HEALING SUBSEQUENT TO A CIRCUMFERENTIAL SUPRACRESTAL FIBROTOMY IN HUMANS

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INTRODUCTION

It is well known that orthodontic relapse may occur subsequent to active tooth movement[1]. This is especially true following rotational corrections. Consequently several procedures have been used clinically to help eliminate rotational relapse. Skogsburg[2] (1927) advocated severing the fibrous and bony structures in the septal area on both sides of the root after rotation. Hallet[3] (1956) drew attention to the idea of immediate correction of rotations, a procedure he referred to as "redressement forcé". Reitan[4] has suggested overcorrecting the rotated tooth and retaining it for a stable period. Following retention some minor relapse is to be expected to allow the tooth to align correctly. In addition, he has proposed that it is beneficial to correct any rotations prior to the complete development of the apical portion of the root. Again, the argument is one of stability. By achieving the desired position early, the root will develop and accept its new environment easily. All of these procedures only approach the problem from the perimeter. Edwards[5,6], on the other hand, has demonstrated that the supracrestal fibers surrounding the tooth are the true instigators of rotational relapse. He has suggested severing these fibers subsequent to the rotational correction. This procedure, circumferential supracrestal fibrotomy (C.S.F.), has sparked mounting interest in the question, "What is the response of the gingival fibers during the healing phase?" "Does the healing response proceed in a characteristic manner?" Also does the epithelial

attachment change as a result of this procedure? Factors that may influence this type of wound healing include marginal gingivitis, age, nutrition, and hormones. However, their contribution is incompletely understood at present.

The purpose of this paper is to determine whether gingival wound healing following the C.S.F. procedure will proceed in a characteristic manner with time. In other words is the ensuing inflammatory response specific for any time period post-surgically? It may be possible to elucidate whether specific cell types act characteristically within the supracrestal structures. Also it is hoped that this study will corroborate the findings that the structural organization of the connective tissue regenerates its original architecture[7].

LITERATURE REVIEW

Since the beginning of man's existence, the healing of wounds has concerned everyone. It has been suggested that minor skin abrasions are man's most frequent medical incident. Historically, there has been little contribution to this area prior to 1950. Arey[8], (1936) was one of the pioneer workers and described the basic wound response as it is known today. However, there still remains an incomplete understanding of many underlying biochemical processes involved. For example, the control or regulation of the sequential stage of healing which ultimately results in repair* or regeneration* of the injured tissue is unknown as well as are many other questions associated with healing. In spite of our limited knowledge of the basic wound response, modern research is attempting to control the rate of wound healing and subsequently to eliminate scar tissue or cicatrix formation.

Most of the research of the periodontium has been carried out in the last twenty years by Reitan[4], Melcher[7,9], Ramfjord[10,11], Stahl[12,13,14], Listgarten[15,16], Edwards[5,6] and others. These investigators have been responsible for the development of the current literature on gingival healing as well.

*Refer to Melcher, A.H., On Repair Potential of Periodontal Tissues. Journal of Perio., 47:256, 1976, for his definition of "repair" and "regeneration".

Recently, Edwards has sparked an interest in the elimination of rotational relapse. The procedure he described is directly concerned with gingival wound healing subsequent to severing the supracrestal structures. However, attention was focused on the supracrestal region as a causative factor in rotational relapse when Fullmer and Lillie[17,18], 1958, demonstrated the presence of oxytalan fibers in this area. These fibers have been implicated in rotational relapse by exerting an elastic-like force on the tissues[5,6]. Further corroboration by Reitan[4], 1959, and Boese[19],1969, confirmed that the supracrestal structures played a major role in determining rotational relapse. But in order to fully understand gingival healing, the basic process of generalized skin healing must be considered concurrently.

Initially, when a wound is inflicted a number of ordered sequences are triggered[20]. The fresh wound will make some attempt at adhering its edges together creating a "plug" to protect itself from the outside environment. This is apparently followed by the release of humoral factors such as leukokinins, histamine, heparin and serotonin. All of these chemical mediators, previously called "trephones"[8], are assumed to be responsible for the ensuing inflammation. Giblin[21], 1966, may have substantiated this localized humoral response when he demonstrated that re-entry wounds in dogs developed a shorter healing time than the initial wound. Epithelialization appeared to be the only response affected by a second wound.

Generally it has been accepted that the healing process is more rapid within the periodontium as compared with that of the skin[7,9].

Engler[10], 1966, confirmed this statement by demonstrating that within two hours following gingivectomy in monkeys swelling occurred concurrently with the arrival of polymorphonuclear leukocytes (PMN's). Thus at this short time interval acute inflammation has been initiated. On the other hand the inflammatory process in skin was usually not fully evident for at least four to six hours[20]. The reason for this difference is unknown. However Melcher[9] suggested that perhaps the moist oral environment may somehow be conducive to more rapid healing. In any case, Ross[20] stated that PMN's arrived at the wound site by extrusion through capillary vessel walls at the site of the injury. Noaves[22], confirmed this localized permeability of the capillary vessel walls by intra-arterial vital perfusion in dogs. Ramfjord[10,11], and other workers[8,12], have suggested that the PMN's, which formed a "polyband" between the wound surface and connective tissue, served mainly as a protective function against invading microorganisms. This persisted until the regenerating epithelium covered the wound. Previously it was accepted that the PMN's may also have a role in initiating the collagen proliferative phase in wound healing[8]. However, Simpson and Ross[23], 1972, disproved this theory. They demonstrated in guinea pigs that neutrophils did not have any appreciable affect on the progression of wound healing. Instead their only important function appeared to be as a local barrier to bacterial invasion. Also Fullmer[24] confirmed the phagocytic nature of PMN's by demonstrating the presence of a specific collagenase. Furthermore, Howes and Hoopes[25], 1977, have suggested that PMN's may generate electronic excited states of molecular oxygen which then act as a bacterial agent as well as a source of proline hydroxylation,

both of which are necessary for wound repair.

Subsequent to the clot formation and short-lived acute inflammatory response, a number of cell changes are noticed. Ramfjord[11] discovered that as little as two days following a gingivectomy in monkeys the predominant cell types are typically chronic. These include macrophages, lymphocytes, and plasma cells. Furthermore the initiation of this chronic phase appeared subsequent to the short life-span of PMN's, namely two days following injury. Ross[20] also confirmed the presence of round cell infiltrate following acute inflammation. Their presence was noted as early as twelve hours in humans. The main purpose of these bloodborne cells was to act as "scavengers" and phagocytose any cellular or necrotic material in an attempt to reorganize the wound.

As stated previously, epithelialization was shown by Engler[10] to occur concurrently with the inflammatory response. Similarly, Mittelman[26], 1964, noticed that epithelial regeneration in human attached gingiva occurred prior to connective tissue repair. In addition, he demonstrated that twenty-four hours was required for some sort of epithelial covering to bridge the wound following primary intention. These epithelial cells did not bridge the wound via mitosis. But instead they showed a direct extension of pre-existing cells. Apparently mitosis did not occur until after keratinization. Also Mittelman[26] and Ramfjord[11] determined that the peak activity of connective tissue proliferation would occur much later than epithelization. Ramfjord[11] based this finding on labelled endothelial cells, lymphocytes and fibroblasts that appeared subsequent to epithelial formation. In other words some sort of epithelial covering was required prior to connective tissue proliferation.

Many theories have been proposed on epithelial regeneration. However the consensus is that the epithelial cells at the wound edges migrate beneath or through the clot until they contact one another[9,10,12,27]. Melcher[9] suggested that fibrin within the clot acted as a "scaffolding" or "contact guidance" for these cells. Ultimately these cells continued to migrate in a centripetal manner from the edges of the wound until they touched one another. At this point epithelial migration ceased (contact inhibition). It has been suggested from "in vitro" studies that contact inhibition may occur from a physico-chemical or an electrical attraction between the cell membrane and its substrate. In addition Ross[58] pointed out that epithelial cells may become phagocytic in order to eliminate any cellular debris obstructing their pathway during epithelial regeneration. Recently the specific pattern of epidermal cell migration has been elucidated using electron microscopy. Krawczyk[30], 1971, using rats, noted two phenomena associated with epidermal healing, namely cell migration and mitosis. This epithelial mitosis was in direct contrast to what was noted by Mittelman. In any case, Krawczyk[30] observed a thin sheet of epithelial cells, of which the most advanced cell extended a long narrow process through or under the clot. Eventually this cellular extension contacted fibrin or a mesenchymal cell and formed a hemidesmosome-like junction. Subsequently the process was repeated by cells located superiorly, namely the prickle cell layer. It also appeared that cell mitosis did not occur at the wound edges as expected, but instead was noticed at some distance back from the actual margin of the wound. Once again this appeared to be a contradiction to Engler's[10] findings of active mitotic cells at the wound margin in the gingiva of monkeys.

During this cell migration one might consider the question, "How is the basement membrane formed?" Listgarten[31], 1966, demonstrated that it was formed by secretory products of the epithelial cells. Other evidence accrued has further implicated epithelial cell participation in basement membrane formation[59]. Using H^3-proline and C^{14} precursors it has been shown that epithelial cells secrete a granular glycoprotein resembling basement membrane. At selected points along the basal membrane the extended pseudopod formed a hemidesmosome which was thought to be an important process in the migrating epithelial cells. Stahl[12] stated that in most instances gingival epithelialization occurred anywhere from seven to fourteen days post-injury in humans. During this period Engler[10] and Weinstein[36] showed that mitotic activity in the epithelial cells was initiated via the basal cell layer. Thus this layer has been established as the progenitor pool for epithelial replication. Once new cells have been formed they are expressed outward to the surface in accordance with specific turnover rates.

A number of factors may determine whether gingival epithelium is keratinized. It was originally thought this was determined by some genetic component. Karring[32], 1975, suggested that differentiation of gingival epithelium is controlled by inductive stimuli within the connective tissue. However this idea was disproved by a number of investigators. Caffese[33] explained that sulcular and oral epithelium are both under the same connective tissue specificity and could not possibly determine epithelial differentiation. Using monkeys, they showed that environment played a major role in determining whether the epithelium is keratinized or not. Similar

findings were seen in humans in a study by Bral and Stahl[14], 1977. They determined that keratinization of the sulcular epithelium may be inhibited by the presence of a constant local inflammation in the sulcus such as plaque. In any case, Lopez and Belvederessi[34], 1977, showed that crevicular epithelium in humans regenerated completely by the seventh day following subgingival scaling. However Engler[10] noticed that local inflammation may affect healing time of epithelialization.

An important sequelae of the C.S.F. procedure should be considered at this time. It may be said that Skogsburg[2] was on the right track when he recommended severing the fibrous structures in the septal area. But what happened to the epithelial attachment? From the work of Edwards[37], it is now accepted that a new reattachment is possible after injury. Furthermore several E.M. studies have been done to determine the fate of the epithelial attachment. It has been shown that after a period of seven to fourteen days following its removal, it will reattach to a level of approximately two to three layers thick[12]. Taylor and Campbell[35], who have done some work in this area with marmosets, demonstrated that the crevicular epithelium reattached to the tooth by hemidesmosomes intermingled within a cementing substance resembling basement lamina. Also Stern[38], 1965, showed that this attachment strongly resembled normal epithelial-connective tissue junction. As pointed out previously by Taylor and Campbell[35], the actual reattachment sequence was initiated in the basal layer as observed in epithelialization. From this point the attached cells migrated coronally in accordance with normal turnover rates of crevicular epithelium. No attachment to the tooth occurred once the cells left the basal layer. Using monkeys, Listgarten[39] was able to duplicate these

findings. However the question still remained, "Did the epithelial attachment locate more apically following injury?" Stahl[12,13] was quick to answer this question. He proposed that when the epithelial-tooth surface contact was broken, the wound edge migrated apically because the tooth surface alone did not provide sufficient "contact inhibition" to the migrating epithelium. In other words apical migration of attachment occurred until the epithelial cells contacted periodontal fibers on the root surface. Subsequent to attachment, "healing by scar" usually occurred[40]. Staffileno[41], 1966, using partial thickness flaps in dogs, confirmed that the epithelial attachment did migrate apically following surgery.

Oral hygiene appeared to have some importance in the healing time of tooth-epithelial reattachment[42]. Taylor and Campbell[35] noted a slight difference in the location of epithelial reattachment if chronic inflammation was present. However Engler[10] previously determined that oral hygiene played a significant part in altering the rate of healing in the gingiva. Nevertheless, in humans, Kohler and Ramfjord[43] could detect no difference in the rate of healing between areas of gingivitis and normal gingiva. Therefore considerable controversy reigns over the influence of oral hygiene on healing.

The next phase of healing, connective tissue proliferation, exemplified collagen synthesis as the major feature during this time. As stated previously, the inflammatory response changed from acute to chronic. Recently it has been suggested that the cells in the connective tissue or the periodontal ligament may originate from undifferentiated perivascular cells. This was substantiated by Gould[44], 1977. Using mice, they determined that following gingival injury, the

majority of dividing cells, namely osteoclasts and fibroblasts, appeared to be associated with blood vessels. But the question remained whether a single progenitor population gave rise to all the various functional cells in the periodontal ligament. Another worker, Karring[32], used monkeys to determine the origin of the granulation tissue in the periodontium. He concluded that along with the associated inflammatory response the granulation tissue originated from a number of areas, i.e., periosteum, adjacent gingiva, and bone marrow spaces. But by far the majority of granulation tissue was derived from the periodontal ligament itself.

Alveolar crestal resorption was a characteristic finding following gingival trauma. It occurred usually within the first week[27]. As to whether the alveolar crest is returned to its original height seemed to be related to the extent of exposure of the periosteal covering. Karring[32] and Pfeifer[45] demonstrated that bone left without its periosteal covering was severely resorbed and appeared not to regenerate to its original height.

The localized cellular and humoral factors probably elicited the bone response to wounding via an osteoprogenitor pool in the periosteum. Initially necrosis and resorption of bone continued during the first week or so. Then active osteogenesis predominated[7,9,27,45].

Localized cementum resorption is another phenomena that can occur coincident with gingival wound healing[7,9,46,47]. Again this resorptive response may be elicited by humoral factors. The progenitor pool in the periodontal ligament may give rise to the cells which develop into cementoclasts and ultimately cementoblasts. However it is now accepted that the majority of cell types in the PDL, namely osteoblasts,

osteoclasts, osteocytes, cementoblasts, etc., may be derived from a progenitor pool of undifferentiated cells. From this pool they can modulate from one type to another depending on the ambient environmental situation[48].

Somehow there must exist an equilibrium between cells of the PDL and periosteum. Melcher[7], Line[49], and Andreasen[46] observed that ankylosis will occur if an area of periodontal injury is repopulated from a source outside the ligament. In addition, Line demonstrated using labelled cells that bone marrow spaces are the major repopulating sites following periodontal injury. Similarly, Line also noticed a transient ankylosis in the PDL. Also Andreasen[46] noted that collagen formation in the PDL preceded cementum formation. This may have important implications in determining whether cementum repair is secondary or at least dependent upon a functional mature collagen fiber attachment. Previously Andreasen[50], 1973, corroborated these findings in humans by stating that the primary function of cementum was to anchor periodontal fibers. Also, Nasjletti[47], using monkeys, confirmed that a functional fiber attachment to tooth preceded cementum formation. Furthermore they observed cementoblastic formation along the tooth surface after a period of twenty-eight days. In any case the whole process of cementoblastic differentiation seemed to occur at a much slower rate than osteoblastic differentiation. The reason for this delay is not fully understood at the present time.

It is now accepted that periodontal ligament fibers will reorganize following removal from the tooth surface. But it is unknown whether the pre-existing collagen fibers are depolymerized to subunits and then re-aggregate into new fibers, or whether the existing

fibroblasts secrete new collagen sub-units referred to as tropocollagen. Through "in vitro" studies, Gross[51] explained that it is possible for these tropocollagen units secreted by the fibroblast to aggregate into a mature collagen fiber with its characteristic periodicity.

Niinikoski[52], 1977, has demonstrated that increasing the oxygen tension in the injury area will enhance the healing process. This was accomplished by hydroxylation of proline groups and increasing the cross-linking. However it is still incompletely understood. It was suggested by Staffileno et al[53], 1962, that collagen fibers were initially parallel, but later were oriented perpendicular to the tooth surface in the PDL. The reason for this phenomenon is unknown. It has been suggested that perhaps regeneration of the free gingival cuff is due to an upgrowth of connective tissue synthesized at the base of the wound[7].

Connective tissue organization and maturation is still incompletely understood. Stahl[12] has suggested that anywhere from ten to thirty days is required for connective tissue maturation in humans. However Dragoo[56], 1976, noticed that maturation of periodontal ligament fibers in humans required anywhere up to eight months. Peacock[55] stated that maturation involved a more firm cross-linking between collagen molecules.

Regeneration of gingival architecture is another phenomenon that has remained a mystery. Goldman and Cohen[57] have suggested that perhaps a process referred to as "collagenation" may be involved. This implied that some sort of underlying genetic stimuli may be present. Also Melcher[9] conjectured that perhaps various environmental forces may regulate the differentiation and orientation of

connective tissue fibers in the gingiva. But it remains for future research to resolve the various factors involved in promoting gingival regeneration.

MATERIALS AND METHODS

Fifteen patients, previously selected for orthodontic treatment, consented to participate in this study. Their ages ranged from eleven to fifteen years and all were apparently in good health. As a necessary part of treatment, all required extraction of the maxillary first premolars. The surgical technique for circumferential supracrestal fiberotomy (C.S.F.) was explained along with its possible complications. In addition, to simulate actual clinical conditions, oral hygiene was not discussed at any time.

The surgical procedure was modelled after Dragoo and Sullivan[56] and Kohler[43] and provided the basis for studying healing characteristics. These investigators demonstrated the removal of a minimal block section of buccal bone and tooth in the maxillary premolar region (Figures 1 and 2). The resulting defect was virtually healed after a two month post-surgical time period.

The maxillary first premolars were utilized for the circumferential supracrestal fibrotomy procedure. Edwards[6] described the C.S.F. technique in some detail. He suggested using a #11 Bard Parker scalpel directed apically within the gingival sulcus to the area of the PDL just below the alveolar crest. This incision was then continued in this manner until the supracrestal fibers of the complete tooth were severed.

At the initial visit one cc. of lidocaine 1-100,000 was administered locally. Subsequently the operator carried out the C.S.F. on

the specified tooth. In contrast to what Edwards advocated, the C.S.F. was confined to the labial surface only. Following the procedure the patient was dismissed without any unusual post-operative instructions. At a later pre-determined day the same procedure was performed on the contralateral premolar. After a specified healing time, both maxillary first premolars were then extracted concurrently (Table I).

Following administration of local anesthetic, vertical parallel incisions were made extending 5 mm apically from the gingival margin. These incisions were placed mesial and distal to the tooth on the buccal surface. A connecting horizontal incision completed the section. In order to remove the soft tissue and alveolar bone intact, a mallet, chisel and high speed fissure bur were used. The tooth was then elevated from the palate side and the resulting defect was finally closed with interrupted 4-0 B.S.S. Post-operative medication consisted of tylenol. However very little discomfort was reported following the procedure (Figures 1,2, and 3). Representative photographs of the surgical site were taken at regular intervals post-surgically.

Initially the above outlined C.S.F. technique was done on the first ten cases. However a problem arose with the specimens taken from the shorter healing times. The soft tissue and bone became detached from the tooth during its removal. Thus a change in the C.S.F. technique was required. This was accomplished by using a dental fissure bur to demarcate the C.S.F. area on the tooth. A vertical line, approximately 2 mm wide mesio-distally, was placed on the buccal surface of the enamel (Figure 1) just occlusal to the gingival margin on the specified tooth. Local anesthetic was administered and a

modified Orban knife was used. The vertical line established the exact area where the scalpel was inserted apically. A fibrotomy encompassing the entire buccal surface was avoided to include only a defined area dictated by the vertical line on the enamel. Since the C.S.F was confined to a minimal area, the chance for detachment was markedly reduced during the surgical removal of the tooth, soft tissue and bone.

Subsequently the specimens were fixed in 4% buffered glutaraldehyde for approximately twenty-four hours. This necessary variation allowed the use of same specimen for both the light and electron microscopy part of the study. During fixation a vertical incision was made on the soft tissue section continuous with the vertical line on the enamel. This was required in order to delineate the C.S.F. area for subsequent sectioning after decalcification. As an aid in reducing decalcification time, the tooth was sectioned in half mesiodistally. Also the major portion of the root was removed excluding the experimental area.

Following fixation, the specimens were decalcified in EDTA for a period of approximately six months. During this time radiographs taken regularly were used as an indicator of the termination of decalcification. With its completion, seven micron sections were removed bucco-lingually from the surrounding area defined by the incision line on the soft tissue (Figures 4 and 5). Slides were prepared using the standard procedure for the various stains. These included hematoxylin and eosin, Mallory's connective tissue stain, Verhoeff elastic fiber stain and Van Gieson connective tissue stain, Wilder's reticulin stain, and Aldehyde-fuschin oxytalin fiber stain.

Subsequent to decalcification, the light microscopy sections were placed in 10% formalin for ten days. This markedly improved the visualization of several stains, including hematoxylin and eosin.

Slides were then examined by two investigators on a double blind selection basis.

FINDINGS

A method of categorizing the prepared sections was devised. Each section was divided approximately in half by an imaginary line running bucco-lingually through the alveolar crest (Figure 6). For example, "Region A" encompassed the area occlusal to this line, while "Region B" encompassed the area apical to this line. Thus a more comprehensive understanding of the sections was then possible.

I. Day 1 H & E

Region A - A major observation at one day was a discontinuous or severed crevicular epithelium. The spinous layer, displaying intensely basophilic epithelial cells, occupied the area of the wound edge within the sulcus. No epithelial remnant appeared to be attached on the tooth side. In other words the specimen was devoid of any crevicular epithelim united to the tooth. The separation between tooth and wound edge was filled with a large hematogenous clot which extended from the gingival sulcus coronally, to the area of the PDL proper apically, near the alveolar crest. Lingually the clot was attached to the Sharpey's fibers, while labially it encompassed the gingival sulcus and entire crevicular connective tissue. Thus the direction of the C.S.F. incision was somewhat defined. Fibrin, RBCs, round cells and some PMNs were the major composition of the clot. In addition no definitive organization of the clot was present as evidenced by an absence of any vascularization.

Connective tissue fiber destruction was extensive, involving primarily the alveolodental and dentogingival collagen fibers.

Furthermore this destruction was defined by the clot and its associated inflammatory cells.

Sharpey's fibers appeared to be absent around the CEJ. However this may have been due to a sectioning artifact (Figure 7).

Region B - The alveolar crest showed a dense eosinophilic staining reaction. Round cell infiltrate extended into the PDL in close proximity to the alveolar crest. Also within the PDL a large area of possible swelling occurred apical to the round cell infiltrate resulting in a generalized loss of cellular elements and dense eosinophilic staining connective tissue fibers.

The alveolar bone appeared normal with minimal periosteal activity. Also the connective tissue fibers on the labial side of the alveolar bone appeared to be mature with no significant difference from the control sections.

No unusual findings were associated with the cementum in these sections (Figure 7).

Mallory's Stain - This stain further corroborates the extent of the inflammation. A circumscribed area of collagen fiber destruction extended from the sulcus coronally, to the PDL proper apically. Again, the alveodental, dentogingival and horizontal fibers were primarily affected. In contrast to the H & E section, Sharpey's fibers remained attached to the entire cementum in the specimen, including the area of the CEJ.

Verhoeff and Van Gieson - No significant differences were noted between this stain and Mallory's Stain.

II. Day 14 H & E

Region A - A major difference was noted between one day and

fourteen days healing. At fourteen days the crevicular epithelium was attached to the tooth at the CEJ and appeared histologically similar to the control section. In other words the crevicular epithelium was completely intact. However these findings did not reveal whether the epithelial attachment reunited to any extent on the enamel, since the area was decalcified for sectioning.

Connective tissue reorganization was extensive. In addition a small circumscribed area of chronic infiltrate (round cells) existed occlusal to the alveolar crest. The alveolodental and dentogingival collagen fibers had an established pattern but did not display as dense eosinophilic staining as the control sections. However no attempt at reorganization was noted within the area of circumscribed round cell infiltrate.

Variable findings existed within the cementum. One specimen displayed definite cemental resorption with cementoclasts present. The resorption covered the majority of the cementum in "Region A", with the exception of the area at the CEJ. The remaining sections, on the other hand, demonstrated an absence of cemental resorption (Figures 8 & 9).

Region B - The alveolar bone showed a marked scalloped appearance on the labial surface near the alveolar crest, thus suggesting possible osteoclastic resorption with Howship's lacunae present. This localized resorption may have indicated the direction of the C.S.F. incision. Round cell infiltrate was in close association to these areas of resorption. Furthermore no unusual cellular activity occurred within the PDL proper.

The cementum appeared normal. The only exception was a single specimen that demonstrated a possible area of artifactual cemental resorption due to pressure necrosis from a separated bony sequestra

associated with the alveolar crest.

Mallory's Stain - Small areas of collagen fiber destruction were observed as in the H & E sections. A semblance of connective tissue reorganization was intermingled within the location of the round cell infiltrate, while elsewhere, an established pattern was present but with a lighter staining reaction.

III. Day 21 H & E

Region A - A significant finding at twenty-one days appeared to be the presence of numerous foci of cemental resorption with associated cementoclasts.

In contrast to the early healing time, distinct, well-oriented connective tissue fiber patterns were present and resembled the control section. However it was difficult to discern any connective tissue maturation except on a gross scale, i.e., lighter vs darker eosinophilic staining being associated with immature and mature connective tissue fibers respectively (Figure 10).

Region B - Osteoclastic activity was the predominant feature within the alveolar bone in this section. Specifically, this osteoclastic activity was mainly centered on the tooth side of the alveolar bone.

Generally no unusual findings were noted within the cementum of the PDL. However one specimen did display a single focus of cemental resorption (Figure 10).

IV. Day 28 H & E

Region A - The specimens appeared to histologically resemble the control section (Figures 11 & 12).

Region B - Osteoclastic activity was associated with the PDL side of the alveolar bone (Figure 16). However considerable variation existed

between several specimens. Some revealed numerous foci of cemental resorption within the PDL while others were totally devoid of any cemental resoption (Figure 11).

V. Day 29 - Day 121 H & E

Region A - The specimen appeared to histologically resemble the control section with no significant differences (Figures 13,14, and 15).

Region B - Cemental resorption was a characteristic finding in the PDL up to period of ninety days, but was absent subsequently. However the presence of minimal osteoclastic activity was a universal finding. Even the control sample showed little osteoclastic activity within the PDL (Figures 13, 14, and 15).

DISCUSSION

Numerous investigators have suggested that the healing response occurs characteristically with time. In essence it is an oversimplification to categorize various stages of healing in a step by step manner. Other factors such as age, sex, and nutrition may be involved in coordinating the complete healing phase. However, as mentioned previously, their contribution is still not completely understood. Consequently it was not the intention of this study to consider all the factors, but rather to accept the true clinical situation and provide any benefit that may be derived from the present findings.

In some respects this study should have corroborated the major findings of previous studies concerning the basic inflammatory response[8,20]. The fundamental problem was one of attempting to compare and relate determined healing responses following a C.S.F. to known healing responses.

Generally a number of characteristic features were interpreted from the selected specimens. At first glance chronic inflammation (round cell infiltrate) appeared to have a predilection for the connective tissue lining the gingival sulcus. Furthermore the quantity of these cells appeared to vary considerably. Oral hygiene, namely plaque level, has been implicated as a possible determining factor. However, a certain number of chronic inflammatory cells were present in all cases. In effect, this may possibly have suggested that other factors in addition oral hygiene contributed to inflammation in crevicular connective tissue.

Melcher[7], a prolific worker in the field, has implicitly stated that the rate of oral healing exceeded that of generalized skin healing. Interestingly the major inflammatory response in primary gingival healing appeared to span a period of approximately twenty-one days following the C.S.F. Any subsequent changes were probably important only to the complete maturation of the tissue. In this respect observed stages of maturation greater than twenty-one days were minimal as compared to the early healing response. One criterion for a generalized maturation may be the amount of eosinophilic staining of the connective tissue fibers. The more aggregated these fibers are, the more dense and mature-like the specimen. Consequently our findings were only able to delineate visible maturation on whether or not the connective tissue fibers accepted the appropriate stains in the prepared sections. In contrast, Dragoo and Sullivan[56], using light microscopy, identified a maturation of connective tissue fibers spanning four to six months following gingival surgery. This discrepancy may have been due to a number of reasons. Firstly Dragoo and Sullivan selected older individuals with existing periodontal disease. The contrasting ages between the two studies may have contributed to a wider variation in the healing response. However the major reason probably involved the various criteria assigned to describe maturation. In this study, basically short term effects of healing were observed. Once the major stages of regeneration or repair of epithelium and connective tissue were completed, then the section was accepted as being mature. In contrast, Dragoo and Sullivan observed long term effects resulting in an entirely different picture. Although minimal changes occurred, their findings appeared to establish a progression of maturation over a prolonged experimental period (8 months) as increased

or denser connective tissue fiber patterns.

As previously stated there was no definite division between the acute and chronic phases. However, one must be able to identify a certain time in the progression of healing. This usually involved the presence of certain cell types with various healing times. For example, Figure 7 demonstrated PMNs intermingled within an extensive clot containing mainly fibrin and RBCs. The very presence of PMNs suggested a very early inflammatory response, i.e., twenty-four to forty-eight hours. Associated with these PMNs was the initiation of massive collagen fiber destruction as demonstrated in the present findings. The alveolodental and free gingival fibers were primarily involved. In addition destruction extended locally from the site of the initial incision to encompass a considerable area of surrounding connective tissue. As healing progressed, a round cell infiltrate usually dominated after twenty-four to forty-eight hours[11]. Subsequent regeneration of the epithelium and connective tissue then followed.

Ramfjord[10] and Stahl[13] suggested that epithelialization is initiated early in healing in order to provide a barrier to any bacterial invasion. The mechanism of its regeneration is still incompletely understood. However, numerous theories have been proposed to explain this phenomenon[9,30]. Whatever the mechanism, Stahl suggested that gingival epithelization was routinely completed within the first seven to fourteen days following the injury. The present findings also confirmed an intact epithelium by fourteen days. However as Table I showed, the present findings lacked suitable specimens of short healing times, especially between one and fourteen days. Undoubtedly this limited our ability to obtain a comprehensive picture of the entire sequence of events during

the early healing response. In any case the recent literature on gingival healing readily demonstrated the early healing stages as most dramatic. Therefore further investigation during this time interval may prove fruitful.

Considerable controversy exists over possible periodontal complications following a C.S.F. "Does this procedure initiate the beginning stages of periodontal disease, i.e., loss of attached gingiva or deepening sulcular depth?" Our findings were inconclusive in that regard. However they appeared to indicate a fibrous reattachment of the sulcular epithelium to the CEJ in the premolar area. This indicated the apical extent of the epithelial attachment. But the fate of the actual epithelial attachment on the enamel was unestablished due to the preparation of decalcified specimens. Perhaps future studies with frozen ground sections may provide the answer. In any case, Listgarten[15], using electron microscopy, provided reassurance that the epithelial attachment did in fact reattach and form an organic union with the tooth surface. However no mention was made of the extent of loss of the attachment, or whether in fact there was any attachment on the enamel at all. Since our findings demonstrated a fibrous reattachment of sulcular epithelium to the CEJ, it seemed likely, in the light of the current literature, to suspect some form of epithelial attachment was possible. Conversely, it may be just as true that the C.S.F. hastened the "passive eruption" of the tooth.

One might have considered how the sulcular epithelium reattached to the area of the CEJ. A determining factor may have been the initial clot. Besides being attached to the CEJ, it also probably acted as a framework or pathway for epithelialization. According to Taylor and Campbell[35], once the sulcular epithelium encountered the cementum it appeared to have

a predilection to migrate occlusally. Shapey's fibers extending along the whole length of the cementum plus the accompanying clot may have contributed to this phenomenon. Furthermore Taylor and Campbell expressed the idea of reattachment to cementum only through the basal cell layer. In this respect these epithelial cells were able to migrate occlusally along the tooth surface in accordance with normal turnover rates. Despite the concept of "healing by scar" the reformed fibrous epithelial attachment appeared to histologically resemble the control section.

"What are the possible complications following a C.S.F. in other areas of the oral cavity?" It was conceivable that variable findings may have existed in other areas of the mouth, especially the lower anteriors where crowding and rotations are a consistent finding in most malocclusions. It is an accepted clinical fact that one must be cognizant of likely periodontal problems with the lower anteriors, especially when considering orthodontic or periodontal treatment. Minimal labial attached gingiva and alveolar bone may be jeopardized if a C.S.F. was performed routinely in the lower anteriors. Therefore one's clinical judgement should be used to assess each situation individually. In contrast, the premolar area may demonstrate a different situation. For example it is known that there is usually sufficient labial alveolar bone and attached gingiva surrounding the tooth in this area. These factors may contribute to more favorable conditions for more ideal attachment.

Interestingly, long term healing (121 days) manifested no detectable histological change from the original fibrous reattachment. Therefore provided conditions are favorable there appears to be an excellent chance that following C.S.F. a fibrous reattachment will occur at the CEJ and

remain unaltered with time.

As stated previously, a collagen fiber regeneration occurred subsequent to epithelialization. By twenty-four to forty-eight hours post-injury, it was known that the round cells were predominant. Interestingly in this study the small sample size created considerable variation in healing times and thus any observed discrepancy from known responses. However collagen fiber destruction invariably involved adjacent areas to the injury. Figure 7 demonstrated a connective tissue involvement of the alveolodental fibers. This collagenolysis was accompanied by the presence of macrophages or monocytes. The current literature stated that collagen fiber regeneration occurred within a span of thirty days. Figure 8 showed a major attempt at alveolodental fiber regeneration by fourteen days. Also these fibers appeared to be functionally oriented within an environment established by the tissue. Although a functional environment may partially dictate the specific direction of the collagen fibers, it is conceivable that genetic factors play an equally significant role.

Since the C.S.F. was carried out in close proximity to the cementum, scavenger cells of the inflammatory process (lymphocytes, macrophages, etc.) became involved with the possible removal and reorganization of Sharpey's fibers. Also a humorally mediated response may have initiated cementoclastic resorption. Figures 9 and 10 exhibited numerous foci of cemental resorption. In the majority of cases this finding appeared to be exclusive to the area extending from the CEJ to just below the alveolar crest in the PDL proper. Generally the observed range of resorption was assessed from mild to moderate depending on the extent of inflammation from the induced injury. Some question still remained over whether or not the observed cemental resorption was the result of iatrogenic

or inflammatory causes. This idea was quickly dismissed as most of the specimens with resorption were associated with cementoclasts or dentinoclasts. Cemental resorption was noticed as early as fourteen days and persisted to approximately ninety days. Furthermore previous studies explained an active cemental resorption as part of the inflammatory response in gingival healing. However one should consider the fate of Sharpey's fibers during cemental resorption. Andreasen[46] has suggested a possible explanation. He pointed out that the collagen fibers in the PDL regenerated prior to cementum formation and cementoblasts. Interestingly it was noteworthy that cemental resorption did not affect the CEJ. Perhaps it was resistant to any significant visible resorption by some form of interaction with its associated crevicular epithelial cells.

Melcher has suggested a possible interaction between cells of the PDL. This statement probably has considerable credibility since in the normal situation there may conceivably be an established equilibrium between all the cell types in the PDL. Although not new, this concept calls into play a progenitor pool. During times of stress any number of cells may be called upon to play very distinct roles. In the case of the alveolar bone, it was noted that osteoclastic resorption was a consistent finding from fourteen days to one hundred twenty-one days. Generally this resorption was found in close association to the ambient inflammation as evidenced by the scalloped appearance and osteoclasts in the bone. However as healing progressed, the extent of osteoclastic resorption was considerably reduced but never appeared to be nonexistent. The dynamic nature of bone suggested the possibility of continual remodeling.

The genuine efficacy of the C.S.F. procedure may have been

enhanced somewhat by this study, but it remains for future research to elicit many unanswered questions concerning gingival healing.

SUMMARY AND CONCLUSIONS

A circumferential supracrestal fibrotomy was performed on the maxillary first premolars of fifteen patients previously selected for orthodontic treatment. Various healing times extending from one to one hundred twenty-one days provided a broad spectrum for intensive study of the major healing period. The premolars were then extracted and prepared with the usual stains for light microscopy.

Based on the present findings, the following conclusions were then made:

1. The observed healing response appeared to occur characteristically with time.
2. Generally the healing response corroborated that of previous studies on gingival healing, although considerable variation occurred with certain healing responses.
3. Primary gingival healing was very rapid, completing its major repair or regeneration by twenty-one to twenty-eight days.
4. Variable round cell infiltrate in crevicular connective tissue was a universal finding.
5. A fibrous epithelial attachment reattached to the cemento-enamel junction in the maxillary premolars and was not altered with time. However no conclusions were formed on the fate of a cohesive epithelial attachment on the enamel.
6. Healing times greater than twenty-eight days did not reveal extensive maturation, especially of connective tissue fibers.

7. Long term connective tissue reorganization following the circumferential fibrotomy appeared to resemble the control sections.

8. Bone was confirmed to be dynamic in nature.

More extensive study of earlier healing times may improve the validity of a future study of this nature.

Finally the long term effects of this procedure should be evaluated by periodontal assessment of completed cases at approximately fifteen years. This assessment, along with an evaluation of tooth position would be beneficial to clinical science.

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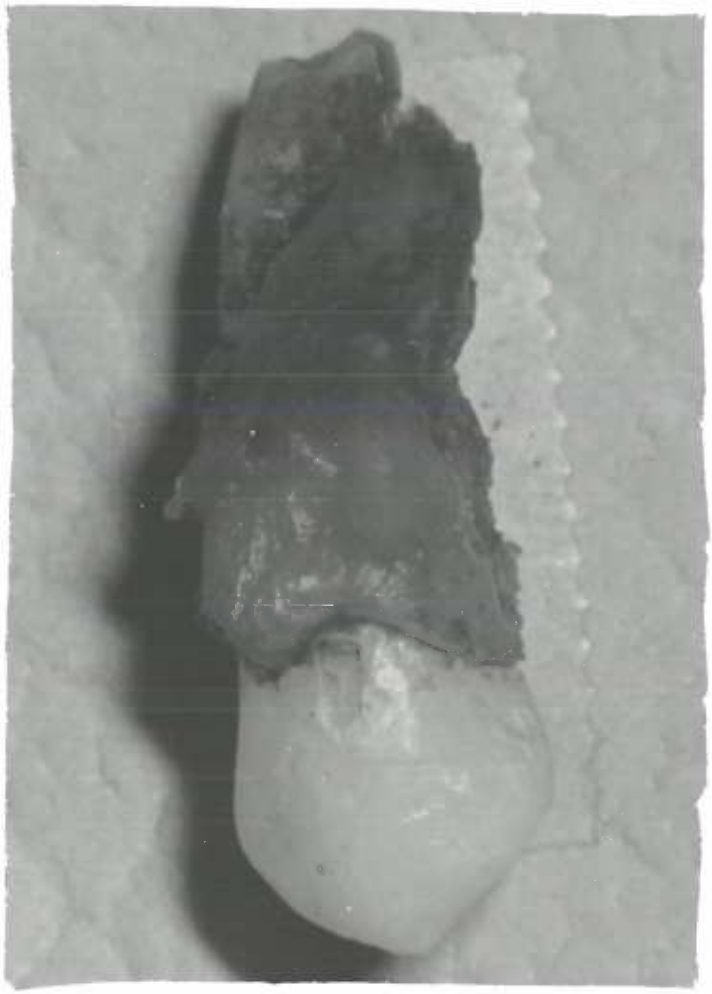

Figure 1 Antero-posterior view of the experimental tooth showing block section of soft tissue and vertical groove on enamel.

Figure 2 An antero-posterior view of experimental tooth.

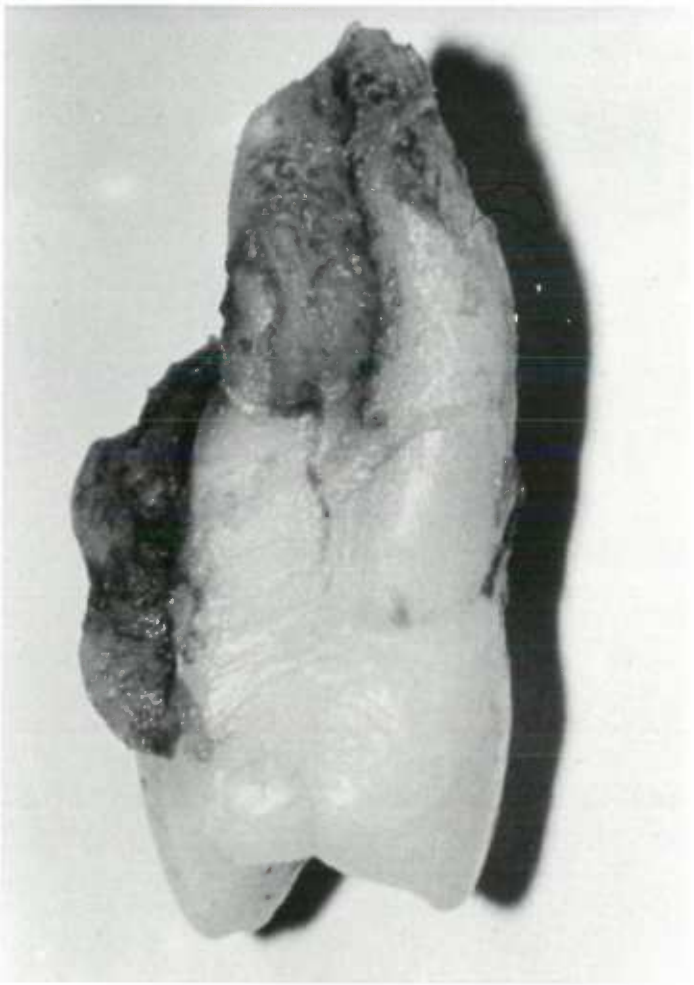

Figure 3 A frontal view of experimental tooth.

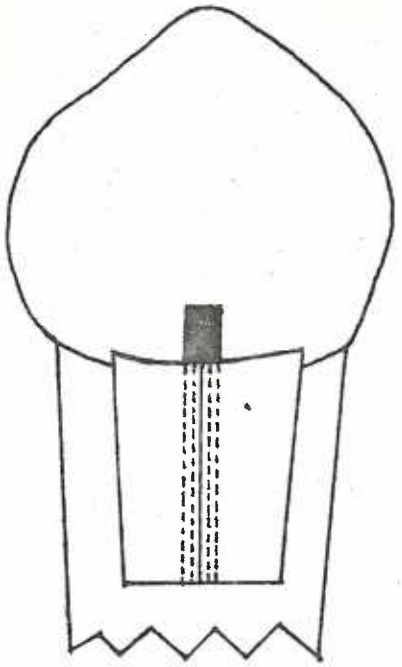

Figure 4 Diagrammatic representation of the soft tissue and tooth section with a vertical reference groove on the enamel. The dark line in the middle of the soft tissue section represents the reference line for sectioning. The dotted lines represent the area of the light microscopy sections.

Figure 5 Hematoxylin and eosin stain showing a frontal overview of the area of study. Scale equals 550μ.

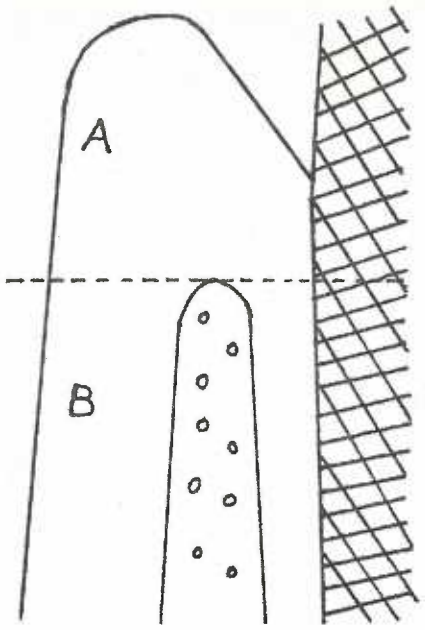

Figure 6 Diagrammatic representation of categorized microscopic section showing "Regions A & B" in a frontal view of the sections.

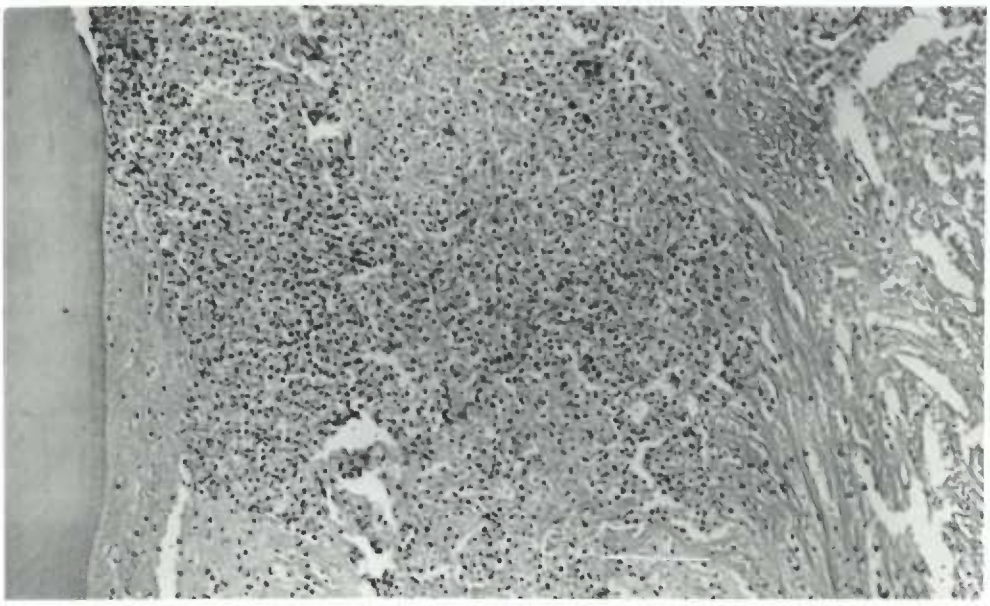

Figure 7 Hematoxylin and eosin at one day showing chronic infiltrate and extensive collagen fiber destruction. Scale equals 100μ.

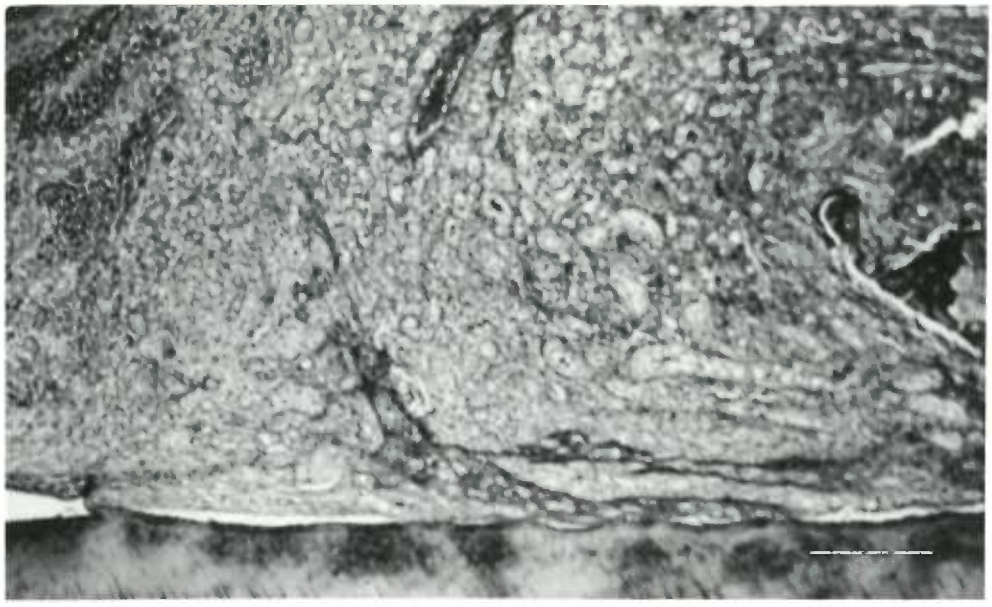

Figure 8 Mallory's stain at fourteen days showing some semblence of collagen fiber regeneration. Scale equals 200μ.

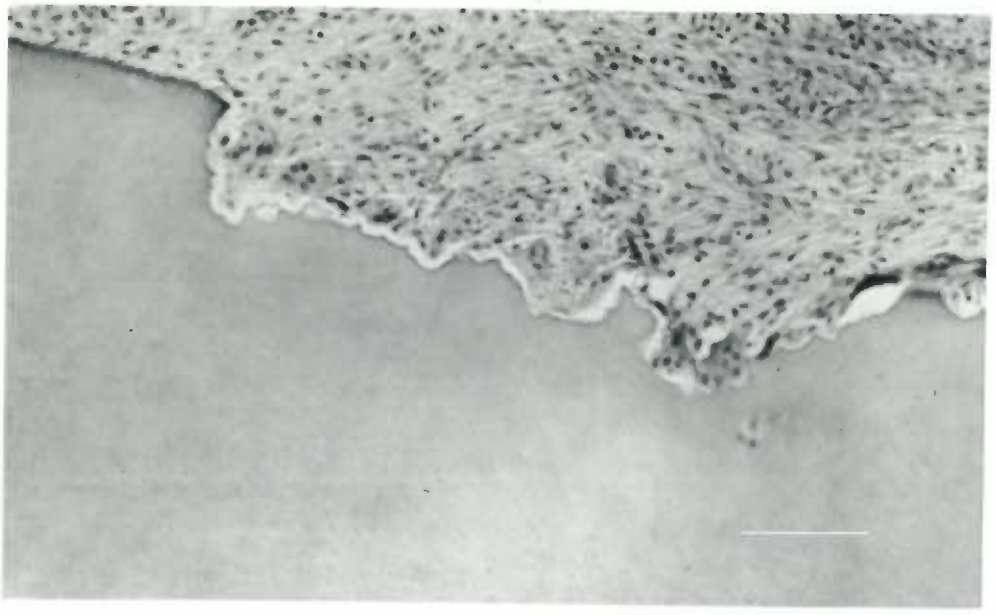

Figure 9 Hematoxylin and eosin stain of "Region A" at fourteen days showing extensive cemental resorption with cementoclasts present. Scale equals 100μ.

Figure 10 Hematoxylin and eosin stain of "Region A" at twenty-one days showing numerous foci of cemental resorption. Also note circumscribed area of chronic infiltrate. Scale equals 150μ.

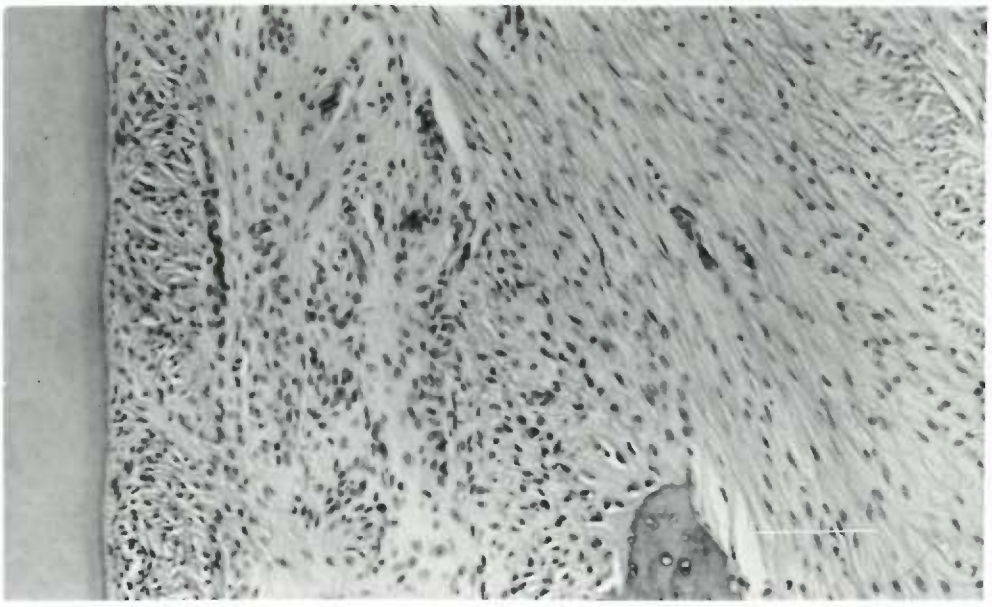

Figure 11 Hematoxylin and eosin stain at twenty-eight days resembles the control section. Scale equals 100μ.

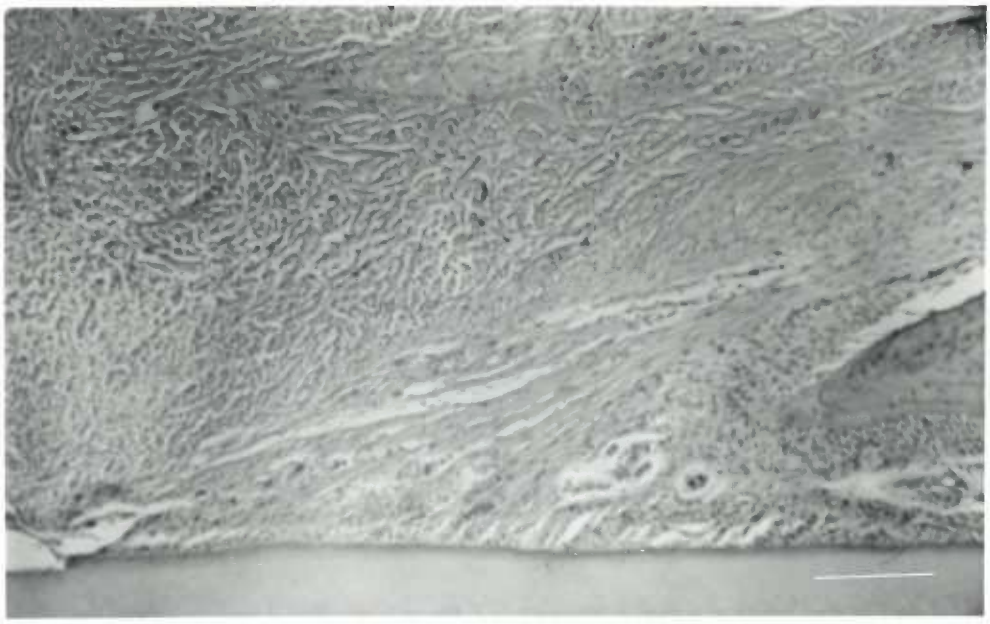

Figure 12 Hematoxylin and eosin stain of the control sample. Scale equals 200μ.

Figure 13 Hematoxylin and eosin stain at ninety days. Specimen appears to resemble the control. Scale equals 200μ.

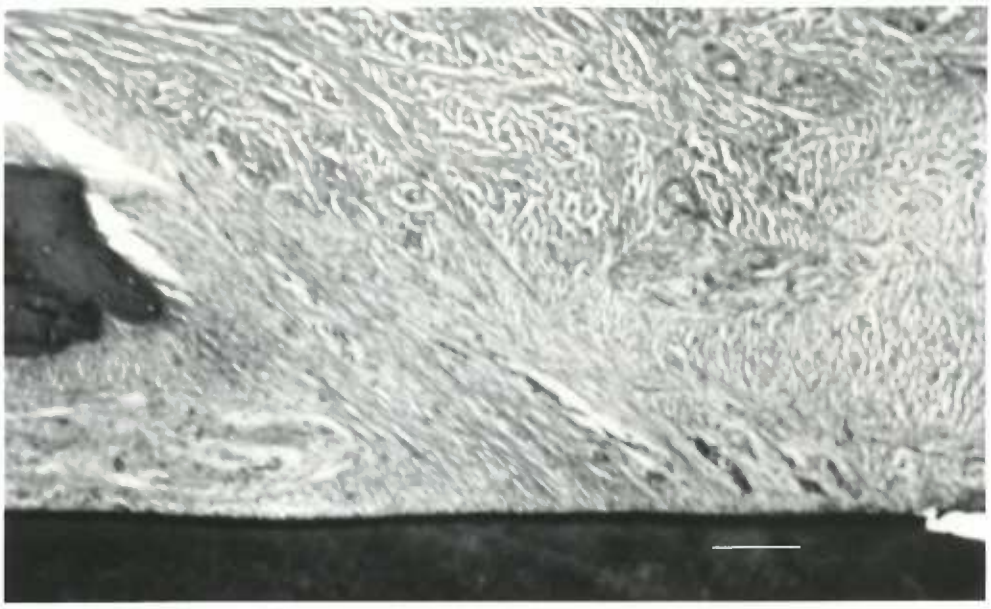

Figure 14 Verhoeff's connective tissue stain at 120 days. Scale equals 150μ.

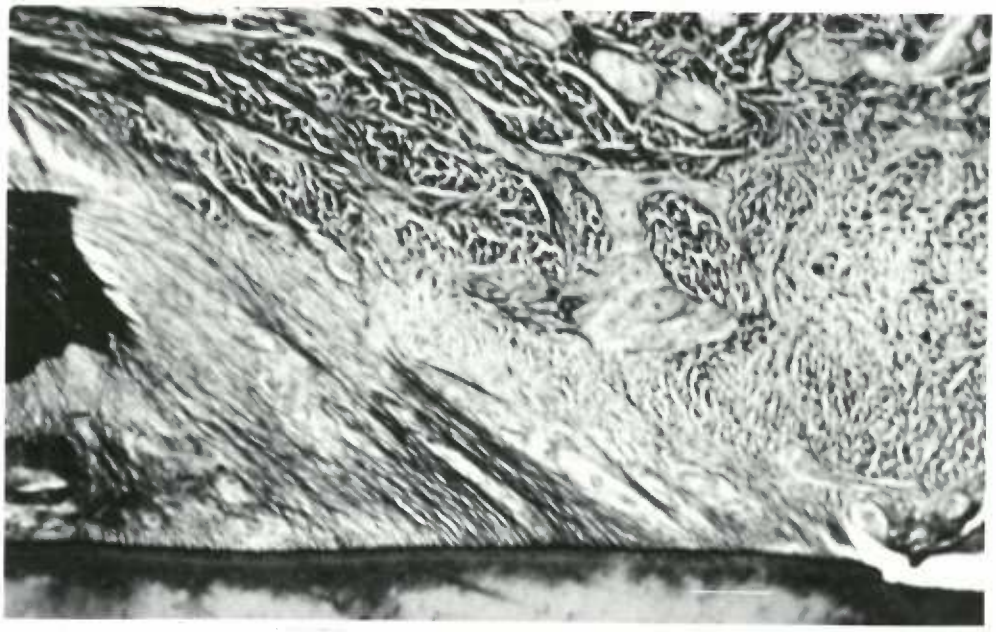

Figure 15 Mallory's stain at 120 days. Scale equals 150μ.

TABLE I Table showing various healing periods subsequent to the C.S.F. procedure performed on maxillary first premolars of fifteen patients.

Case #	Healing Time After C.S.F. (days)	
	Maxillary Right First Premolar	Maxillary Left First Premolar
1	Control	121
2	Control	121
3	90	56 *
4	90	56 *
5	28	14
6	14	28
7	7 *	28
8	21	7 *
9	1	4 *
10	1 *	4 *
11	56	No Specimen
12	4	1
13	7	4
14	4	31
15	11	7

* Unable to use specimen since block section of soft tissue and bone became detached from the tooth during its extraction.

APPENDIX

Slide Identification	Healing Time (days)	Patient Sample
E X & E Z	1 day	Sandra Lowe
D A X & D A Z	14 days	Terri LeGore
16 A X & 16 A W	14 days	Elizabeth Hateli
B A X & B A Z	21 days	Tammy Jo May
02 A X & 02 A W	28 days	Roy Neal
18 B Z & 18 B X	28 days	Elizabeth Hateli
C A X & C A Z	28 days	Terri LeGore
K B X & K B Z	56 days	Tammy Taylor
M X & M Z	56 days	Marcia Fergison
L B X & L B Z	90 days	Tammy Taylor
I B X & I B Z	90 days	Amy Dinkler
2 A Z & 2 A W	120 days	James Betts
6 A X & 6 A Z	120 days	Lisa Hansen
4 A Z & 4 A W	Control	James Betts
8 B X & 8 B Z	Control	Lisa Hansen