A HISTOLOGICAL STUDY OF GINGIVAL IMPLANTS INTO
THE DENTAL PULP AND CONNECTIVE TISSUE OF DOGS

By

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CURRICULUM VITAE

ABSTRACT
INTRODUCTION
The vulnerability of the dental pulp is strikingly different from that of loose connective tissue elsewhere in the body, as pulpal injury frequently results in progressive and irreversible damage. This vulnerability to injury has persistently baffled the dental profession, and has led to the production of myriad therapeutic pulp capping methods and materials.

Glass and Zander, in reviewing the dental literature, reported the following agents have been prepared to be effective in promoting pulpal healing: asbestos, plaster of Paris, powdered ivory, aseptic sponge, vulcanized rubber, gutta percha, cork, iodoform powder, formaldehyde, lactophosphate of lime, borax, zinc oxide, zinc oxide and eugenol, zinc oxide and thymol, calcium phosphate, and zinc phosphate cement, and a variety of commercial products. Currently antibiotics, and antibiotic-steroid combinations are being investigated.

To date, therapeutic agents containing calcium hydroxide have been more promising than any others in affecting pulpal healing. However, only limited success with calcium hydroxide therapy has been achieved and these results are dependent upon a meticulous, aseptic technic. A growing body of information
indicates that minimal pulpal injury is prerequisite to success with calcium hydroxide. Seltzer and Bender concluded that the recovery or death of an injured dental pulp depends more upon the extent of cellular damage than upon healing properties of a given medicament.

Reports of histological studies of dental pulps treated with agents containing calcium hydroxide concur that calcium hydroxide is a tissue necrotizer. Pulpal healing, when it occurs, does so beneath a necrotic zone which results from the contact of calcium hydroxide with pulpal tissue. A questionable feature of calcium hydroxide therapy is the fact that the calcific repair which it induces may continue unchecked, thereby producing deleterious effects on the dental pulp. The need for a biologically acceptable pulp "capping" agent is apparent.

According to Orban, the fibroblasts and defense cells of the dental pulp are identical to those found in other loose connective tissues in the body. If this is true, the rigid confinement of the pulp, the relative lack of collateral circulation, and its lack of direct association with ectodermal derivatives all may be factors peculiar to the dental pulp which hinder its defense mechanism.
Epithelium plays an important role in wound healing. References to the significance of epithelium in healing of the dental pulp are sparse as epithelium is not normally associated with pulpal tissue. Stratified squamous epithelium has been shown to cover the surfaces of some exposed pulps in chronic hyperplastic pulpitis. These pulps remain vital and asymptomatic under their epithelial covers.

Since nature occasionally resorts to epithelialization as a method of pulp protection, and since epithelialization of wound surfaces is an important factor in successful healing of other tissues, experimental investigation of the possibility of grafting oral epithelium to exposed surfaces of the dental pulp is warranted.

The present study is concerned with observing pulpal reactions to autogenous gingival epithelium, in an effort to evaluate its effectiveness as a possible biological pulp "capping" agent.
REVIEW OF LITERATURE
In 1929, Orban described two situations in which epithelial rests are seen in the dental pulp, and proposed a theory for the occurrence of each.

Epithelial rests may occur in a pulp which appears to have normal functioning odontoblasts. In such an instance true denticles are often a concurrent finding. To illustrate this phenomenon, a photomicrograph of the pulp of a newly erupted tooth in a young monkey was presented. There were numerous pulp stones and four to five degenerating epithelial rests. Orban explained the occurrence of the epithelial rests in this tooth to be analogous to epithelial rests in the periodontal membrane. The cells became separated from Hertwig's sheath and entered the pulp while the tooth was developing.

The occurrence of pulp stones in teeth with epithelial rests was proposed to be the result of epithelial induction of odontoblastic proliferation in the pulp causing dentin formation. The calcifications described were morphologically similar to dentin, with its associated odontoblasts. A photomicrograph of a squirrel's molar in which epithelial rests were noted at either pole of a dentine was presented to further support this theory.

The second type of epithelial rest in the dental pulp occurs in adult teeth. Odontoblasts are noticeably absent in the vicinity of these epithelial rests; however, the
coronal pulps of these teeth may show normal odontoblasts. When epithelial rests occur in such teeth, the connective tissue at the root apex is not the loose connective tissue of the normal pulp canal, but resembles the fibrous connective tissue of the periodontal membrane. Orban proposed that the periodontal membrane grows up into the apex, carrying epithelial rests with it. Resorption and deposition of cementum on the wall of the apical canal may accompany this phenomenon.

Muller\textsuperscript{10} reported an example of the latter type of epithelial rest in the distal pulp canal of a cariously exposed human lower second molar.

In a histological study of a pulp polyp, Boulger,\textsuperscript{11} considered epithelial rests in the pulp a possible source of the stratified squamous epithelium covering the exposed pulp. The pulp, in Boulger's specimen, rested entirely on dentin, and had no contact with surrounding soft tissue. In an area of the pulp in which there was purulent material, epithelialization was not present. It is interesting to note that Boulger's specimen contained large areas of secondary dentin, which were in contact with odontoblasts along the walls of the pulp canal. Decalcified masses of degenerated pulp were embedded in this secondary dentin. Boulger did not allude to Orban's
hypothesis of epithelial induced dentin formation to explain these findings, but considered the occurrence of secondary dentin as "nature's effort to seal the pulp from outside irritants."

Radden\textsuperscript{12} ruled out epithelial rests in the pulp as the origin of the epithelial covering of pulp polyps. In a clinical and histological study of pulp polyps, he found no evidence to support this theory. Radden proposed that if epithelial rests were a source of the external covering of an epithelialized pulp polyp, they would have to traverse the connective tissue of the pulp to reach the surface. In no specimen was there evidence of epithelium in any area of the pulp other than on the surface.

The concept of epithelial rests as a source of the epithelium covering pulp polyps persisted in the French literature until 1950 when Jansky\textsuperscript{13} reported them as the only probable source of epithelialization. He came to this conclusion after attempting to transplant gingival mucosa to the denuded surfaces of 20 human pulp polyps. None of the epithelial grafts took. Jansky proposed that epithelialization of the dental pulp occurred in the same way that epithelial proliferation occurs in the periodontium. Chronic inflammation provides a stimulus for proliferation of epithelial rests which cover the pulp polyp.
Dolomore, in 1923, speculated that pulp polyps became epithelialized either by direct growth from adjacent gingivae, or from free epithelial grafts from the cheek or tongue. The concept of the oral mucosa as the source of epithelialization of hyperplastic dental pulps has gained additional support from subsequent investigators.

Radden tried to demonstrate that the source of the epithelial covering of a pulp polyp was the oral mucosa. He selected eight first permanent molars having large pulp polyps which clinically appeared to be devoid of an epithelial covering. Into each of these polyps he implanted a piece of epithelium (one millimeter wide by two to three millimeters long) obtained by scalpel excision from the alveolar mucosa. He tried to procure epithelium free of underlying connective tissue. The mucosal tissue was implanted two to three millimeters into an incision in the pulp polyp. Hemostasis was achieved by compressing the sides of the incision until hemorrhage ceased.

Serial histological sections revealed evidence of the epithelial implant in only one of the eight polyps, after an implantation time of five days. Unfortunately Radden chose not to report his histological findings in the unsuccessful cases. In the single pulp in which the implant was observed, the cells in the central portion
of the epithelium showed degeneration. Cells on the immediate outside edge of the implant were well preserved. Those cells which normally would have been desquamated showed morphologic and staining characteristics of the stratum germinativum. Radden concluded that the implant was viable after five days, and predicted that it may have assumed one of three fates if left for a longer period of time: retrogression and complete absorption of the implant, cyst formation, or proliferation possibly covering the outside of the polyp.

Radden interpreted the viability of cells which would have been desquamated under normal circumstances, as a significant finding. These cells had previously been considered to have passed their normal life span, and to be in the process of degeneration. The potential viability of desquamating cells would suggest them as a possible source of epithelialization.

The morphological characteristics of the epithelial coverings on polyps in deciduous teeth led Frey to suggest the gingivae as the source of cells for epithelialization. Many of the specimens in Frey's sample showed an arrangement of papillae which was typical for gingiva. Frey suggested that the difference in polyps covered by epithelium and those which were not is probably related to a stage of development rather than to differences in the essential nature of the hypertrophied pulp tissue.
A recent study of the fine structure of human pulp polyps by Dixon and Peach in 1965, has established that the ultrastructure of the epithelium of the pulp polyp is markedly similar to that of other areas of the oral mucosa. This study did not establish the origin of the epithelium. Comparing the dimensions and cellular characteristics of the various strata described by Dixon and Peach, with those of other electron microscope studies of the oral mucosa, the findings of Dixon and Peach seem to be very comparable to those obtained with human gingiva, observed by Listgarten, and by Kurahashi and Takuma.

Experimental Epithelialization of the Dental Pulp

Intentional epithelial covering of the dental pulp was reported in 1948 by Mezl. He believed the dental pulp capable of healing spontaneously without asepsis as do other parts of the body. To demonstrate this he used dogs in which he laid a gingival flap, drilled a hole through the alveolar bone and dentin to expose the radicular pulp. Mezl then sutured the flap back into place and histologically demonstrated healing at the end of four to eight weeks. The pulp tissues were confluent with the overlying gingiva, and there was no inflammatory infiltrate in the specimens. The epithelial surface was intact. Mezl explained his success on the basis of having provided a biologically acceptable environment for healing.
He contended that in order to overcome an inflammatory response, an injured pulp needs space in which to expand, and a collateral circulation. Both of these conditions were achieved in his experiment by covering the exposed pulp with full thickness gingiva.

Mézli proposed that the excellent healing of oral injuries would indicate that oral microorganisms are in biologic equilibrium and therefore are inoffensive. He questioned the aseptic technic and the practice of sealing an injured pulp off from the oral environment which may in fact contribute to the healing process.

Mézli's concept of the necessity of a collateral circulation was further substantiated by Jansky in 1950 when he produced pulp polyps in young dogs by widely exposing the pulps to the oral cavity. The production of polyps was only successful in multirooted teeth; the pulps of all single rooted teeth became necrotic. Jansky did not report epithelialization of the surfaces of his experimentally produced pulp polyps.

Epithelialization was recently seen in experimentally produced pulp polyps in miniature swine. In 1966, Bennett and McKeen produced pulp polyps in miniature swine by applying Terramycin to the surface of the exposed bleeding pulps. Hyperplasia developed within a week and persisted for six to 20 weeks. Epithelialization was seen on only those
pulps in contact with gingiva. The histological appearance of the epithelium was not described.

Epithelialization of the dental pulp was a serendipitous finding in a study by Baker\(^2\) in which he was testing an antibiotic pulp capping agent in the teeth of monkeys. The source of the observed epithelium was unknown, but the cavity preparations were Class V type; therefore, gingiva was suspect.\(^{24}\) The epithelium was three to four cells thick, and covered the surface of the exposure site. There were no papillary projections as occur in epithelialized polyps.

Calcific Repair in Epithelialized Pulps

Glass and Zander\(^1\) defined healing as the restoration of tissue to its normal structure and function. Since the normal dental pulp is surrounded by dentin with an adherent odontoblastic layer, this condition must obtain in order to consider a disturbed dental pulp healed.

In a discussion on pulp healing, Masterton\(^6\) pointed out that the element of protection afforded by epithelialization of pulps is secondary since protective calcification is not usually found in conjunction with epithelialized pulp polyps. He concluded that ameloblasts and calcium hydroxide "possess the essential induction factor for dentinogenesis, a factor which is not possessed by gingival epithelium."
The literature on pulp polyps includes examples of epithelium covered pulps in which the process of walling off of the exposed pulp chamber is apparent.

In 1959, Shroff\textsuperscript{25} reported calcific bridging of pulp polyps with and without epithelial coverings. In each case atubular dentin developed below the hyperplastic proliferation.

Thoma and Goldman\textsuperscript{26} presented a case in which the pulp in the root canal is walled off by osteodentin which covers the floor of the coronal pulp chamber under a hyperplastic epithelialized pulp. They speculated that the vitality of the hyperplastic tissue was maintained by vascular connections, through the calcified barrier, from the radicular pulp to the polyp. No such vascular connections were demonstrated in the section shown.

Stones et al.\textsuperscript{27} presented a photomicrograph showing a calcific deposit partially closing a pulp exposure below an epithelialized pulp polyp.

Calcific repair in teeth with pulp polyps is not a surprising finding in view of the fact that Pisanti and Sciaky\textsuperscript{28,29} have established that the calcium incorporated in calcific bridging is derived from the blood stream, and not from available calcium ions in calcium hydroxide dressings.
Cyst Formation in the Dental Pulp

Cystic degeneration of oral and/or odontogenic epithelium is a common periodontal occurrence. One case of an epithelial lined cyst within the dental pulp was reported by Pritz\textsuperscript{30} in 1964. The condition was seen in a deeply carious lower right second bicuspid. Dentin resorption occurred where the cyst wall was in contact with the dentinal wall. Pritz refers to epithelial rests as described by Orban\textsuperscript{9} and by Muller\textsuperscript{10} as a possible source of such epithelium lined cysts. He speculated that the inflammatory condition of the involved pulp would favor endodontic cyst formation similar to the periodontal occurrence of this same phenomenon.
STATEMENT OF PROBLEM
The most frequently reported successes in healing exposed dental pulps have been associated with the use of calcium hydroxide, a known tissue necrotizing agent. A more innocuous healing mechanism should be sought. The current investigation is concerned with observing pulpal reactions to implanted autogenous gingival epithelium with the goal of evaluating implanted gingiva as an inducer of pulpal healing.

The introduction of oral microorganisms with the gingival implants was unavoidable. Systemic antibiotics were administered to the experimental animals to minimize the deleterious effects of the contaminating organisms, since bacterial contamination has been shown by Kakebashi et al.\(^{31}\) to be a principal deterrent to pulp healing in rats.

A number of studies of rat connective tissue reactions to dental materials have been conducted at Indiana University School of Dentistry.\(^{32-36}\) In the present study, gingiva was used as a pulp capping agent. A decision was made to implant gingiva into the connective tissue of the dogs' backs for correlative study with the pulps. The fate of implanted autogenous skin has been established in rats by Baker and Mitchell\(^ {37}\) and by Fast and Mitchell.\(^ {38}\) Skin implants were used in the present study as controls for the connective tissue studies.
EXPERIMENTAL PROCEDURE
Young dogs, one male, two female, approximately six months of age, were used for this investigation. The procedure was performed initially on two dogs intended for pilot studies. Twenty-four permanent teeth were operated in each dog, and the animals were sacrificed seven and 30 days postoperatively. Histopathologic study of the pulps of the pilot projects were rather disappointing and seemed to indicate that shorter implantation periods should be considered.

Three dogs were subsequently operated and sacrificed after 24, 48 and 96 hour intervals. Gingival implants into the connective tissue of the backs of the 24, 48 and 96 hour animals were made for correlative study with the treated dental pulps.

Antibiotic Administration

Each animal was injected intramuscularly with five milligrams of tylosin* per pound body weight (24 hours before surgery, the morning of surgery and 24 hours after surgery).

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*Tylocine®, Corvel Division, Eli Lilly Company, Indianapolis, Indiana.
Preparation of Animals for Surgery

Anesthesia was obtained by intravenous injections of sodium pentobarbital* (10 mg./lb. body weight) into a hind leg. The needle and syringe were left in place and taped to the animal's leg in the event that more anesthetic agent would be required during the surgical procedure. The animal was placed on its side on the operating table with the head in a suitable position for surgery. The labial and buccal surfaces of the teeth were wiped with 70 percent alcohol prior to operating on each quadrant. The use of a rubber dam was sometimes difficult because of tooth anatomy, but it was always used where it could be retained. Saliva contamination was minimized by packing the animal's mouth with gauze sponges in those instances when rubber dam application was impractical. Generally, the dogs salivated very little.

Gingival Implants into the Dental Pulp

Experimental: Class V cavities were prepared on the labial and buccal surfaces of 22 teeth in each dog with a friction grip No. 557 fissure bur in a Borden Air Rotor Handpiece. A No. 2 round bur in a conventional handpiece was used to expose the dental pulps. An effort

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*Nembutal Sodium, Abbott Laboratories, Chicago, Illinois.
was made to minimize dentin chip introduction into the pulp after the exposures were made. Hemorrhage was controlled with the aid of sterile paper points.

It was necessary to use magnifying optical loops during the following procedures. Rectangular pieces of gingival epithelium were excised using two Bard Parker blades as shown in Figure I. The epithelium thus obtained was placed into fresh sterile saline solution, on a watch glass, and cut into several very small pieces. For each pulp exposure, a piece of epithelium was picked up on the sharpened point of a No. 23 dental explorer and placed onto the exposed surface of the pulp. The exposure site was covered with a sterile piece of aluminum foil,* pre-cut to the approximate size of the cavity. The cavity was sealed with zinc oxide-eugenol.

Control: The control teeth were operated and treated exactly as previously described except that no epithelium was placed into the pulp exposures. Eight teeth were operated in each animal.

*Reynolds Wrap, Reynolds Metals Company, Richmond, Virginia
Obtaining Specimen

The animals were sacrificed at the designated times by intravenous injection of an overdose of sodium pentobarbital.

A gingival flap was laid for each quadrant, and the operated teeth were removed using a high speed bur, bone chisel and forceps as necessary. The teeth, upon removal, were immediately placed in 10 percent formalin. After all teeth were removed, each was trimmed flat on one surface with a high speed bur and water coolant, until an outline of the pulp cavity could just be seen beneath the remaining dentin. The teeth were then replaced in the formalin solution and allowed to fix for one week, after which time they were decalcified in five percent formic acid, dehydrated, and embedded in paraffin for histologic sections. Serial sections were cut through the cavity preparation and underlying pulp of each tooth. A few sections of each specimen were stained by the Brown and Brenn method for the demonstration of Gram-positive and Gram-negative organisms in tissue sections, and a one step Gomori trichrome stain. The remainder of the slides were stained with hematoxylin and eosin.
Gingival and Skin Implants into Connective Tissue

An area of the dog's back between the sacral and scapular regions was shaved, washed with surgical soap,* rinsed with ether, then tincture of merthiolate. The cleaned surface of the animal's back was dried with gauze sponges. Using a Bard Parker blade, three 1.5 cm. horizontal incisions, approximately two inches apart, were made on either side of the midline. Each incision was made through full skin thickness, and bluntly dissected with a hemostat. Sterile gauze sponges were used to control hemorrhage.

Autogenous Gingival Implants: The gingival sulcus of a maxillary canine was deepened with a No. 15 Bard Parker blade. Surgical silk was drawn through the marginal gingiva of the deepened sulcus and knotted to form a single loop at the periphery of the gingiva. The canine tooth was then gingivectomised to obtain pieces of gingiva approximately 2X2X5 mm., with a loop in one corner of each piece of gingiva. For each animal, three gingival implants were obtained thusly and sutured into previously made individual openings into the animal's back. The gingiva was placed approximately one cm. cephalad of the

incision in each case, and the incision closed with surgical silk.

**Autogenous Skin Implants:** Surgical silk was drawn through the edge of one flap of an incision and knotted as described for the gingival implants. A strip of skin, approximately 2x2x5 cm., was trimmed from the edge of a given incision, and sutured one cm. cephalad of the incision as was the gingiva. All incisions were closed with surgical silk.

**Obtaining Specimen:** The connective tissue implants were excised and placed in 10 percent formalin, after anesthesia, prior to sacrifice of each animal. The tissue was fixed in 10 percent formalin for two days, then dehydrated and embedded in paraffin for histological sections. The histological sections were stained with hematoxylin and eosin, the Brown and Brenn stain, and Gomori's connective tissue stain as were the teeth.
RESULTS
Gingival Implants into the Dental Pulp

**Twenty four hour experimental teeth:** All 20 specimens showed severe inflammatory responses with pulpal destruction manifested as marked hyperemia, extensive abscess formation and vacuolation of the pulpal tissue. Irreversible pulpal damage was seen in 12 specimens, 11 of which were single rooted anterior teeth. Figure 3 shows a typical example of a 24 hour specimen with an abscessed exposure site and a large vacuole within the pulp chamber.

Recognizable epithelium was seen in one maxillary canine. The epithelial cells were within an abscess, and appeared to be in the process of degenerating.

**Forty eight hour experimental teeth:** The 22 specimens obtained at 48 hours were considerably healthier than those at 24 hours. The inflammatory response was confined to the area of injury. Pulps were generally hyperemic in the uninjured areas, but were only mildly hyperemic when compared to the 24 hour teeth. There were no irreversibly damaged pulps in the 48 hour group.

Figure 4 and Figure 5 show the two examples of recognizable epithelium observed in this group of specimens. In Figure 4 a few epithelial cells can be seen within an abscess in the vicinity of the exposure site. Figure 5 shows an exposure site covered by a partially epithelialized
cyst-like structure.

**Ninety six hour experimental teeth:** The 96 hour teeth histologically resembled the 48 hour specimens except that the inflammatory response generally appeared to be milder. No recognizable epithelium was seen in the 96 hour group. Figure 6 and Figure 7 show 96 hour experimental and control teeth respectively.

**Control teeth:** The controls for each time interval showed a milder inflammatory response than the experimental teeth. The inflammatory tissue was confined to the site of injury and the pulps in general showed no devastating damage.

**Gingival Implants into Connective Tissue**

**Twenty four hour implants:** The implanted gingiva was surrounded by an inflammatory exudate consisting mainly of polymorphonuclear leukocytes, in close association with dissociated cells from the surface of the gingival implant. The epithelial surface of the implant appeared to be disintegrating. This dissociative activity was prominent in the granular and prickle cell layers; whereas, the more viable basal cells maintained their association with the underlying connective tissue. The remainder of the stratum germinativum was intact for a few cell layers.

Large masses of spindle shaped bodies were seen
adjacent to the disrupted implant. Some of these structures were anuclear and stained as keratin with the Gomori stain. It appeared that dissociated cells became progressively flattened and "degenerated" at this stage, as dissociated cells having intermediate staining properties could be seen between the intact epithelium of the implant and the disconnected spindle shaped aggregates. The Brown and Brenn stain revealed no remarkable accumulations of bacteria.

Forty eight hour implants: The inflammatory response of the host tissue appeared to be more circumscribed than it was around the 24 hour gingival implants. The integrity of the implants was lost at this stage. The bases of a few rete pegs were discernible in one specimen. Aggregates of large vacuolated cells were seen in addition to the keratin staining bodies described in the 24 hour specimens. All gingival derivatives were within an abscess. The majority of the gingival cells were collected around the periphery of the abscess. The Brown and Brenn stain again revealed no demonstrable microorganisms.

Ninety six hour implants: These specimens were grossly purulent. Microscopically there were few recognizable gingival cells. The detectable gingival derivatives were mainly spindle forms aggregated at the periphery of the abscess. A few large pale staining vacuolated cells could be seen within the body of the abscess with the Gomori stain.
Two of the specimens were cut in a plane which showed
the abscess perforating the skin of the dog.

Skin Implants into Connective Tissue

**Twenty four hour implants:** The 24 hour skin implants
were lost in histological preparation.

**Forty eight hour implants:** The host inflammatory
reaction was considerably milder than for any of the gingival
implants. The implanted skin remained intact and showed
no remarkable signs of degeneration at 48 hours. The
most severe foreign body reaction demonstrable in these
specimens was elicited by the hair follicles. Proliferation
of epithelium occurred at the cut edges of one 48 hour
specimen.

**Ninety six hour implants:** All 96 hour skin implants
were grossly purulent as were the gingival implants for
the same time interval. The amount of host tissue
disturbed by the inflammatory response was considerably less
than for the analogous gingival specimens. Abscess
formation was seen surrounding the implanted skin which
was recognizable and essentially intact. There was
inflammatory invasion of the connective tissue of the implant.
The keratinized epithelial surface of the implant was not
disturbed although surrounded by inflammatory cells.
Epithelial proliferation at the edges of one 96 hour implant
was observed.
TABLES AND FIGURES
Abbreviations Used in Following Tables

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ab</td>
<td>abscess</td>
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<td>mi inf</td>
<td>mild inflammatory responses</td>
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<td>mo inf</td>
<td>moderate inflammatory response</td>
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<td>sev</td>
<td>severe inflammatory response</td>
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<td>hem</td>
<td>hemorrhage in pulp chamber</td>
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<td>hyperemia</td>
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<td>necrotic</td>
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<td>ur</td>
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<td>nd</td>
<td>not demonstrable</td>
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<td>absent</td>
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<td>V</td>
<td>vacuole in empty space</td>
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**upper right, upper left** - scheme for designating teeth by quadrants

**lower right, lower left** - scheme for designating teeth by quadrants

Numerical References Correspond to the Following Groups of Teeth

- 1-3 - incisors
- 4 - canines
- 5-8 - premolars
- 9-10 - molars
## TABLE I
Pulpal Reactions to 24 Hour Gingival Implants

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Response at Exposure Site</th>
<th>Condition of Remainder of Pulp</th>
<th>Epithelium</th>
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<td>n, v, id</td>
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* Bacteria in cavity preparation
TABLE IA
Pulpal Reactions of 24 Hour Control Teeth

<table>
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<th>Tooth</th>
<th>Response at Exposure Site</th>
<th>Condition of Remainder of Pulp</th>
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TABLE II
Pulpal Reactions to 48 Hour Gingival Implants

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*Bacteria in cavity preparation
### TABLE IIA

Pulpal Reactions of 48 Hour Control Teeth

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TABLE III
Pulpal Reactions to 96 Hour Gingival Implants

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### TABLE IIIA

Pulpal Reactions of 96 Hour Control Teeth

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## TABLE IV

Histologic Results of Subdermal Connective Tissue Implants

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Figure 1. Method for excising gingival epithelium.
A Bard Parker blade is pressed on the surface of alveolar gingiva so that the mucosa rises slightly above the opening in the blade surface. The gingiva is obtained by drawing a second blade along the surface of the first, excising the gingiva in its path.
Figure 2. Various stages of cavity preparation
1: prepared, cavity
2: exposed pulp
3: cavity restored with zinc oxide and eugenol.
Figure 3. Typical 24 hour experimental pulp.

A: abscess formed at exposure site,

V: large "vacuole" or empty space, at exposure site, probably resulting from disruption of necrotic center of abscess in tissue preparation. Hematoxylin and eosin stain. Original magnification 100X
Figure 4. Forty eight hour gingival implant showing epithelial cells within an abscess near the exposure site. (arrow) Hematoxylin and eosin stain. Original magnification 100X
Figure 5. Forty eight hour gingival implant showing epithelial cells partially lining a cyst-like structure at the exposure site. (arrows) Hematoxylin and eosin stain. Original magnification 100X
Figure 6. Ninety six hour gingival implant showing a moderate inflammatory response at the exposure site. (F=remnant of aluminum foil) Hematoxylin and eosin stain. Original magnification 100X
Figure 7. Ninety six hour control showing a mild inflammatory response at the exposure site. Hematoxylin and eosin stain. Original magnification 100X
Figure 8. Bacteria in cavity preparation.
Bacterial cells are seen in debris in cavity preparation and in dentinal tubules (arrows). Brown and Brenn stain. Original magnification 1000X
Figure 9. Twenty four hour subdermal gingival implant.
E: gingival epithelium, D: dissociating epithelial cells, B: intact basal cell layer, S: suture, dissociated keratin staining bodies within rectangle. Hematoxylin and eosin stain. Original magnification 40X

Figure 10. Higher magnification of area enclosed within rectangle. K: keratin staining bodies, D: dissociated epithelial cell. Gomori trichrome stain. Original magnification 450X
Figure 11. Forty eight hour subdermal gingival implant. An abscess has formed at the implantation site. (arrows) Hematoxylin and eosin stain. Original magnification 26X

Figure 12. Higher magnification of abscess in Figure 11 showing epithelial cells within the abscess (arrows). Hematoxylin and eosin stain. Original magnification 450X
Figure 13. Ninety six hour subdermal gingival implant showing perforation of the skin by purulent exudate. Hematoxylin and eosin stain. Original magnification 28X
Figure 14. Forty eight hour subdermal skin implant showing proliferation of epithelium at the edge of the implant (arrows). Hematoxylin and eosin stain. Original magnification 100X

Figure 15. Ninety six hour subdermal skin implant showing proliferation of epithelium at the edge of the implant. Hematoxylin and eosin stain. (arrows) Original magnification 100X
DISCUSSION
The unavoidable introduction of microorganisms into the dental pulp and connective tissue along with the implants was a hazard in the present study. The use of systemic penicillin as an adjunctive agent with calcium hydroxide pulp capping has rendered favorable clinical results in humans. In this investigation the antimicrobial agent tylsin was administered prophylactically in an endeavor to minimize the deleterious effects of any implanted microorganisms.

Similar to penicillin, tylsin has an essentially Gram-positive antibacterial spectrum. Tylsin is also active against pleuropneumonia like organisms, certain spirochetes, large viruses, and a few Gram-negative organisms. The drug is recommended for combating infections associated with surgery or injuries in domestic animals.

Except for the 24 hour specimens, the majority of the pulps of the operated teeth, both control and experimental, appeared to be quite healthy, excluding the immediate area of injury. It would probably be safe to attribute this lack of extensive degeneration to the prophylactic antibiotic therapy.

Failure to demonstrate microorganisms within the pulps and connective tissue by the Brown and Brenn stain does not necessarily mean that the organisms were not present. It is possible that the antimicrobial action of
the drug in some way altered the cell surfaces of the organisms so that they did not accept the dye. It has been established that an intact cell surface is essential for Gram staining.

The dental pulps of the 24 hour animal were noticeably more damaged than the pulps for the 48 and 96 hour intervals. The dogs used for the 48 and 96 hour intervals were obtained from the same source and had a known negative medical history, good diet, and veterinary care. The 24 hour dog was a mongrel of unknown history and surmised age. This difference in animals is indeed unfortunate, as three comparable animals might have provided greater insight into the mechanism by which implanted gingival epithelium is destroyed in the dental pulp.

It is possible that a previous history of illness, malnutrition, or both could have contributed to the relative debilitation of the pulps in the 24 hour animal. Systemic influences on non-curious dental pulps of experimental animals, have been discussed in the literature by Glickman and Shklar. These authors suggested that pulpal response to local factors could be modified by systemically induced pulpal degeneration. Starvation was among the phenomena observed to cause degeneration.

Cells which could be indisputably recognized as epithelial cells were observed in a total of three of the
63 experimental specimens. Many experimental teeth exhibited what appeared to be degenerating epithelial cells. There was no reliable method of identifying these cells as such; they were therefore reported as having no recognizable epithelium. It should also be noted that the conditions under which the epithelium was seen, namely within an abscess or associated with what appeared to be a cyst, were consistent findings for the entire sample.

The gingival implants into the dogs' backs provided the opportunity to observe the fact that implanted gingiva undergoes a rapid dissociation resulting in abscess formation and total destruction of the epithelial elements. The large incidence of abscesses in the experimental pulps in addition to the fact that the inflammatory response in these teeth was demonstrably more severe than the inflammatory response in the control teeth, would perhaps suggest a similar fate of gingival epithelium in the dental pulp.

The incompatibility of gingiva with the abnormal environment provided by this experiment may be related to the microorganisms introduced with the gingival tissue, or to a foreign body reaction of the host tissue to the gingival cells. The solution to this speculation is beyond the scope of the present study.

The demonstrated lack of viability of implanted gingiva
in dental pulps and connective tissue may account for the rarity of gingival cysts. There are numerous reports of traumatic or surgical implantation cysts of epidermis or cornea, in the literature. Traumatic or surgical implantation of gingiva resulting in cystic degeneration has not been a popular concept. Ritchey and Orban, \(^{45}\) in 1953, studied 350 gingival biopsies in which they found six gingival cysts. Traumatic implantation was one of the three sources of epithelium proposed as a result of this study. The other two sources suggested by these authors were heterotopic duct glands and remnants of the enamel organ. Bhaskar and Laskin, \(^{46}\) in 1955, also suggested traumatically displaced oral epithelium as a source of gingiva, in a report of three cases. A more recent study by Bhaskar \(^{47}\) in which he histologically observed 29 gingival cysts and eight fetal beards, led him to suggest that gingival cysts are derived from remnants of odontogenic epithelium left in the oral mucosa during odontogenesis. This proposition is in keeping with that of Kreshover \(^{48}\) who histologically examined maxillae and mandibles of 17 infants, and observed 65 cysts below the surface of the gingival epithelium. All of the cysts observed by Kreshover developed from the cells of the dental lamina. He proposed that many of these would be lost in the process of eruption, accounting for the negligible clinical observance of these cysts.
The foreign body reaction to autogenous gingiva in the present study, and the fact that Fast and Mitchell observed no evidence of oral mucosal implants in rat and monkey connective tissue after a ten day implantation interval, would suggest that implanted autogenous gingival mucosa is not viable in connective tissue. These observations in addition to the many references to glandular and embryonic tissue remnants in gingiva make the concept of implanted gingiva as a source of gingival cysts suspect.

The skin controls in the connective tissue study were much like the skin implants into rats' backs described by Baker and Mitchell, in that they elicited a moderate inflammatory response and remained viable for the 96 hour implantation period. These specimens were also similar in that an increasing host inflammatory reaction occurred with longer implantation intervals. Baker and Mitchell reported evidence of epithelial proliferation in their 24 hour implants. In the current study this phenomenon was observed in one 48 hour and in one 96 hour skin implant. No 24 hour specimens were observed in this study. The viability of the skin controls dramatically demonstrated the difference in epithelial survival of implanted skin and gingiva.

The failure of gingival proliferation in connective tissue is perhaps fortuitous in view of the numerous
opportunities for gingival implantation. The inflammatory response that ensues would perhaps implicate traumatically implanted gingiva as an etiologic agent in pyogenic granuloma rather than gingival cysts. Shafer et al. report that it is generally agreed that pyogenic granuloma arises as a result of minor trauma providing a pathway for the invasion of non-specific organisms. Epithelial cells could probably enter by the same pathway. Implanting gingival epithelium into gingival connective tissue might shed some light on this speculation.

The results of the current study do not support the use of gingival epithelium as a possible biologic pulp capping agent. It must, however, be admitted that epithelialized exposed pulps seem to be clinically more viable than those which have no epithelial covering. Perhaps the condition of inflammatory hyperplasia is conducive or prerequisite to epithelialization of the dental pulp. It would be interesting to conduct an experiment along these lines. Repetition of the current experiment in gnotobiotic animals, if technically possible, could clarify the influence of microorganisms on the results obtained in this experiment.
SUMMARY AND CONCLUSIONS
An experiment was conducted on three young dogs in an effort to evaluate the possibility of using autogenous gingival implants as pulp "capping" agents.

Each dog was injected, prophylactically, with maximum recommended doses of the antibiotic Tylocine, the day before, the day of, and the day after surgery. Class V cavity preparations were made on the buccal surfaces of 30 permanent teeth in each animal. Small pieces of autogenous gingival epithelium, excised from the alveolar mucosa as shown in Figure 1, were placed into pulp exposures in 22 of the 30 operated teeth. Aluminum foil was used to cover the exposures, and the teeth were restored with zinc oxide eugenol. Eight teeth in each animal served as controls. No epithelium was placed in the pulp exposures of the control teeth, otherwise the procedure was identical to the procedure for the experimental teeth. The implantation intervals were 24, 48 and 96 hours.

The greatest amount of pulpal damage was observed after the 24 hour implantation period, lessening at the later intervals. There were many teeth exhibiting what appeared to be degenerating epithelial cells within abscesses, however in only three teeth was there recognizable epithelium in the dental pulps. The recognizable epithelial cells were within abscesses in
one 24 hour specimen, and in one 48 hour specimen. Epithelium partially lining a cyst-like structure at the exposure site was seen in one 48 hour tooth.

The control teeth for each implantation period showed less inflammatory response and pulpal damage than the corresponding experimental teeth.

These results of the study do not favor the use of autogenous gingival implants as a pulp "capping" agent. The observed failure of epithelial survival in the dental pulp, in most instances, corresponds to the results of some previous attempts at epithelialization of the dental pulp described in the review of the literature. Whether the rejection of autogenous gingiva by the dental pulp is related to the introduction of microorganisms or whether it is a reaction to the gingival epithelial cells per se, is speculative.

Full thickness gingival implants into the animals' backs were made for each implantation period, for correlative study with the dental pulps. Skin was used for control implants for this subdermal connective tissue study. Within 24 hours the gingival cells were seen to dissociate from the implanted gingival tissue. The dissociated cells appeared to change to a flattened keratin staining form. By 48 hours definite abscesses were formed. Dissociated epithelial cells could be observed within the abscesses
and gross suppuration was observed at 96 hours. Microscopically, perforation of the animal's skin by purulent exudate was seen.

The gingival implants into subdermal connective tissue correlated with the gingival implants into the dental pulp in demonstrating that implanted gingiva is not viable. The inability of gingiva to survive implantation deserves further study. The possibility of gingival cysts not being of gingival origin has been discussed in this connection.

The skin implants remained recognizable and viable for each implantation period; thus demonstrating that implanted gingiva and skin have markedly different fates.

The present study has shown that implanted autogenous gingiva causes an acute inflammatory response in dental pulps and in subdermal connective tissue, with loss of viability of the implants. Autogenous skin implanted into subdermal connective tissue remained viable and proliferated during a 96 hour implantation period.

From this investigation it may not be concluded that implanted gingiva can be used to induce pulpal healing. The possibility of cyst formation from traumatic implantation of gingiva is questionable in light of the fact that implanted gingival cells became non-viable within 96 hours.
REFERENCES


42. Corvel Division, Eli Lilly Co. Product literature.


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July 5, 1936
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June 1957-Sept. 1961
Sept. 1961-June 1965
Sept. 1965-Sept. 1967

Born: Brooklyn New York
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Graduate student,
Department of Pedodontics,
Indiana University School
of Dentistry

Organizations
American Dental Association
American Society of Dentistry for Children
ABSTRACT
A Histological Study of Gingival Implants
into the Dental Pulp and Connective Tissue of Dogs

By: Millicent Henry
Indiana University School of Dentistry
Indianapolis, Indiana

This investigation was undertaken to evaluate autogenous gingival implants as possible inducers of pulpal healing.

The microscopic study of autogenous gingival implants into the dental pulps of dogs, injected with a systemic antibiotic, revealed that the implants produced an acute, severe inflammatory reaction resulting in abscess formation at the implantation sites. This phenomenon was observed after 24, 48 and 96 hour implantation intervals. The inflammatory response for the control teeth was generally mild to moderate.

Subdermal connective tissue implants of full thicknesses of autogenous gingiva provided a clearer picture of the fate of the implanted gingival cells. Within 24 hours, the epithelial cells became dissociated from the implant. Definite abscess formation was observed in the 48 hour specimens. Perforation of the animal's skin by purulent material was seen after 96 hours. Full thicknesses of skin obtained from the edges of the dermal incisions were used as control implants for the subdermal connective tissue study. The implanted skin remained viable and proliferated for the duration of the experiment. These results would not favor gingival implants as inducers of pulpal healing.