibres. (Hematoxylin and Eosin, original magnification x 100).

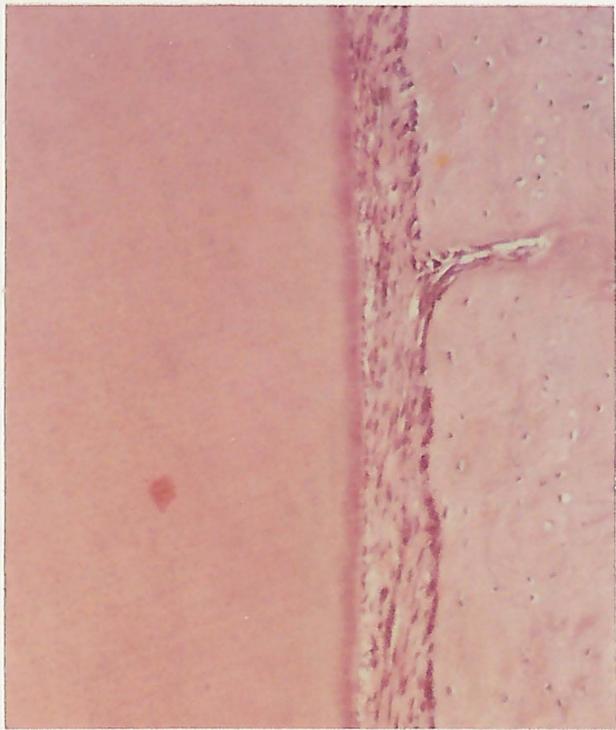


FIGURE 9 Photomicrograph demonstrating an area of external root resorption (Hematoxylin and Eosin, original magnification x 35).

FIGURE 10 Photomicrograph demonstrating an area of external root resorption with ankylosis. Internal root resorption is also seen in this section (black arrow) (Hematoxylin and Eosin, original magnification x 35).

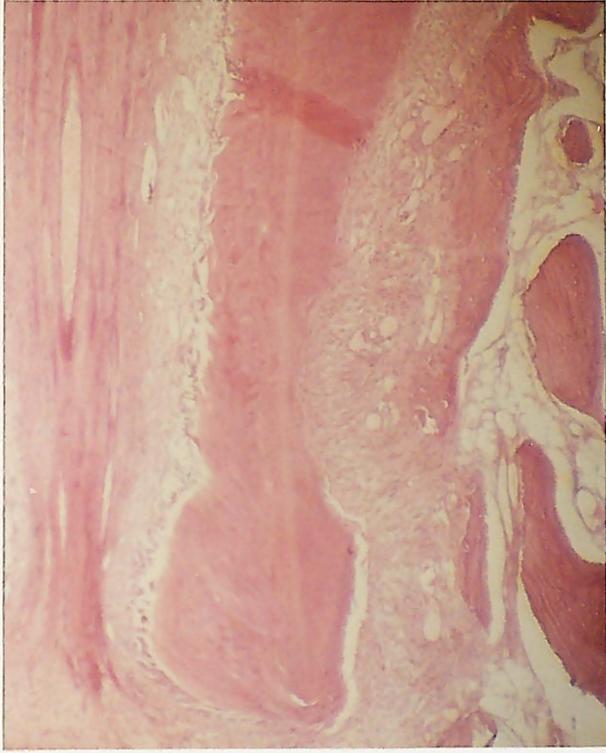


FIGURE 11 Photomicrograph of the upper left cuspid illustrating the revascularization which has taken place. Notice the line of demarcation between the primary dentin and the newly deposited irregular dentin (black arrow). An area of external root resorption is also evident (white arrow) (Hematoxylin and Eosin, original magnification x 35).

FIGURE 12 Photomicrograph illustrating pulpal necrosis in an apically modified tooth (Hematoxylin and Eosin, original magnification x 35).



FIGURE 13 Higher magnification of Figure 12 showing necrotic pulp tissue (Hematoxylin and Eosin, original magnification x 100).

FIGURE 14 Photomicrograph illustrating the ingrowth of granulation tissue which has taken place in an apically modified tooth (black arrow). Notice the apical and lateral granulomas present (white arrows). (Hematoxylin and Eosin, original magnification x 35).

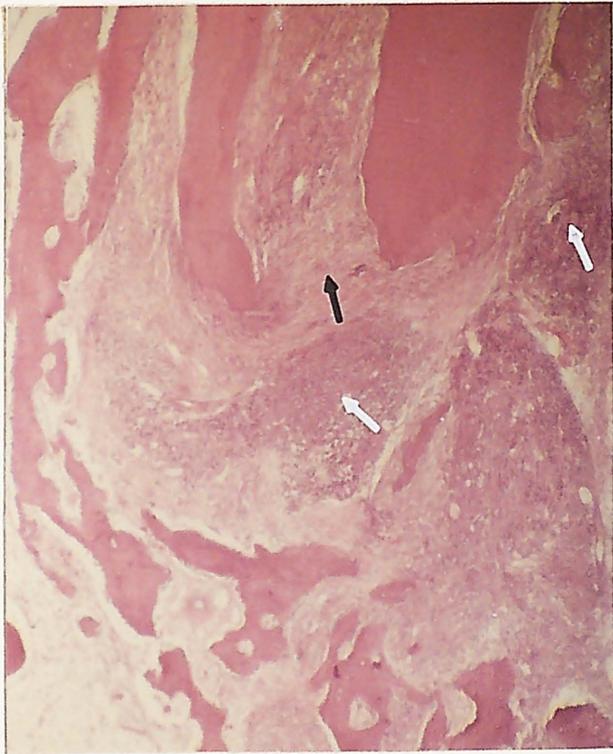
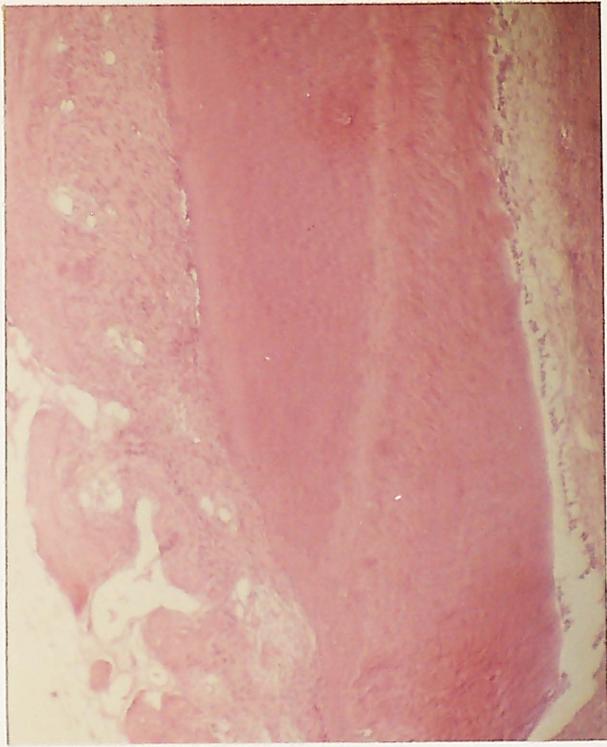


FIGURE 15 Photomicrograph of the upper left cuspid illustrating the deposition of irregular dentin which has taken place with continued root development and narrowing of the root canal (Hematoxylin and Eosin, original magnification x 35).

FIGURE 16 Photomicrograph demonstrating the presence of bacteria in the pulp of a necrotic tooth. The dentin and pulpal remnants stain yellow while the bacteria stain purple (Brown and Brenn, original magnification x 250).



DISCUSSION

The histological results showed that the experimental technique was not a success. All of the apically modified teeth became necrotic, while the pulp of three of the control teeth resumed a vital appearance following replantation. The most likely cause of the pulp necrosis is damage to the pulpal blood vessels during the apical modification procedure. Alternatively, the teeth could have been contaminated, before replantation. The most likely sources for contamination would have been the water coolant which was not sterile, or the ingress of bacteria from the gingival or buccal tissues during replantation. However, the Brown and Brenn stain failed to demonstrate a large number of bacteria, which would have been expected if significant contamination had taken place. Bacteria which were found in the sections could have resulted from the anachoretic effect as reported by Robinson and Boling.⁹⁴

When a nerve is severed Wallerian degeneration of the distal portion of the nerve occurs with swelling, fragmentation and disintegration of the axis cylinders and myelin sheaths. Repair of the nerve begins with proliferation of the axis cylinders, the cells of the neurilemmal sheaths and the endoneurium of the proximal segment of the severed nerve. Regeneration is facilitated by the persistence of the neurilemmal tubes of the distal segment since new fibers proliferate through them and Schwann cells multiply around them with subsequent myelination.⁹⁵ Regeneration usually occurs unless some obstruction such as a blood

clot or a fibrous scar impedes the proliferating proximal end. In other words, the proximal and distal ends of a severed nerve must be in close proximity and in proper alignment in order for regeneration to occur. In the present study the apically modified teeth were out of their sockets for approximately one minute longer than the control teeth. During this time some hemorrhage into the socket may have taken place. This may have exerted hydrostatic pressure on the experimental replanted tooth, thus tending to increase the distance between the proximal and distal ends of the severed nerve. In this way the potential for reinnervation could have been considerably reduced. Alternatively, damage to the pulpal nerves during the apical modification procedure will reduce the potential for reinnervation.

An interesting finding which merits some discussion was the ingrowth of granulation tissue seen in 10 of the replanted teeth. This ingrowth of granulation tissue was similar to that reported by Berger⁹⁶ in his study of the formocresol pulpotomy reported in 1965. He noted that three weeks after formocresol pulpotomies on primary molars in children an ingrowth of granulation tissue took place through the apical foramen. He stated that this could best be described as healing by secondary intention and that unless specimens were viewed over representative periods of time, it would be easy to consider the mature connective tissue in the apical region as normal pulpal tissue. It is interesting to hypothesize that if the present study period had been longer, the entire pulp canal might have been filled with this connective tissue which may be mistaken for mature pulp.

In this experiment the rhesus monkey had some disadvantages for the study of replanted teeth. First, the rhesus monkey was difficult to handle. Any protracted procedures such as the radiographic examination had to be performed under general anesthesia. Second, the bruxing habit of this species made it difficult to provide a long-lasting splinting mechanism. This latter observation was demonstrated by the fact that the splints were repaired on three separate occasions. Third, the necessity of placing the monkey in a collar to prevent it from interfering with the splints played a part in the monkey being anorexic for four days at a time when the general health of the monkey was of prime importance. Alternatively, the anorexia may have resulted from the pain which followed the sixteen extractions.

In the present study adjacent teeth were extracted and replanted in the anterior region to provide a sample of adequate size. A more suitable approach would have been to use a larger number of animals, since attempts to stabilize mobile adjacent teeth led to difficulties during the splinting procedures. Initially it was planned to use interdental wiring reinforced with a composite resin to splint the teeth. This type of stabilization was discarded because adjacent teeth required immobilization. The acid etch technique was then used but the short clinical crown height, especially of the molars, made this a difficult procedure. Fewer experimental teeth per animal and the fabrication of a precast splint might achieve a better result.

The apical modification technique could be improved by the use of an operating microscope. The method of creating the groove was not precise, hence any magnification of the operating area at the apex would enhance the visibility and precision of the technique.

SUMMARY AND CONCLUSIONS .

A study was conducted on the effects in a rhesus monkey of increasing the surface area of pulpal tissue available for revascularisation and regeneration of nerves following replantation of permanent teeth. Four of the monkey's teeth were replanted and used for controls. Two of these controls had incomplete apical development. Twelve teeth with completed apical development were immediately replanted following apical modification to increase the surface area of exposed pulp. The technique consisted of creating a facial and lingual groove in the apical region and exposing additional pulpal tissue. Radiographs were taken periodically and the replanted teeth were stable during the study period. The monkey was sacrificed after 93 days.

The first hypothesis, that the apically modified teeth would sustain a healthy periodontium clinically for a period of ninety days, was disproved.

Histological sections of the teeth and their supporting tissues were prepared. The results showed that varying amounts of root resorption and ankylosis were evident. All of the apically modified teeth showed pulp necrosis, while three of the four control teeth had re-established blood and vascular supplies and presented a vital appearance. Ingrowth of granulation tissue was seen in the replanted teeth with pulp necrosis including one control tooth which had failed to revascularise. It was significant that the two control teeth with incomplete apices had a vital appearance on histological examination.

The second hypothesis, that the apically modified teeth would demonstrate significantly more apical revascularisation and nerve regeneration than the control teeth, was also disproved.

Several problems with the experimental apical modification technique undoubtedly influenced the results. These included the difficulty of creating the apical grooves without traumatizing the exposed pulp. In addition, the use of adjacent teeth for extraction and replantation produced problems in achieving a satisfactory splint.

In conclusion, the apical modification technique as performed to increase the area available for regeneration of nerves and vessels was not a success. As expected, however, immediate replantation of avulsed teeth resulted in reestablishment of periodontal membrane attachments.

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ABSTRACT

THE EFFECTS OF APICAL MODIFICATION OF THE VITALITY
OF REPLANTED PERMANENT MONKEY TEETH

by

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A study was conducted on the effects in a rhesus monkey of increasing the surface area of pulpal tissue available for revascularisation and regeneration of nerves after replantation of permanent teeth. Four teeth were replanted and used for controls, including two with incomplete apical development. Twelve teeth with completed apical development were replanted immediately after apical modification to increase the surface area of exposed pulp. The technique consisted of creating a facial and lingual groove in the apical region and exposing additional pulpal tissue. Radiographs were taken periodically and the replanted teeth were stable during the study period. The monkey was sacrificed after 93 days.

The first hypothesis, that the apically modified teeth would sustain a healthy periodontium clinically for a period of ninety days, was disproved.

Histological sections of the teeth and their supporting tissues showed varying amounts of root resorption and ankylosis. All of the apically modified teeth showed pulp necrosis, while three of the four

control teeth had reestablished blood and vascular supplies and appeared vital. Ingrowth of granulation tissue was seen in the replanted teeth with pulp necrosis including one control tooth which had failed to revascularize.

The second hypothesis, that the apically modified teeth would demonstrate significantly more apical revascularisation and nerve regeneration than the control teeth, was also disproved.

In conclusion, the apical modification technique as performed to increase the area available for regeneration of nerves and vessels was not a success. As expected, however, immediate replantation of avulsed teeth resulted in reestablishment of periodontal membrane attachments.