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Wound Healing in the Tissues of the Periodontium following Periradicular Surgery. II. The Dissectional Wound

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Wound healing responses of the tissues of the periodontium following periradicular surgery in rhesus monkeys were evaluated by light microscopy. Part II of this investigation reports the responses of mucoperiosteal and osseous tissues to blunt dissectional wounding resulting from the reflection of triangular or submarginal rectangular flaps.

Healing of the dissectional wound is rapid, although slower than the incisional wound. Granulation tissues replaces the fibrin clot in the wound site as early as 4 days after surgery, and is replaced by fibrous connective tissue by 14 days. Minimal differences were found in the temporal and qualitative dissectional wound-healing responses to the two types of flap designs. The periosteum does not survive the flap reflection procedure. The cells of the cambium layer are destroyed and the collagen of the fibrous layer undergoes depolymerization. It is postulated that the depolymerized periosteal collagen plays a role in rapid reattachment of flapped tissues to cortical bone.

Periradicular surgery involves three basic types of surgical wounding: incisional, dissectional, and excisional wounding. A previous report (1) detailed the wound-healing responses of the mucoperiosteal tissues of the periodontium to *incisional* wounding in periradicular surgery. *Dissectional* wounding results from the elevation of a mucoperiosteal flap from cortical bone during flap reflection. The blunt dissectional wound is created by insertion of a periosteal elevator between cortical bone and overlying mucoperiosteal tissues, and separation of the soft tissues from the cortical surface to provide surgical access to periradicular tissues. The dissectional wound involves surgical trauma to the mucoperiosteal tissues and cortical bone. The purpose of Part II of this report is to describe the wound-healing responses of the mucoperiosteal tissues and cortical bone to dissectional wounding at postsurgical periods of 1 through 4, 14, and 28 days.

MATERIALS AND METHODS

Four mature rhesus monkeys (*Macaca mulatta*) served as research models for this investigation. Surgical procedures were scheduled to provide six mucoperiosteal flaps in each animal for a total of 24 experimental flaps. Two flap designs were used: a triangular flap with an intrasulcular incision and a single vertical releasing incision, and a submarginal rectangular (Ochsenbein-Luebke) flap with a horizontal incision in attached gingiva and vertical releasing incisions at each periphery of the horizontal incision. Surgical sessions with animals under general anesthesia, supplemented by infiltration injection of 2% liodcaine with 1:50,000 epinephrine, provided two flaps of each design at postsurgical periods of 1 through 4, 14, and 28 days.

Flaps were reflected for 15 min with frequent irrigation of the tissues with sterile, physiological saline, then reapproximated. Wounds were closed and stabilized with interrupted plain gut sutures. Tissues were compressed for 3 min by firm finger pressure on saline-soaked gauze placed over the surgical site.

Details of the preparation of experimental material for light microscopic examination were presented in Part I of this report (1). Evaluation of the responses to dissectional wounding was based on anticipated biological events as extrapolated from wound-healing studies of both oral and nonoral tissues (1–5). These events included clotting and inflammation, and connective tissue healing. Specific biological events were graded using the following criteria.

CLOTTING AND INFLAMMATION

Clot Formation

Clot formation was graded as follows: (#) absent = no fibrin clot, coagulum present; (+) evident = fibrin clot formed, areas of coagulum; (++) very evident = fibrin clot occupies wound site; and (##) event completed = clot partially or totally replaced by reparative tissue.

Polymorphonuclear Leukocytes

Polymorphonuclear leukocytes (PMN) were graded: (#) absent = no PMN infiltrate in wound site; (+) evident =

PMN infiltrate evident in wound site; (++) very evident = heavy PMN infiltrate, PMN clearly the predominant inflammatory cell type; and (##) event completed = PMN infiltrate no longer present, scattered PMN in wound site.

Macrophages

Macrophages (MP) were graded: (#) absent = no MP infiltrate in wound site; (+) evident = MP infiltrate evident in wound site; (++) very evident = heavy MP infiltrate, MP clearly the predominant inflammatory cell type; and (##) event completed = MP greatly reduced in number, MP no longer the predominant cell type in wound site.

CONNECTIVE TISSUE HEALING

Fibroblast Migration

Fibroblast migration was graded: (#) absent = no fibroblast migration into wound site; (+) evident = fibroblasts evident in wound site; (++) very evident = fibroblasts and fibroblast-like cells most predominant reparative cells in wound site; and (##) event competed = reduced number of fibroblasts in wound site, no evidence of continued migration to wound site.

Collagen Synthesis

Collagen synthesis was graded: (#) absent = no collagen fibers in wound site; (+) evident = type III collagen fibers present in wound site; (++) very evident = type I and III collagen fibers present in wound site; and (##) event completed = remodeling and maturation of collagen in wound site.

Granulation Tissue Formation

Granulation tissue formation was graded: (#) absent = no granulation tissue in wound site; (+) evident = granulation tissue apparent in wound site; (++) very evident = granulation tissue apparent throughout wound site; and (##) event completed = granulation tissue maturing or replaced by fibrous connective tissue.

Fibrous Connective Tissue Formation

Fibrous connective tissue formation was graded: (#) absent = no fibrous connective tissue in wound site; (+) evident = early maturation of granulation tissue into fibrous connective tissue in areas of the wound site; (++) very evident = maturation of granulation tissue into fibrous connective tissue throughout wound site; and (##) event completed = fibrous connective tissue in wound site undergoing maturation and remodeling.

OTHER OBSERVATIONS

Cortical Resorption (Osteoclastic Activity)

Cortical resorption was graded: (#) absent = no osteoclastic activity on cortical surface; (+) evident = osteoclastic activity in isolated areas on cortical surface; (++) very evident = osteoclastic activity along entire cortical surface; and (##) event completed = osteoclastic activity replaced by osteoblastic activity, no evidence of continued osteoclastic activity.

Cortical Repair (Osteoblastic Activity)

Cortical repair was graded: (#) absent = no osteoblastic activity on cortical surface; (+) evident = osteoblastic activity in isolated areas on cortical surface; (++) very evident = evidence of osteoblastic activity along entire cortical surface; and (##) event completed = no evidence of continued osteoblastic activity.

Cortical Necrosis

Cortical necrosis was graded: (#) absent = no necrosis of cortical surface lamellae resulting from dissectional wounding, vital osteocytes in lacunae; (+) evident = necrosis of cortical surface lamellae in areas devoid of cortical-retained periosteal tissues, empty lacunae; (++) very evident = cortical surface lamellae necrosis throughout dissectional wound site, including areas with cortical-retained periosteal tissues; and (##) event completed = necrotic cortical surface lamellae replaced by osteoblastic activity.

Cortical-Retained Periosteum

Cortical-retained periosteum was graded: (#) absent = no periosteal tissues retained on the cortical surface after dissectional wounding; (+) evident = isolated areas of periosteal tissues retained on the cortical surface following dissectional wounding; (++) very evident = periosteal tissues retained on the entire cortical surface exposed during dissectional wounding; and (##) event completed = cortical-retained periosteal tissues incorporated into connective tissue reattachment to cortical surface.

Reflected Periosteum

Reflected periosteum was graded: (#) absent = no periosteum in reflected tissues; (+) evident = periosteum in isolated areas of reflected tissues; (++) very evident = periosteum lining entire inner surface of reflected tissues; and (##) event completed = reformation of new functioning periosteum replacing periosteum destroyed by reflective forces.

RESULTS

The 48 block specimens provided two samples of dissectional wounds of each flap design at each postsurgical evaluation period. Table 1 shows the light microscopic evaluation of wound-healing events that occurred in the dissectional wounds of the two flap designs.

Dissectional Wound: Triangular Flap

At 1 day, a thin fibrin clot was evident in the wound site, with fibrin strands parallel to the plane of the wound. The

TABLE 1. Microscopic evaluation of wound healing events occurring in the dissectional wounds of two flap designs at varying postoperative periods

	Flap Design											
	T*	SR	T	SR	T	SR	T	SR	T	SR	T	SR
	Post-operative Day 1	Post-operative Day 1	Post-operative Day 2	Post-operative Day 2	Post-operative Day 3	Post-operative Day 3	Post-operative Day 4	Post-operative Day 4	Post-operative Day 14	Post-operative Day 14	Post-operative Day 28	Post-operative Day 28
Clotting and inflammation												
Clot formation	++†	++	++	++	++	++	##	++	##	##	##	##
PMN	++	++	++	++	+	+	##	##	##	##	##	##
MP	+	+	+	+	++	++	++	++	##	##	##	##
Connective tissue healing												
Fibroblast migration	#	#	+	+	+	+	++	++	##	##	##	##
Collagen synthesis	#	#	+	+	+	+	++	+	##	##	##	##
Granulation tissue	#	#	#	#	#	#	+	#	##	##	##	##
Connective healing	#	#	#	#	#	#	#	#	++	++	##	##
Other observations												
Cortical resorption	#	#	#	#	#	#	#	+	+	+	+	+
Cortical repair	#	#	#	#	#	#	#	#	+	+	+	+
Cortical necrosis	+	+	+	+	+	+	+	+	+	+	+	+
Cortical periosteum	+	+	+	+	+	+	+	+	##	##	##	##
Reflected periosteum	#	#	#	#	#	#	#	#	##	##	##	##

* T, triangular flap design; SR, submarginal rectangular flap design.
† # = absent; + = evident; ++ = very evident; ## = event completed.

clot had formed between cortical bone and the base of the flapped tissues (Fig. 1). PMN were the predominant inflammatory cell type. Surface lamellae of the cortical bone denuded of periosteal tissues evidenced necrosis, with empty lacunae. However, isolated areas of the cortical (periosteal) surface had retained periosteal tissues which lacked microvasculature and vital cells, and had depolymerized collagen fibers. These cortical-retained periosteal tissues were associated with Sharpey's fibers incorporated into vital bone. Unlike cortical surface lamellae without retained periosteal tissues, surface lamellae with cortical-retained periosteal tissues showed lacunae filled with apparently vital osteocytes, indicating that bone necrosis had not occurred.

There was no evidence of a periosteum at the base (periosteal surface) of the flapped tissues. The base of the flap adjacent to the fibrin clot occupying the dissectional wound site was essentially acellular and without a functioning microvasculature, and was characterized by depolymerized collagen. The lamina propria of the flapped attached gingiva exhibited a normal architectural pattern with minimal inflammation, unlike the lamina propria of alveolar mucosa which was highly inflamed and had a disrupted architectural pattern.

At 2 days, reduction of PMN activity was indicated by a decrease in number, but they remained the predominant inflammatory cell in the wound site, although the number of MP had increased markedly as compared with 1 day specimens. Fibroblasts and undifferentiated ectomesenchymal cells were evident along the fibrin strands. Reticulin staining showed type III collagen fibers had formed within the wound site. Cortical-retained periosteal tissues were seen in isolated areas along the cortical surface. These tissues were acellular, avascular, and comprised of depolymerized collagen associated with Sharpey's fibers (Fig. 2). The underlying cortical surface lamellae showed lacunae filled with osteocytes. Cortical bone from which the periosteum had been elevated had empty lacunae in the surface lamellae.

The base of the flapped attached gingiva showed no evidence of a periosteum and was characterized by a mass of depolymerized collagen without normal tissue fibroblasts (Fig. 3), although migrating fibroblasts, macrophages, and undifferentiated ectomesenchymal cells were observed in this structure. With the exception of the base or periosteal surface of flapped tissue, the lamina propria of attached gingiva showed minimal response to surgical dissectional wounding, exhibiting a normal architectural pattern of collagen fibers, bundles, and fascicles with a normal fibroblast population. Mild perivascular inflammation was evident, but only scattered inflammatory cells were seen elsewhere in the lamina propria. The overlying epithelium was intact and contained no inflammatory cells. In contrast, the lamina propria of alveolar mucosa showed marked inflammation, extravascular erythrocytes, collagen degradation, and general architectural disruption.

No osteoclastic or osteoblastic activity was observed on the cortical surface. Several bone slivers were noted in the base of the flapped tissues of the attached gingiva. These slivers were apparently dislodged by the periosteal elevator during reflection of the flap.

At 3 days, the observations were essentially the same as at 2 days, except that more type III collagen was present in the wound site. MP were now the predominant inflammatory cell, and fibrinolytic changes were noted. Bone slivers lodged in flapped tissues and cortical surface gouging defects were also seen.

At 4 days, fibrinolytic activity was very evident, the number of PMN markedly reduced, and the MP continued to be the predominant inflammatory cell in the wound site. The number of fibroblasts and fibroblast-like cells had greatly increased, angiogenesis and the synthesis of type I collagen fibers were evident, and granulation tissue had formed in some areas of the wound site. All other observations were essentially the same as the earlier postsurgical intervals.

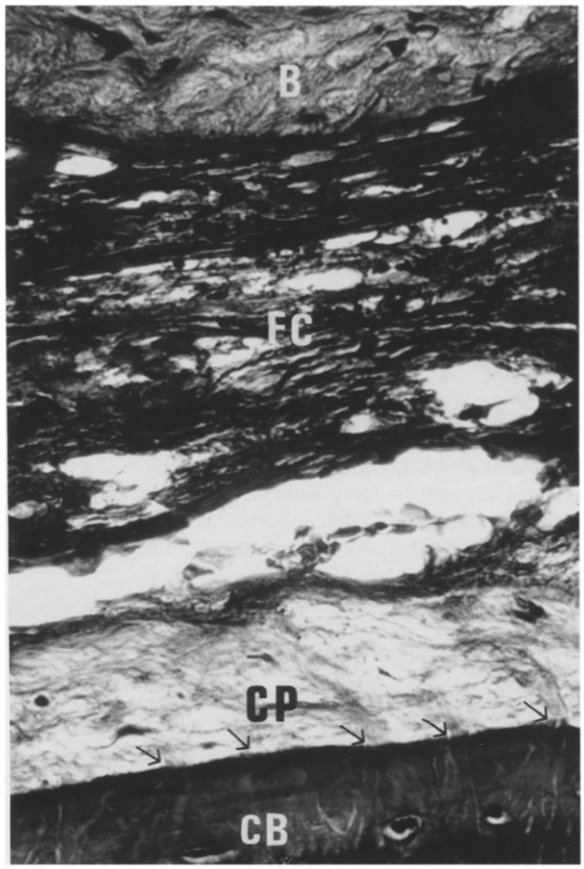


FIG 1. Dissectional wound site 1 day after surgery. A thin fibrin clot (*FC*) occupies the site and separates the base (*B*) of the flapped tissues and cortical bone (*CB*). Cortical-retained periosteal tissues (*CP*) are evident on the cortical surface and are associated with intact Sharpey's fibers (*arrows*). Vital cells and microvessels are not seen in the base of the flapped tissues or in the cortical-retained periosteal tissues. Surface lamellae of the cortical bone contain vital osteocytes (hematoxylin and eosin; original magnification ×132).

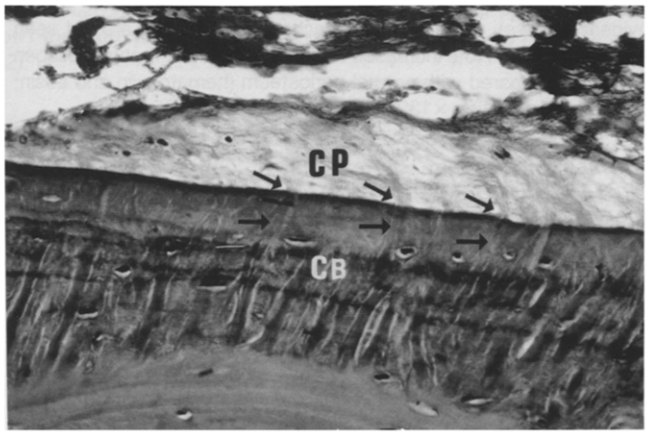


FIG 2. Cortical-retained periosteal tissues (*CP*) with depolymerized collagen and associated Sharpey's fiber attachments (*arrows*) to vital cortical bone (*CB*) 2 days after surgery. Note presence of normal-staining, vital osteocytes in lacunae of the surface lamellae of bone. The acellular, avascular cortical-retained periosteal tissues were always associated with vital bone (surface lamellae) in contrast to the necrotic bone observed in areas denuded of these tissues. *FC*, fibrin clot (hematoxylin and eosin; original magnification ×132).

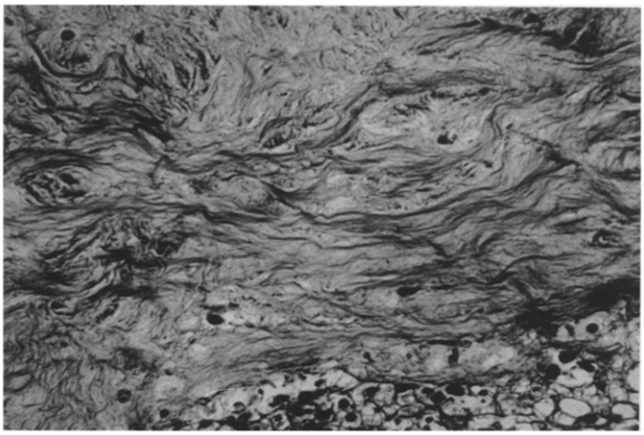
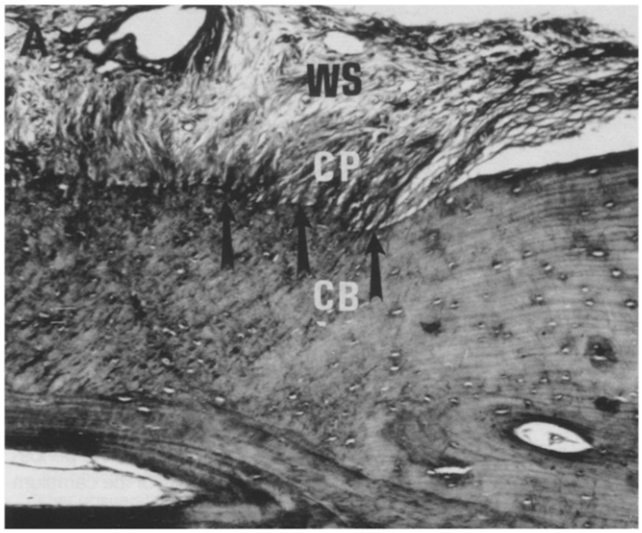


FIG 3. Base of the flapped tissues 2 days after surgery. The periosteum did not survive the forces of flap elevation. Cells of the cambium layer were destroyed and collagen of the fibrous layer has become depolymerized, leaving an acellular, avascular mass of depolymerized collagen along the base of the flapped tissues (hematoxylin and eosin; original magnification ×132).

There was no evidence of a periosteum at the base of the flapped tissues, but the depolymerized collagen layer now contained a large population of undifferentiated ectomesenchymal cells, fibroblasts, and fibroblast-like cells. A similar cell population was noted in the cortical-retained periosteal tissues. New collagen fibers in the granulation tissue formed in the wound site appeared to be intertwined with the polymerized collagen of the cortical-retained periosteal tissues (Fig. 4). Cortical surface necrosis, indicated by empty lacunae in the surface lamellae, was noted in all areas except where cortical-retained periosteal tissues were present (Fig. 5). Although no osteoclastic or osteoblastic activity was seen on the cortical surface, limited osteoclastic activity was noted on the periodontal ligament surface of crestal bone.

At 14 days, fibrous connective tissue had replaced the depolymerized collagen layer at the base of the flapped tissues. A new periosteum had formed along the cortical surface, differing from normal periosteum in its higher cellular content, greater vascularity, and thinner, less mature collagen fibers (Fig. 6). In most areas the collagen fibers of the new periosteum were oriented parallel to the cortical surface with no evidence of fibrous attachment to cortical bone. However, in isolated areas, some collagen fibers were oriented perpendicular to the cortical surface and connected to bone by Sharpey's fibers. In the latter areas, there was no evidence of recent osteoclastic or osteoblastic activity and the underlying surface lamellae were vital. These were apparently areas where cortical-related periosteum, with its associated Sharpey's fibers, were present, and new collagen fibers and repolymerized collagen subunits had been remodeled into a functional attachment unit between lamina propria and cortical bone (Fig. 7).

Osteoclastic activity was present along some of the areas of the cortical surface which had empty lacunae. The clastic activity involved only the outer periosteal surface lamellae and the osteoclasts were primarily mononuclear cells rather than large multinucleated cells. In a few resorptive defects, early osteoblastic activity was noted. Mild osteoclastic activity also was seen on the cortical surface of crestal bone but the



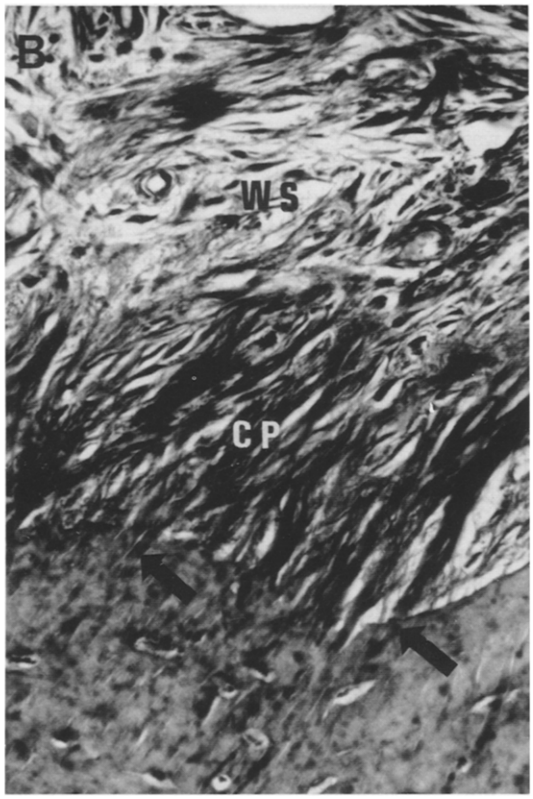


FIG 4. *A* and *B*, Cortical bone (*CB*) with cortical-retained periosteal tissues (*CP*) 4 days after surgery (triangular flap design). Newly formed collagen of the granulation tissue in the wound site (*WS*) appears to be intertwined with repolymerizing collagen of the cortical-retained periosteal tissues which are apparently associated with Sharpey's fiber attachments (*arrows*) to cortical surface lamellae (Masson's trichrome; original magnification *A*, ×33; *B*, ×132).

periodontal ligament surface showed full osseous repair of resorptive defects, as evidenced by reversal lines and new bone deposition. There was no microscopic evidence of a change in crestal height.

The lamina propria and epithelium of both attached gingiva and alveolar mucosa showed no evidence of inflammation and exhibited a normal architectural pattern. Bone slivers were observed in the base of the lamina propria and were apparently well tolerated (Fig. 7).

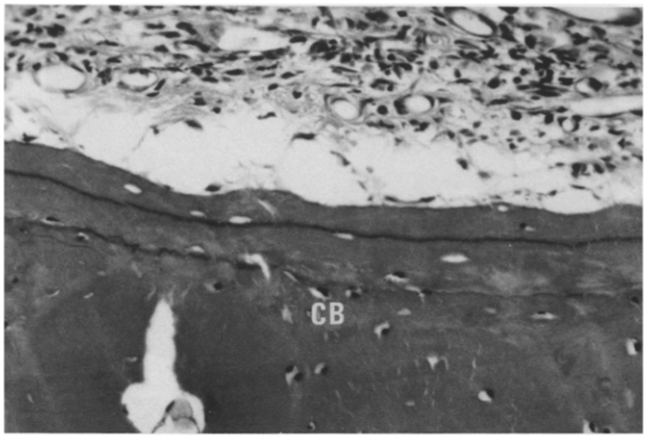


FIG 5. Cortical bone (*CB*), denuded of periosteum by the flap elevation procedure, 4 days after surgery. Note the absence of vital osteocytes in the surface lamellae of cortical bone, indicating necrosis of the cortical surface (hematoxylin and eosin; original magnification ×100).

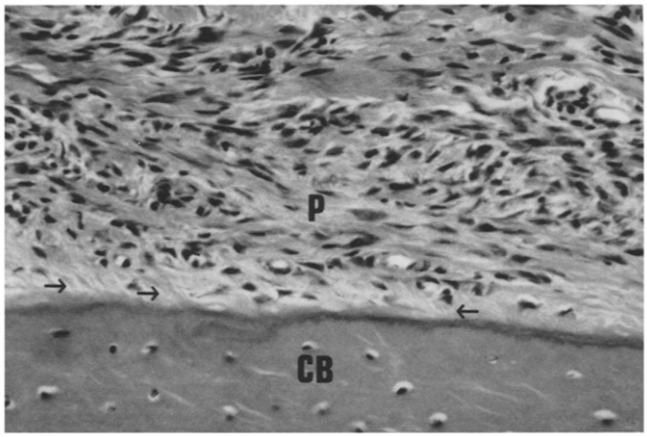


FIG 6. Reforming periosteum (*P*) on the cortical surface 14 days after surgery. Vitality of surface lamellae of cortical bone (*CB*) indicates that this was an area associated with cortical-retained periosteal tissues. Fibers oriented perpendicular to the cortical surface are evident (*arrows*), although fiber attachments into bone are not seen in this specimen. Note increased cellularity and fewer mature collagen fibers as compared with normal periosteum (hematoxylin and eosin; original magnification ×132).

At 28 days, the flapped tissues were completely normal. In the wound site, the new periosteum had taken on a more normal appearance but increased cellularity remained and collagen fibers had not completely matured. Both osteoclastic and osteoblastic activity were present in isolated areas along the cortical surface. Although some resorptive areas had been repaired, others showed no evidence of osteoblastic activity. Resorptive defects were limited to the outer three to four lamellae. An increased number of collagen fibers were oriented perpendicular to the cortical surface and attached to bone by Sharpey's fibers. This was evident in areas where new bone had repaired resorptive defects and in areas in which cortical-retained periosteal tissues were present. The former areas were easily distinguishable from the latter by the presence of new bone in an area of previous resorption and thin, sparse Sharpey's fibers confined to newly deposited bone and not extending to the depth seen in areas associated with cortical-retained periosteal tissues.

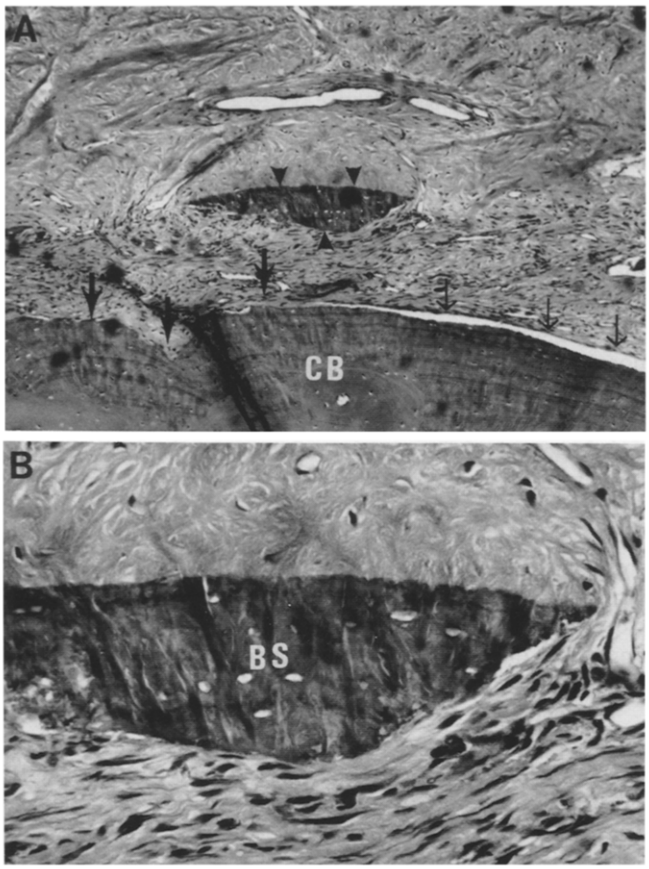


FIG 7. Reforming periosteum on the cortical surface 14 days after surgery. *A*, Periosteum (*large arrows*) on the left is attached to vital bone by Sharpey's fibers, indicating that cortical-retained periosteal tissues were present in this area. Periosteum (*small arrows*) on the right remains unattached (separation is artifactual) to necrotic surface lamellae of cortical bone (*CB*), indicating that the osseous surface was denuded of periosteum during flap reflection. Note bone sliver (*arrowheads*) lodged in base of flapped tissue. *B*, Higher magnification of same specimen showing well-tolerated necrotic bone sliver (*BS*), with empty lacunae, dislodged during flap reflection and now separating uninflamed lamina propria (*above*) and reforming periosteum (*below*) (hematoxylin and eosin; original magnification *A*, ×33; *B*, ×132).

Crestal bone showed complete osseous repair of resorptive defects on the periodontal ligament surface and partial repair on the cortical surface. There was no evidence that crestal height had been altered.

Dissectional Wound: Submarginal Rectangular Flap

As shown in Table 1, there were few differences in dissectional wound healing between the triangular and submarginal rectangular flap designs. In the latter, at 4 days, the fibrin clot was essentially intact with little fibrinolytic activity and no evidence of type I collagen or granulation tissue in the wound site, indicating a slower rate of healing.

At 14 and 28 days. no discernible differences were noted between the flap designs in any of the evaluated events.

Variations between the two samples of the submarginal rectangular design at the earlier intervals followed the same pattern as found in the incisional wound. In specimens associated with greater gaping of the horizontal incisional wound, the dissectional wound-healing events were delayed when compared with the other specimen at the same postoperative period. This intersample variation was particularly evident at the 2-, 3-, and 4-day intervals.

All observations regarding cortical osteoblastic and osteoclastic activity, cortical necrosis, cortical-retained periosteal tissues, and reflected periosteal tissues were essentially the same as described for the triangular flap. However, unlike the triangular flap, mild crestal osteoclastic activity on both the cortical and periodontal ligament surfaces was observed in both 4-day specimens. Similar to the triangular flap, mild osteoclastic activity on the cortical surface of crestal bone was seen at 14 and 28 days, indicating that crestal bone resorption is not prevented by using a submarginal rectangular flap. Crestal resorption on the periodontal ligament surface was completely repaired at 28 days.

At the 1- through 4-day intervals, the flapped attached gingiva contained a considerably greater number of inflammatory cells than seen with the triangular flap design. Alveolar mucosal tissues were highly inflamed with edema, extravascular erythrocytes, collagen degradation, and general disruption of the normal architecture of the lamina propria and submucosa.

Bone slivers and gouged cortical surfaces were seen in the 2- and 4-day specimens. The slivers were lodged in the deep tissues of the lamina propria, but were apparently well tolerated.

DISCUSSION

Healing of the dissectional wound is rapid, but slower than the incisional wound. Several factors may contribute to this slower rate. First, healing of the dissectional wound involves wound edges of dissimilar tissues, cortical bone and lamina propria, with only the latter contributing to the early reparative process (2). Second, dissectional wounding is less precise and causes greater damage to tissues than incisional wounding. However, it is the extremely rapid incisional wound healing that provides the peripheral seal (epithelial and connective tissue) from the noxious elements of the oral environment, allowing the more complicated dissectional wound-healing events to proceed at a slower pace without added insults. This study indicates that healing of dissectional wounds is very advanced at the 4th postsurgical day and essentially complete by the 14th day, with remodeling and maturation of soft and osseous tissues continuing through the 28th day.

Of particular interest is the observation that the periosteum elevated with the flap is destroyed and no longer exists as a microscopically discernible tissue within 24 h after flap reflection. This confirms the findings of Klingsberg and Butcher (3) and Melcher and Accursi (4) that the periosteum does not survive the reflective forces of flap elevation in surgical dissectional wounding. The resting periosteal cells of the cambium layer are destroyed during flap elevation (4), and the collagen of the fibrous layer undergoes rapid depolymerization within the first 24 h (3). The destruction of the periosteal cells of the cambium layer may explain, in part, the delay in osteoblastic and osteoclastic activity after flap reflection, because these cells are the precursors of osteoblasts and osteoclasts which function in physiological bone remodeling. Osteoclastic and osteoblastic activity on the periosteal cortical

surface were not observed until the 14th postsurgical day with triangular flaps. However, recent evidence suggests that osteoclasts also may form from plasma-derived monocytes (5). If this is correct, there is apparently some factor or factors which delays osteoclastic activity by these cells.

The imprecise dissectional wound results in periosteal remnants remaining attached to cortical bone in irregular, isolated areas. These avascular and acellular remnants, referred to as cortical-retained periosteal tissues, show collagen depolymerization similar to that observed in the reflected periosteal tissues. However, the depolymerized collagen maintains a microscopically identifiable structure and apparently exerts some protective role against necrosis of the surface lamellae of underlying cortical bone. No osteoclastic activity was observed in areas with cortical-retained periosteal tissue. Whether this is a result of cortical surface viability or antiosteoclastic influence of the cortical-retained periosteal tissues is not known.

The base (periosteal surface) of the flapped tissues forms one wound edge and the cortical surface, with some cortical-retained periosteal tissues, forms the opposing wound edge. At 24 h, the former wound edge is lined with a layer of depolymerized collagen which contains no tissue fibroblasts but maintains a structural identity easily identifiable by light microscopy (Figs. 1 and 3). The cortical-retained periosteal tissues on the opposing wound edge presents the same microscopic appearance, with the exception that the depolymerized collagen apparently remains attached to Sharpey's fibers entrapped in bone (Figs. 1 and 2). The presence of these opposing depolymerized collagen masses may be important factors in the early reattachment of flapped tissues to bone. It is postulated that these masses represent deaggregated collagen in the form of collagen subunits as described by Klein and Weiss (6). These investigators demonstrated that injured collagen, under appropriate conditions, may not undergo total degradation but may depolymerize into collagen subunits, remain in the wound site, and rapidly reaggregate (polymerize) to form new collagen which may connect or splice with severed collagen fibers. Although the mechanism by which severed fibers become reattached has not been defined, this concept would explain the rapid reattachment of fibers observed in the dissectional wound (Fig. 4). This pool of collagen subunits may effect a rapid splicing of severed collagen fibers in the base of the flapped tissue with newly synthesized collagen fibers in the wound site and Sharpey's fibers at the cortical surface, thus providing areas of rapid reattachment of flapped tissues to bone without the necessity for osteoclastic and osteoblastic activity to establish a new attachment. Other investigations (7, 8) suggest that the splicing concept is valid.

In the present study, cortical-retained periosteal tissues were seen in all specimens but in varying amounts along the cortical surfaces, indicating that the dissectional wound is imprecise and that the amount of periosteal tissue remaining on the cortex is unpredictable. No effort was made to preserve this tissue during flap reflection because the potential advantage of early reattachment of flap to bone was not considered at the time of surgery. This has interesting clinical implications because cortical-retained periosteal tissues are likely intentionally destroyed, albeit unknowingly, during most surgical procedures. Reflection of the flap usually results in exposure of a cortical surface that evidences some areas of bleeding tissue tags along the otherwise bare, whitish surface. It has been common practice to curette these bleeding sites, which effectively burnishes the microvasculature against bone, plugging the vessels, and reducing the annoying source of bleeding. This may be an unwise practice because these bleeding tissue tags are probably cortical-retained periosteal tissues. Such sites are particularly prevalent in the depressions between prominent root eminences. Curettement of bleeding tissue tags from the cortical surface following flap reflection may be counterproductive because it removes cortical-retained periosteal tissues and thus eliminates sites of early reattachment of flapped tissues to cortical bone. Clinically, it is observed that these sites will stop bleeding within a few minutes after flap reflection, without curettement, probably due to normal intravascular clotting mechanisms.

Further discussions regarding the cortical-retained periosteal tissues raised the question of the potential advantage of reflecting a split-thickness flap to the level of cortical penetration to gain surgical access to the periapical tissues. This is not recommended because split-thickness flaps cause greater crestal bone loss than full-thickness flaps (9), probably by disrupting the supraperiosteal vessels. Disruption of the major blood supply to these tissues may result in collagen degradation rather than depolymerization, eliminating the protective mechanism that prevents surface lamellae necrosis.

The evidence of type III (reticular) collagen formation in the dissectional wound site at 2 days is significant. These fibers form a framework or matrix in which type I collagen is apparently induced to form (10). The function of reticular fibers in mature connective tissues is unknown, but they may contribute to the mechanical properties of the lamina propria by maintaining the architecture of collagen bundles when they are subjected to shearing forces. Collagen metabolism is greater in oral tissues than skin, because the oral tissues exist in a state of continuous wound healing and repair. On the basis of animal studies, gingival collagen appears distinct in that its turnover rate is much faster than that of collagen in alveolar mucosa or the lining mucosal tissues (11), although not as fast as in the periodontal ligament tissues (12). In response to surgical wounding, the speed with which collagen macromolecules enter the extracellular environment in the wound site is important for several reasons. First, this triggers the release of angiogenesis factors from macrophages and other sources, stimulating the migration of endothelial and smooth muscle cells into the wound site and the subsequent formation of new microvessels (angiogenesis) in the area (13, 14). Second, angiogenesis will not proceed toward the center of the wound site unless collagen macromolecules continue to be synthesized and released into the extracellular environment in the path of the advancing healing front of new microvessels (2, 15). Third, the new vessels are highly inefficient, with tortuous routes, turbulent blood flow, and excessive permeability. Maturation of the endothelial cells is required to transform these new microvessels into an efficient microvascular system and this, too, is dependent upon continuous collagen and matrix synthesis by fibroblasts in the wound site (13).

Crestal bone loss is considered an inevitable result of full mucoperiosteal flap reflection. In this investigation, crestal osteoclastic activity after triangular flap reflection was observed only on the periodontal ligament surface at 4 days. By 14 days, osteoblastic activity had repaired the resorbed crestal bone on this surface. Osteoclastic and osteoblastic activity

were noted on the periosteal surface of crestal bone at 14 and 28 days. There was no light microscopic evidence that the height of crestal bone was altered, although the thickness was evidently being remodeled. Human studies indicate that the mean loss of crestal bone height from full mucoperiosteal flap procedures in periodontal surgery is about 0.5 mm (9, 16). But, in extrapolating these findings to endodontic surgery, it is important to note that these studies involved apically positioned flaps and intentional removal of root-attached epithelium and connective tissues and, thus, depended on epithelial and connective tissue new attachment rather than reattachment. It is logical to assume that the preservation of supracrestal root-attached epithelial and connective tissues, and flap reapproximation (repositioning) rather than apical positioning, would result in little or no permanent crestal bone loss. This contention can be strongly supported by evidence from periodontal studies (17, 18). Hiatt et al. (17) reported complete restoration of early crestal bone loss after 4 weeks when root-attached epithelium and connective tissues were preserved during full mucoperiosteal flap procedures in dogs. Caffesse et al. (19) concluded that flap reapproximation to its original position, even with reverse bevel incisions and curettement of root attached tissues, produced only superficial crestal bone loss which promptly underwent complete repair by osteoblastic activity.

Osteoclastic activity was observed at 4, 14, and 28 days on the cortical (periosteal) and periodontal ligament surfaces of crestal bone after reflection of submarginal rectangular flaps. This occurred although the horizontal incisional wound was located several millimeters apical to the crestal bone height. Similar osteoclastic activity in control specimens was not seen, suggesting this was not a result of normal physiological remodeling. Osteoclastic activity on the periosteal surface of crestal bone at 4 days can be explained by the presence of a periosteum with vital precursor cells of osteoclasts in the cambium layer. Full repair of resorption on the periodontal ligament surface was seen at 28 days. As with the triangular flap, cortical bone height did not appear to be altered.

The minimal degree of irregular, isolated cortical bone resorption and early evidence of osseous repair indicates that osteoclastic activity following periradicular surgery is of no clinical significance and probably serves mainly as a mechanism for new attachment of collagen fibers from the reforming periosteum and overlying lamina propria into bone as Sharpey's fibers.

Damage to the alveolar mucosa was obvious with both flap designs. The contrast between the excellent response of attached gingival tissues and the poor response of alveolar mucosal tissues with the triangular design was especially evident. It may be that the more delicate, less fibrous alveolar mucosa does not have the structural strength to withstand the forces of flap reflection. However, it is more likely that retractive rather than reflective forces caused this damage. Photographs taken during the surgical procedures revealed that the retractor was frequently resting on soft tissue rather than on bone. Thus, the surgeon should ensure that the retractor does not impinge on mucosal tissues during surgical procedures.

Reflective forces often result in gouging of the cortical plate which dislodges bone slivers and causes them to become firmly lodged deep in the flapped tissues. Apparently, some of these slivers remain in the deep lamina propria and are not removed by copious irrigation. This finding confirms the observation by Melcher and Accursi (5) that mechanical forces applied with a periosteal elevator during flap reflection can dislodge boney slivers from the cortical surface. In dissectional wound healing, these slivers become surrounded by new connective tissue with a normal fiber architecture and cellular content, showing no evidence of inflammation or fibrous encapsulation (Fig. 7). The clinical significance, if any, of these slivers is not known. However, it can be speculated that this may explain reports by patients that a "sensitivity to finger pressure" develops in the surgical site several weeks after surgery. This could result from pressing a small ectopic bone sliver against the sensitive, newly formed periosteum (20).

CONCLUSIONS

Within the parameters of this study, the following conclusions are drawn:

1. Few differences exist in dissectional wound healing between triangular and submarginal rectangular flaps. However, intersample variations in temporal and qualitative healing at the earlier postsurgical intervals were greater with the submarginal rectangular flap.
2. The elevated periosteum is destroyed by the reflective forces of dissectional wounding. The cells of the cambium layer do not survive the flap reflection procedure and the collagen of the fibrous layer becomes depolymerized, but is microscopically identifiable as a structure on the base of the flapped tissues during the early phases of wound healing.
3. Cortical-retained periosteal tissues remain on the cortical surface in variable and unpredictable amounts after flap reflection when efforts are not made to protect or preserve these tissues. Periosteal cells of the cambium layer do not survive in the cortical-retained tissues and the collagen becomes depolymerized but remains attached to the cortical bone and associated Sharpey's fibers.
4. Cortical-retained periosteal tissues apparently exert some protective influence which prevents necrosis of surface lamellae in underlying cortical bone.
5. Crestal bone osteoclastic activity occurs following reflection of both triangular and submarginal rectangular flaps. However, osteoblastic repair occurs and crestal bone height is not altered.
6. Reflective forces exerted with a periosteal elevator cause dislodgement of bony slivers from the surface of cortical bone that may become embedded in the base of flapped tissue.

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The Way it Was

Those involved in scientific or academic presentations are constantly barraged with exhortations to brevity. Those wearied by such nattering will rejoice in the story of the student who was assigned to write an essay which touched on all of three such weighty subjects as divinity, nobility and chastity—and to keep it short. He wrote only eleven words. "My God," said the Duchess, "take your hand off my knee."

Zachariah Yeomans