Reverse Bevel Periodontal Flaps in Monkeys*

by

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"REVERSE BEVEL FLAP"[1] is the name presently used for a surgical procedure first described by Widman[2] in an English language publication in 1918. After new modifications by Nabers (1954),[3] Ariaudo and Tyrrell (1957),[4] Ramfjord (1959),[5] and Friedman and Levine (1964),[1] Widman's modification of Neumann's[6, 7] classical periodontal flap has become increasingly popular during the last decade. It has been used often for the elimination of deep periodontal pockets and in the management of mucogingival problems. The most recent publication by Morris (1965)[8] which stipulates close and careful flap adaptation to the root surfaces of the operated teeth, buccolingually and interproximally, returns almost completely to the original Widman flap.

Clinical[9–11] and histologic studies[10, 12–16] both in animals and humans have indicated complete healing of all periodontal structures following periosteal flap separation initiated at the bottom of the gingival crevice. A few clinical[7, 9] and histologic[13, 17] studies of reverse bevel flaps, have also been reported but details of tissue reaction and healing are still very incomplete.

Several aspects of reverse bevel flap surgery still are controversial. The accumulation of further information on the behavior of the periodontal tissues following specific modifications of surgical procedure relative to the extent of apical or coronal repositioning of the flap, bone exposure, treatment of tooth surfaces and mucogingival problems should resolve some of the controversy.

The purpose of the present study was to investigate healing of reverse bevel flaps. The effect on healing of commonly used variations in positioning of the flaps was emphasized.

In order to obtain adequate histologic material and to include autoradiographic techniques, the investigation was done on Rhesus monkeys.

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MATERIALS AND METHODS

Four adult male Rhesus monkeys with complete dentitions were used for the experiment. The weight of the four monkeys was respectively 17, 17, 17 and 18 pounds. Their age was unknown. Initially one monkey had severe chronic gingivitis with abundant supra and subgingival calculus, while the other three animals had only mild chronic gingivitis and moderate amounts of calculus. The depth of the gingival crevice did not exceed 3 mm and the bottom of the epithelial attachment, as indicated by probing with a thin probe, did not extend more than 1 mm apically to the cementum-enamel junction in any area.

To create as uniform an experimental and control environment as was possible in the monkeys, all teeth were scaled and polished 2 weeks prior to the surgery.

Only posterior segments (premolars and molars) were used for the experiment. Each surgical procedure included one of three posterior segments, and the fourth segment (different in each monkey) was left unoperated for control. A total of 12 experimental surgical procedures were carried out at the following time intervals prior to sacrifice of the animals: 2 hours, 13 hours, 1, 2, 3, 5, 7, 9, 14, 21, 35 and 72 days.

Reverse bevel flaps with a scalloped gingival incision[1] were raised on both the buccal and lingual sides in the operated segment. The gingival incision was parallel to the long axis of the teeth, about 0.5 to 1 mm from the surface of the teeth, including the interproximal surfaces, and extending to the alveolar process. A vertical buccal and lingual incision extending 4 to 6 mm down on the alveolar process was made at the mesial aspect of the first premolar to release the flap for repositioning at the mesial aspect. The flaps were separated from the bone with mucoperiosteal elevators for a distance of 3 to 4 mm from the teeth. All remaining soft tissues around the teeth buccolingually and interproximally were removed with curettes, and the surfaces of the teeth were planed with curettes. A slight amount of bone was removed by a chisel or a bone file on the buccal aspect of the alveolar crest and extending for about 1 mm apically on the alveolar process.

The flaps were sutured together by interrupted interproximal sutures (atraumatic Ethicon needle and 0000 silk sutures). The distal aspects of the flaps were sutured to approximate closely the necks of the teeth, while the sutures at the mesial aspect were tied loose enough to allow a slight apical repositioning of the flap (1-2 mm). No dressings were placed and the sutures were removed after 7 days, if the animals had not been sacrificed before that time.

The surgery was performed as aseptically as possible. Aspiration was used, and the animals were not given any

antibiotics. The usual monkey diet pellets were used exclusively for food throughout the experiment and the animals were kept under controlled stable conditions of heat and humidity.

One hour prior to sacrifice (at the same time, 10:00 a.m., to avoid diurnal variations), the monkeys were given an intravenous injection of H^3 thymidine (1 microcurie per gram of body weight, specific activity 5 curies per millimole).*

The animals were sacrificed by exsanguination and histologic and autoradiographic slides prepared according to the methods previously described.[17]

Some of the longer term specimens were transferred to 5 percent formic acid since decalcification with EDTA was slow. This resulted in autoradiographs of very poor quality, not suitable for inclusion in the present report. The histologic sections; however, were entirely satisfactory.

At the time of necropsy, specimens were obtained from the viscera. Histologic examination of this material did not reveal any pathologic changes except a mild infestation with a lung fluke (Pneumonyssus simicola) which does not seem to affect the monkey's general health to any appreciable extent.

FINDINGS

The following is a combined report of the histologic and autoradiographic observations from the control and experimental specimens, arranged on the basis of the time interval from the experimental surgery to the sacrifice of the animal. The autoradiographs recorded the labeling about 1 hour prior to death so there is a discrepancy of 1 hour between the histologic and autoradiographic time intervals of postsurgical observation.[18] Thus the autoradiographs of a 2-hour specimen show the labeling that took place when the isotope was given 1 hour earlier (1 hour postsurgically).

Control Specimens

Mild chronic gingivitis is seen around all of the teeth. The epithelial attachment extends not more than 0.5 to 1 mm apically to the cementum-enamel junction. The gingival inflammation is located mainly at the junction of the crevicular epithelium and the epithelial attachment.[19] This area shows a high degree of labeling of both epithelial and connective tissue cells. The rest of the periodontal structures are normal with average distribution of labeled cells when compared with previous reports on rhesus monkeys. There is fatty bone marrow with only a few hematopoietic cells.

Two Hours After Flap Surgery

The surgical separation of both the buccal and palatal flaps extends 3 to 4 mm in an apical direction. The crevicular epithelium and the epithelial attachment are missing completely (Fig. 1A) and the surgical surfaces of the flaps show a narrow zone of necrosis covered by fibrin and coagulated blood. A few polymorphonuclear cells appear in the junction between the flaps and the

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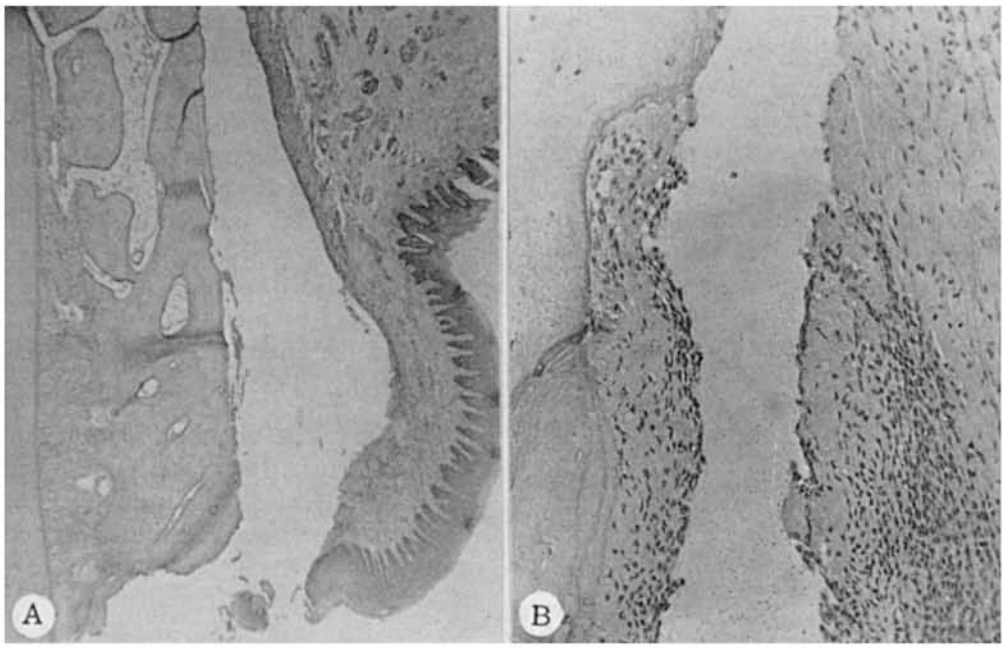

FIGURE 1. *(A) Two hours after surgery. Separation of buccal flap from alveolar process at line of surgery. Slight surgical alteration of buccal aspect of alveolar process. Original magnification X30. (B) High magnification from same specimen as (A). Focal area of polymorphonuclear cells at bottom of picture. Small strip of residual periosteum attached to alveolar process. Original magnification X270.*

underlying tissues. In areas of close flap adaptation, there are very few such cells, but in other areas there are small aggregations of polymorphonuclear cells (Fig. 1B), possibly as a result of surgical infection or the introduction of other irritants during the surgery.

There is less labeling of the basal cells of the epithelium covering the flaps than in the adjacent epithelium or in corresponding epithelium from the control specimens.

The flap separation has stripped the periosteum almost completely from the surface of the bone and in the interproximal areas there are only small scattered strips of connective tissue attached to the alveolar process.

Early necrotic changes are present on the surface of the crestal extension of the periodontal membrane, and in part of the specimen, the usual arrangement of cementoblasts is missing along the root surface for about 0.5 mm from the surgical separation.

The surface of the alveolar process on the buccal side is rough and has lost its normal cortical lamination as a result of the bony surgery (see Fig. 1A). In this area there are some empty lacunae toward the very surface indicating superficial bone necrosis, while on the palatal aspect (no bone removal) the osteocytes are viable to the surface of the bone.

Interproximally, where the flaps did not cover the bone completely, there is a thick layer of fibrin and coagulated blood with a large number of polymorphonuclear cells at the borders of the flaps and over the surface of the bone.

The periodontal structures apical to the alveolar crests are normal. A few labeled osteoblasts and cementoblasts are seen, mainly in the bifurcation and the periapical areas. A few diffusely spread labeled angioblasts and fibroblasts appear in the rest of the periodontal membrane, but there is no indication of an "intermediate plexus."

Thirteen Hours After Flap Surgery

The surgical borders of the flaps are even and well defined (Fig. 2A). All crevicular epithelium has been removed.

The buccal flap is thin and there is no labeling 0.5 to 1 mm from the surgical margin of the flap, but there is a high degree of labeling of epithelial cells beyond the area involved in flap separation (Fig. 2B). There is a thin layer of necrotic tissues and fibrin with a few polymorphonuclear cells on the surgical side of the flap.

The palatal flap is thicker but did not completely extend to the root surface of the teeth following the surgery (Fig. 2C). Coagulated blood fills the space between the flap and the teeth. The epithelial cells show minimal or no labeling for about 0.5 mm from the margin of the flap (Fig. 2D). However, the rest of the epithelial cover of this thick flap shows normal labeling. The flap is closely adapted to the palatal alveolar process and only a narrow zone of polymorphonuclear cells and slight hematoma indicates the line of incision.

There is no evidence of necrosis of bone and the cementoblasts have been disrupted for only a narrow zone apically from the alveolar crest. The rest of the periodontal structures are normal, with the same pattern of labeling as seen in the control and 2-hour specimens.

One Day After Flap Surgery

Most sections indicate inadequate adaptation of the buccal flap to the teeth and a thick blood coagulum fills the space between the flap and the teeth (Fig. 3A). There is a fairly thick band of polymorphonuclear cells and superficial necrosis on the surgical surface of the flap (Fig. 3B).

The epithelial cells have not started to migrate over the surgical margin of the flap (Fig. 3C). While there is only minimal labeling of epithelial cells toward the border, there is a slight increase in labeling starting about 0.5 mm from the margin. The palatal flap also is short of contact with the teeth (0.1 to 1 mm). There is no labeling at the border of the palatal flap, but there is a marked increase in labeling of a narrow band of palatal mucosa about 0.5 mm from the margin of the flap.

The bone appears to be vital to the margin of the surgery even in the areas of bone removal. Where the adaptation of the flap to the bone is close, only a few polymorphonuclear cells are seen, while in other small areas of less close adaptation there are numerous such cells. The rest of the periodontal structures are normal.

Two Days After Flap Surgery

Both the buccal and the palatal flaps end at a slight distance from the teeth and the intervening areas between the flaps and the teeth are filled with coagulated blood and a large number of polymorphonuclear cells. Epithelial cells have started to migrate over the border of the flaps and cover 0.1 to 0.2 mm of the inner surface (Fig. 4A). The regenerating epithelium is migrating between necrotic and living connective tissue under the surgical surface of the flap. However, these initial projections of epithelium are much shorter than those which were reported 2 days following gingivectomy. Only a few epithelial cells are labeled in the flaps.

A large number of labeled angioblasts and fibroblasts are seen at the surgical opening of the periodontal membrane on the buccal side at the alveolar crest. A thin layer of bone has been removed on the buccal aspect of the alveolar crest, and there are some empty lacunae in

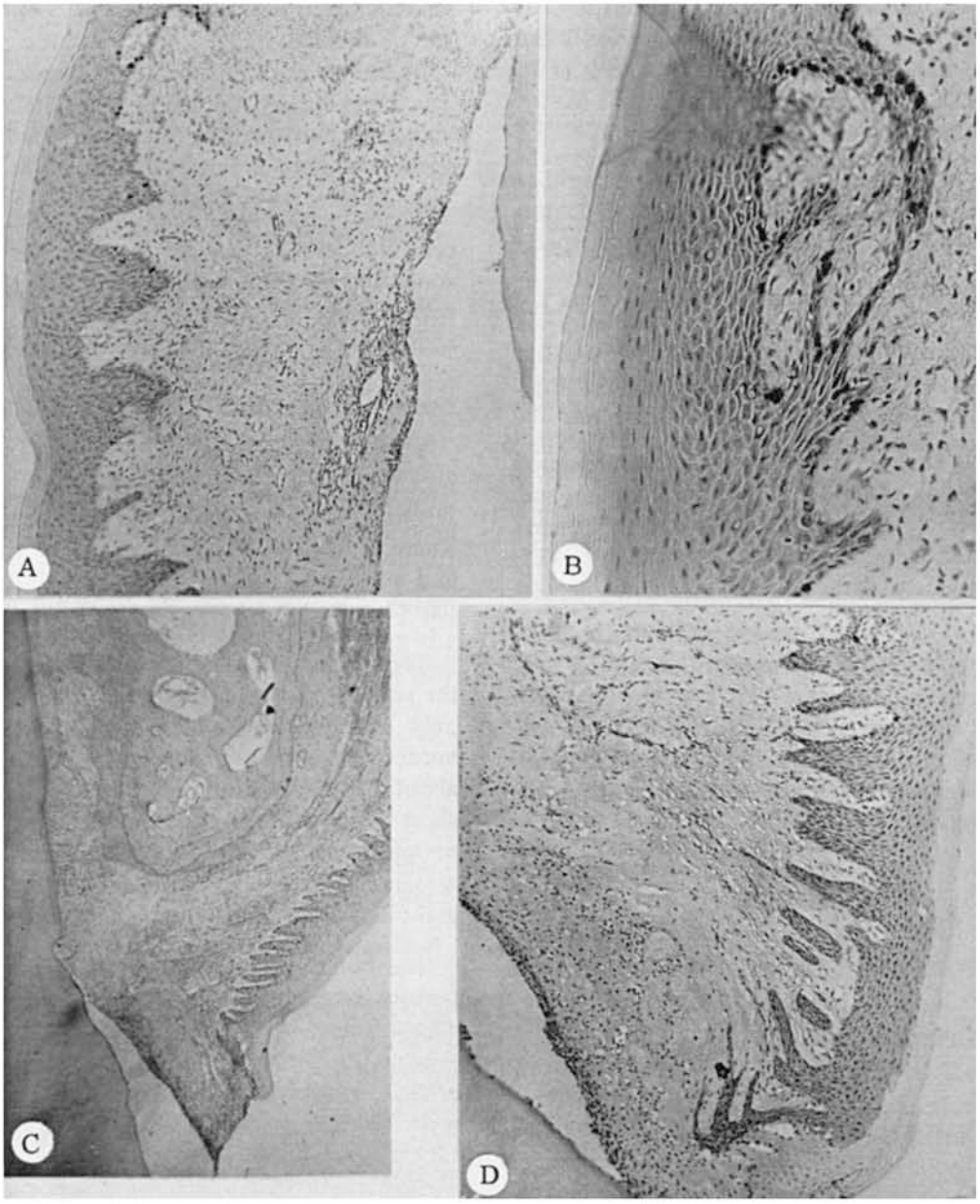

FIGURE 2. *(A) Thirteen hours after surgery. Buccal flap. Decrease in labeling toward gingival border of flap (bottom of picture). Original magnification X92. (B) High magnification from top of (A), toward junction of attached gingiva and alveolar mucosa. More labeling of epithelial cells at top of picture (area not involved in flap surgery) than at bottom (part of flap). Original magnification X218. (C) Palatal flap from same specimen as (A). Flap short of contact with tooth, and intervening space filled with coagulated blood. Good flap adaptation to alveolar crest. Original magnification X34. (D) From same specimen as (C). No labeling for about 0.5 mm from surgical border of flap. Original magnification X85.*

the bone close to the surface indicating necrosis. The rest of the alveolar process has viable osteocytes. There is beginning superficial fibrosis of the marrow spaces close to the wound. The rest of the bone marrow is normal fatty bone marrow with a few hematopoietic cells.

On the palatal side both the periodontal membrane and a narrow strip of bone are not covered by the flap (Fig. 4B). In this area there is necrosis of the bone and of the surface of the periodontal membrane extending from the alveolar crest for about 0.5 mm into the periodontal space. The cementoblasts are deranged for about 1 mm from the wound surface. In the interproximal areas the bone is covered by coagulated blood, and there is beginning surface necrosis of the bone. The epithelium has started to migrate down toward the bone at the borders of the flaps (Fig. 4C).

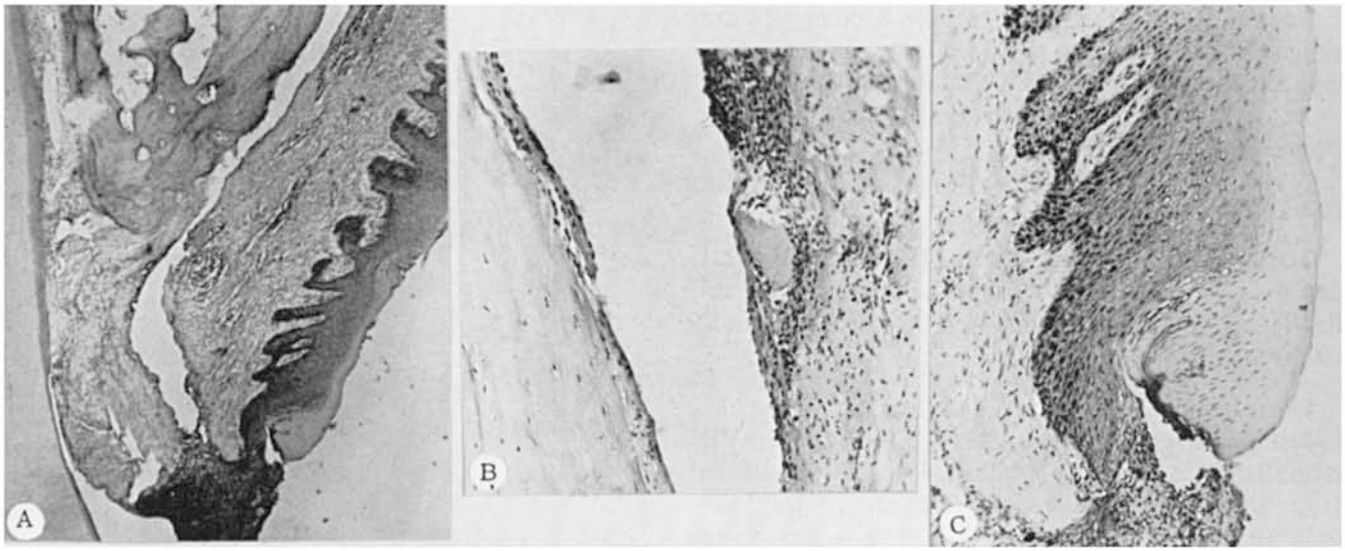

FIGURE 3. *(A) One day after surgery. Buccal flap. Incomplete adaptation to tooth. Artifactual separation between flap and alveolar process. Original magnification X41. (B) High magnification from (A). Flap lined by fibrin and polymorphonuclear cells. Small fragment of bone. Bone of alveolar process vital. Original magnification X194. (C) High magnification from (A). No appreciable migration of epithelial cells over border of flap. Original magnification X138.*

Three Days After Flap Surgery

The specimens are from the mandible and at the second and third molars both the buccal and lingual flaps are closely adapted to the teeth, while at the anterior there is an apical repositioning of the flaps (0.5 to 2 mm from the flaps to the premolar teeth). The inflammatory reaction is much less severe and the healing much more advanced in the areas of close flap adaptation than in the areas of apical repositioning of the flaps. In the areas of close contact between the flaps and the teeth (Fig. 5A), there is a high degree of epithelial labeling toward the border of the flaps. The epithelium is making contact with the surface of the teeth and there is only a very thin blood clot between the connective tissue of the flaps and the teeth.

Some labeled angioblasts and fibroblasts are seen at the junction between the flaps and the underlying connective tissue and bone (Fig. 5B). The tip of the alveolar crest is necrotic and the cells on the very surface of the crest are not labeled. Also there are no labeled cells close to the small areas of superficial bone necrosis along the separation between the flaps and the alveolar process.

Where the flaps have been apically repositioned, coagulated blood and numerous polymorphonuclear cells are covering the exposed periodontal membrane and bone. There is minimal labeling of the epithelial cells of the flap until about 2 mm from the margin of the buccal flap where there is a marked increase in labeling (Fig. 5C). The thicker lingual flaps have intensive labeling of epithelial cells almost to the surgical border (Fig. 5D). The epithelium has started to migrate over the border of the flaps for a very short distance. None of the migrating cells show any labeling.

The uncovered crestal opening of the periodontal membrane shows acute inflammation and numerous labeled angioblasts (Fig. 5E). Granulation tissue is proliferating into the covering blood clot.

There is a shallow zone of bone necrosis both under the buccal alveolar crest where some superficial bone removal had been done and on the palatal aspect (no bone removal but denudation after the surgery). The normal arrangement of cementoblasts has been disturbed for a distance of 0.5 to 1 mm from the crests.

Where the flaps are closely adapted to the alveolar process there is only minimal inflammation, while other areas with poor adaptation show severe acute inflammation. In most areas the periosteum has been completely separated from the bone during the surgery, but in spots there are some periosteal cells adherent to the surface of the bone. There is labeling of angioblasts and fibroblasts on the inside of the flaps toward the bone and the periosteal cells remaining on the bone surface show some labeling. Collagen fibers within the flaps are undergoing degeneration (Fig. 5F).

The interproximal areas are filled with coagulated blood and polymorphonuclear cells cover the necrotic surface of the bone (Fig. 5G). Granulation tissue formation is starting from the marrow spaces. There is slight epithelial migration at the interproximal borders of the flaps, partly due to folding of the edges and partly due to epithelial migration. The epithelium extends about

0.5 mm down from the border of the flaps toward the bone. This represents an early tendency for separation between flap and blood clot which apparently may lead to a temporary, partial separation between flaps and regenerating granulation tissue.

Five Days After Flap Surgery

The specimen is from the mandible. While the buccal flap has been closely adapted to the teeth, the lingual flap has been apically repositioned up to as much as 2 mm from the teeth.

Part of the epithelial adaptation to the teeth on the buccal side is apparently a result of folding under the surgical margin of the still keratinized gingival epithelium (Fig. 6A) at the time the flap was pulled toward the teeth by the sutures. However, there is regeneration of epithelium at the margin of this keratinized epithelium, and labeling extends into the narrow zone of regenerated epithelium (Fig. 6B). The regenerating epithelium is proliferating under a superficial necrotic zone on the inside of the flap and does not establish full contact with the surface of the adjacent tooth. Thus a new epithelial attachment is not yet present.

At the crestal aspect of the periodontal membrane and over the alveolar crest there is granulation tissue. There are some osteoclasts at the crest and extending for 1 mm over the periodontal membrane side of the alveolar bone (Fig. 6C). This is the shortest time interval after the surgery in which osteoclasts were observed. The cementoblasts were missing apical to the crest for a distance of 1 mm.

The adaptation of the buccal flap to the alveolar process was close and a number of young fibroblasts cover the bone. There are numerous labeled cells along the entire junction between the flap and the underlying tissues. In the areas of closest adaptation there are practically no inflammatory cells present, but there is subacute inflammation at the alveolar crest.

The apically repositioned lingual flap shows beginning migration of the epithelial cells over the connective tissue at the border of the flap (Fig. 6D). There is very extensive labeling of epithelial cells at the border of the regenerating epithelium, and marked labeling also in the connective tissue in the same area. The exposed and superficially necrotic alveolar crest and the opening of the periodontal membrane are covered by necrotic debris, fibrin and numerous polymorphonuclear cells. Vascular granulation tissue showing a high degree of labeling has proliferated out of the periodontal membrane. A few spiculae of dead bone are being sequestered.

Seven Days After Flap Surgery

The specimen is from a mandibular segment.

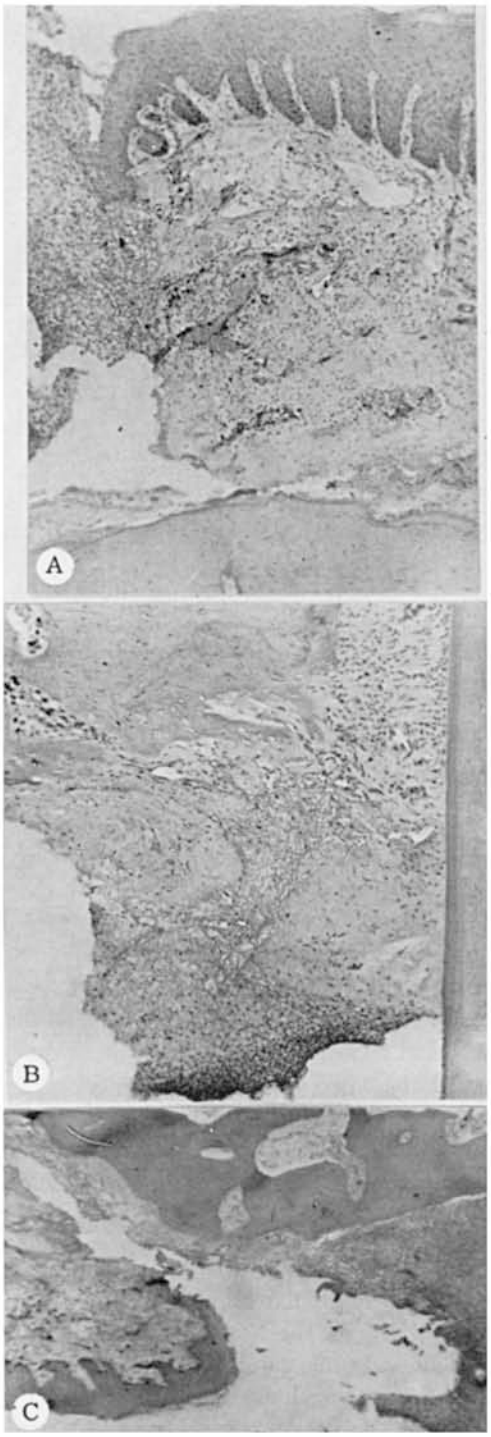

FIGURE 4. *(A) Two days after surgery. Buccal flap over alveolar process, short of contact with tooth. Beginning migration of epithelial cells toward alveolar crest at border of flap. A few labeled epithelial and connective tissue cells. Original magnification X85. (B) Palatal area from same specimen as (A). Wound over periodontal membrane not covered by flap. Surface necrosis. Partial loss of cementoblasts. Labeling of fibrotic bone marrow at upper left corner. Original magnification X97. (C) Interproximal area from same specimen as (A) and (B). Alveolar process covered by coagulated blood. Epithelial extension toward alveolar process. Shrinkage of flap due to fixation. Original magnification X51.*

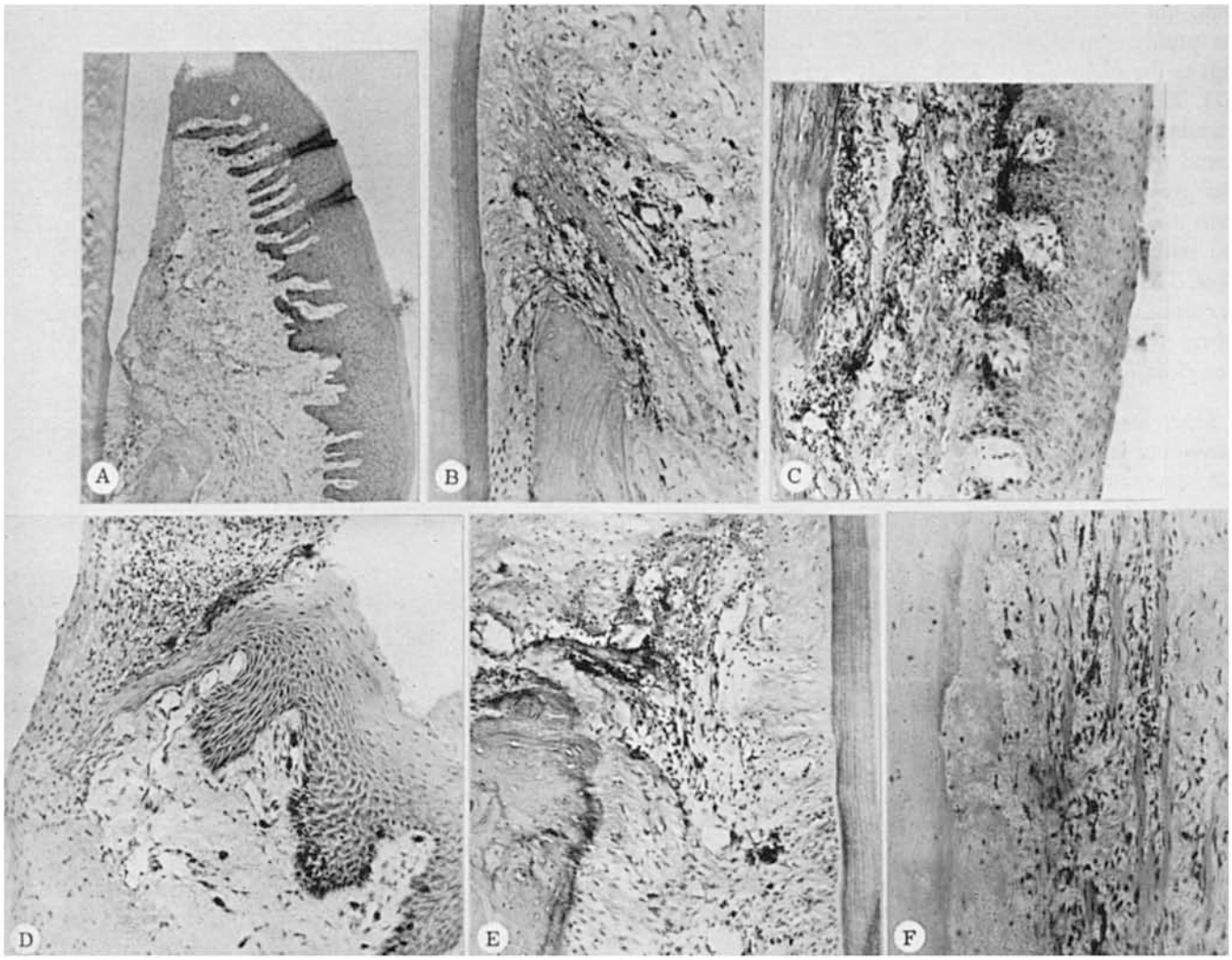

FIGURE 5. *(A) Three days after surgery. Buccal flap with close adaptation to tooth. Intensive labeling toward border of flap. Fibrin and thin blood clot between flap and enamel space. Original magnification X55. (B) From same specimen as (A). Intensive labeling of angioblasts and fibroblasts at alveolar crest on both sides of flap incision. Empty lacunear spaces at alveolar crest. Loss of cementoblasts till bottom part of picture. Original magnification X152. (C) From same specimen as (A). Intensive labeling of epithelial cells. Some inflammation and labeling at junction of flap and alveolar process. Original magnification X147. (D) Lingual flap from same specimen as (A). Slight epithelial migration at border of flap under blood coagulum. Heavy labeling of epithelium almost to border of incision. Original magnification X208. (E) From same specimen as (A). Acute inflammation and loss of cementoblasts at alveolar crest. Labeled angioblasts. Original magnification X126. (F) From same specimen as (A). Junction of flap and alveolar process. Degeneration of collagen fibers. Inflammation and labeling in periosteum. Original magnification X218.*

The posterior parts of the flaps have been closely adapted to the molar teeth, while the anterior aspects have been apically repositioned as much as 1 mm from the surface of the premolars.

Where the flaps have been closely adapted to the teeth on the buccal aspect, there is a beginning formation of an epithelial attachment to enamel (Fig. 7A). This has not reached the cementum-enamel junction yet and there are numerous inflammatory cells adjacent to the bottom of this incomplete epithelial attachment. On the lingual side the epithelium is approaching the tooth, but there is no evidence of epithelial attachment (Fig. 7B).

The buccal flap is adhering to the alveolar crest and the alveolar process by means of numerous young fibroblasts (Fig. 7C), but there also are some osteoclasts present at the crest. They apparently have originated from the marrow spaces rather than from the granulation tissue on the surface.

Under the lingual flap there is severe resorption of the alveolar crest from both the periodontal membrane side and from the lingual side. A sequestrum is evident toward the surface of the granulation tissue.

In the areas of flap repositioning, granulation tissue has partially filled the space between the flaps and the

teeth, but the epithelium from the margin of the flaps has proliferated down along the inside border of the flaps to the surface of the exposed alveolar process (Fig. 7D). Thus, it has partially separated the flaps from the granulation tissue which has proliferated from the periodontal membrane. However, strands of epithelium are also proliferating into this granulation tissue. In some areas these strands have reached the surface of the tooth and initiated the formation of a new epithelial attachment. The vascular granulation tissue on top of the alveolar crest is covered by polymorphonuclear cells and debris. Bacterial plaques are attached to the tooth surface close to this granulation tissue.

There is severe osteoclastic activity on the periodontal membrane surface of the alveolar bone on the buccal side, extending from 2 to 3 mm into the periodontal membrane, and associated with inflammation. On the lingual side there is a less severe bone reaction and at the most apical aspect of the flap, a thin layer of young connective tissue covers the bone and joins the flap to the bone.

An epithelial lined cyst is seen on the buccal aspect of the alveolar process associated with the separation between the flap and the bone about 2 mm apically to the alveolar crest (Fig. 7E). The cyst is about 2 mm wide and filled with precipitated protein material and polymorphonuclear cells.

The origin of this cyst could not be assessed, but it might have been the result of epithelial implantation during the flap surgery.

Nine Days After Flap Surgery

The buccal flap has been adapted closely to the molar teeth and repositioned apically 1 to 2 mm in the area of the premolars. The palatal flap did not quite contact the teeth after the surgery.

In the area of close adaptation, the gingival crevice is epithelialized and a new epithelial attachment has formed (Fig. 8A). However, there is severe chronic inflammation at the bottom of the epithelial contact with the tooth indicating persistent irritation in his area and thus lack of protection from a normal physiologic epithelial attachment.

Severe osteoclastic activity is seen at the alveolar crest (Fig. 8B) and along the alveolar bone for 2 to 3 mm apically to the crest. There is also some cementoclastic activity in this area, and vascular granulation tissue with subacute inflammation has replaced the periodontal fibers.

Areas in which the flaps did not contact the teeth are filled with vascular and inflamed granulation tissue. Proliferating epithelium from the borders of the flaps extends over the granulation tissue toward the teeth, making contact with the teeth in some instances. Epithelial projections also extend along the previous surgical surfaces of the flaps.

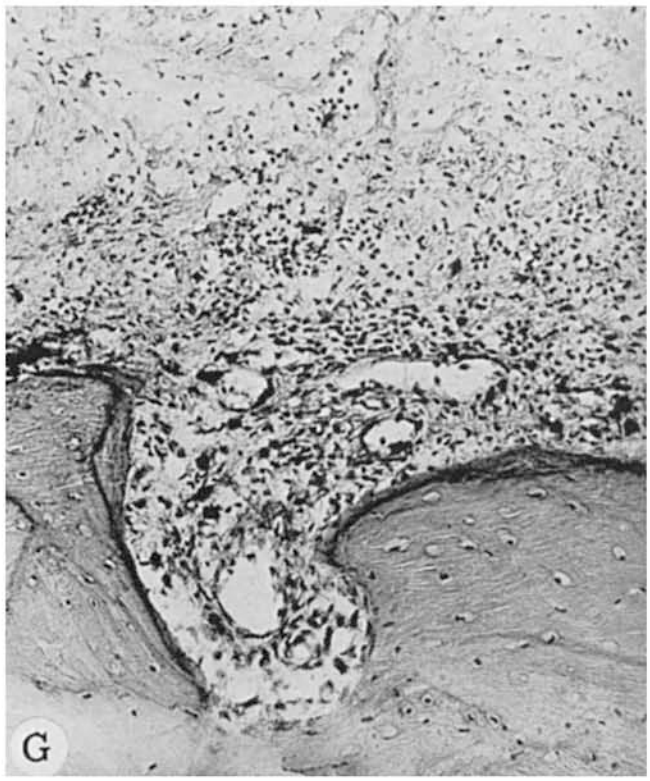

FIGURE 5G. *Interproximal area from same specimen as Fig. 5A. Labeled angioblasts and fibroblasts extending from marrow space opening on surface of alveolar process. Bone necrotic on surface. Original magnification X145.*

The granulation tissue that fills the previous void between the flaps and the teeth apparently has proliferated out from the periodontal membrane. There is no epithelialized gingival crevice.

Mild to severe osteoclastic activity is seen at the alveolar crest. The healing line of the previous incision is easily visible as a band of young connective tissue following the surgical borders of the flap separation through the gingiva and over the alveolar process. There is bacterial plaque on the supragingival surfaces of the teeth.

Fourteen Days After the Flap Surgery

A thin new epithelial attachment to cementum is present on the buccal surface of one tooth (Fig. 9A). However, a deep and wide epithelial projection extends from the apparent margin of the flap down toward the alveolar crest, and parallel with the new epithelial attachment. Between these two epithelial structures the gingiva is made up of vascular granulation tissue with subacute inflammation.

At the buccal aspect of another tooth there is a very

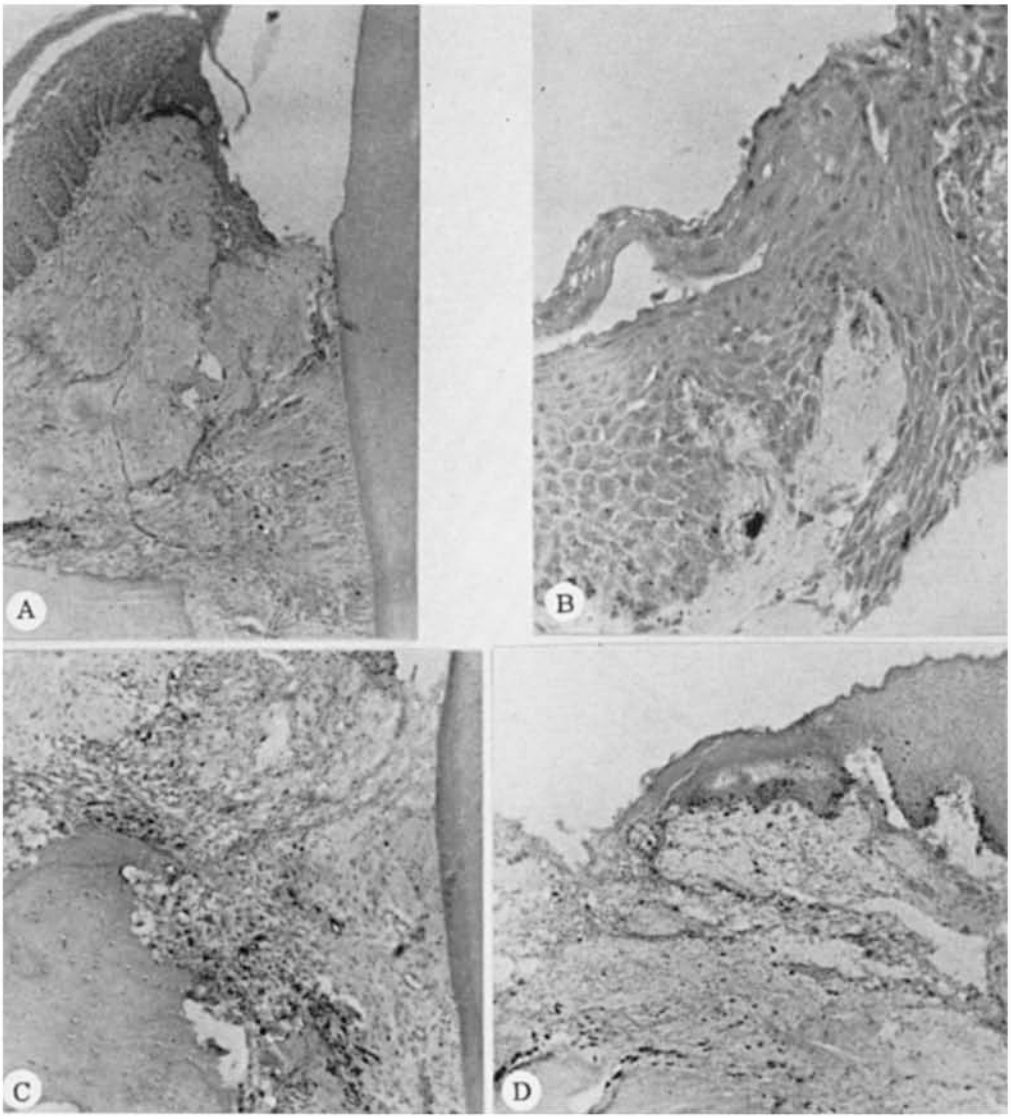

FIGURE 6. *(A) Five days after surgery. Buccal flap closely adapted to bone and margin slightly folded against tooth. Some labeling along line of surgery. Original magnification X83. (B) From same specimen as (A). Epithelial extension at border of flap. Few labeled cells. Original magnification X378. (C) Uncovered wound from same specimen as (A). Osteoclastic activity. Heavy labeling indicating formation of granulation tissue in periodontal membrane. Original magnification X97. (D) Apically repositioned lingual flap from same specimen as (A). Intensive labeling of epithelial cells toward border of wound. Also labeling of connective tissue and heavy labeling on bone surface. Original magnification X97.*

heavy crevicular lining of squamous epithelium with parakeratosis (Fig. 9B and 9C). This may be the result of a downward fold of the margin of the flap at the time of suturing. Sections of a third tooth show what appears to be a normal crevicular lining and a normal epithelial attachment.

The junction between the flaps and the underlying tissues can be followed as a band of young connective tissue. There are still some lacunae on the buccal aspect of the alveolar process and alternating osteoclastic and osteoblastic activity. The new periosteum in the line of healing is made up of young connective tissue cells (Fig. 9D) surrounded by collagen which has not reached the maturity seen in normal periosteum.

A few sections show a sequestrum about 0.3 mm above the alveolar crest.

In some instances the gingival fibers have a functional arrangement, but most of the free gingiva is made up of vascular granulation tissue without functional orientation.

Twenty-one Days After Flap Surgery

A fully epithelialized gingival crevice with a well defined epithelial attachment appears on the buccal aspect

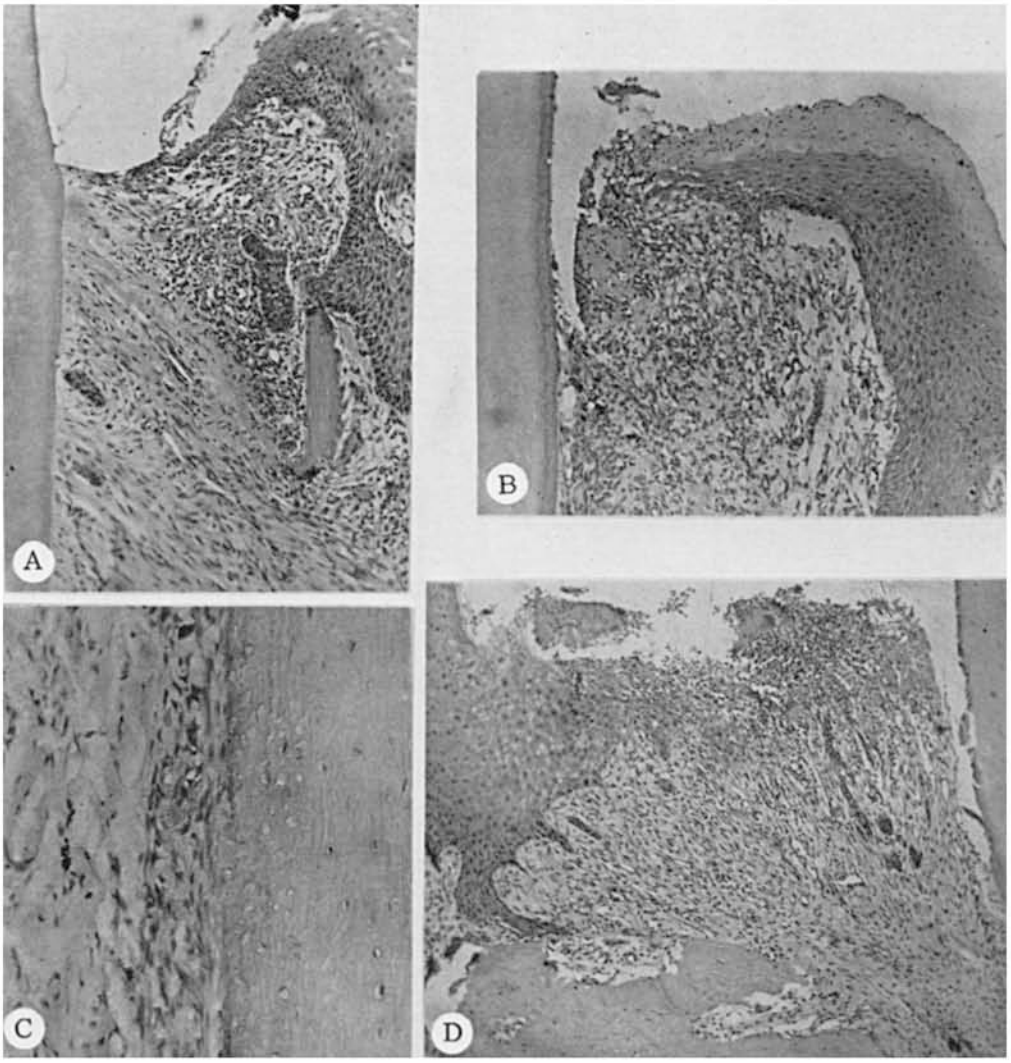

FIGURE 7. *(A) Buccal flap 7 days after surgery. Epithelium extending almost to tooth. Sequestrum. Original magnification X118. (B) Lingual flap from same specimen as (A). No epithelial lining of new gingival crevice. Proliferating granulation tissue. Original magnification X83. (C) Buccal flap from same specimen as (A). Young fibroblasts adhering to partially necrotic alveolar process. Original magnification X230. (D) Apically repositioned lingual flap from same specimen as (A). Epithelium extending to alveolar process at border of flap. Granulation tissue growing out of periodontal membrane and some epithelial strands projecting into the granulation tissue. Osteoclasts at alveolar crest. Original magnification X77.*

of the teeth. However, similar to the findings 14 days after surgery, there is a deep ridge of epithelium running parallel to the epithelial attachment (Fig. 10A). The space between these two bands is filled with chronically inflamed connective tissue.

The entire gingival crevice is on cementum.

There is beginning functional arrangement of the immediate supracrestal fibers. The periosteum on the buccal aspect of the alveolar process still has not fully matured. There is evidence of osteoblastic activity at the alveolar crest and on the periodontal membrane side of the alveolar bone (Fig. 10B). Cementoblasts are lined up in a regular fashion without interruption to the bottom of the new epithelial attachment.

The flap on the lingual side probably was adapted closer to the bone at the time of surgery than the buccal flap was, since the lining of the crevicular epithelium on the lingual side is more regular. However, part of the crevicular lining resembles surface epithelium rather than normal crevicular epithelium. There is abundant chronic inflammation close to the crevicular lining and extending down to the bottom of the new epithelial attachment. Occasional osteoclasts are present at the alveolar crest, but in most instances there is marked osteoblastic activity and the crest is covered by young connective tissue with only a limited amount of collagen.

Thirty-five Days After Flap Surgery

The buccal flap apparently had not been well adapted

FIGURE 7E. *Cyst on buccal aspect of alveolar process in line of flap surgery. Same specimen as Fig. 7A. Original magnification X46.*

to one of the teeth at the time of surgery. A new epithelial attachment is present, but the entire free gingiva is penetrated by numerous epithelial strands. There is severe chronic inflammation extending toward the alveolar crest, but osteoblastic activity is seen at the crest (Fig. 11). The periosteum has not yet matured to normal periosteum on the buccal aspect of the alveolar process.

The epithelial attachment and the gingival adaptation is normal for the other teeth. A couple of resorption lacunae are seen in the supracrestal cementum and dentin. There is some fibrosis of the marrow spaces associated with rapid regeneration of bone at the alveolar crest.

On the lingual aspect there is a fairly normal gingival crevice extending to the cementum-enamel junction. Chronic inflammation is seen in the entire gingiva. There is marked osteoblastic activity at the crest and the periosteum is still made up of young fibroblasts without a well defined fiber arrangement.

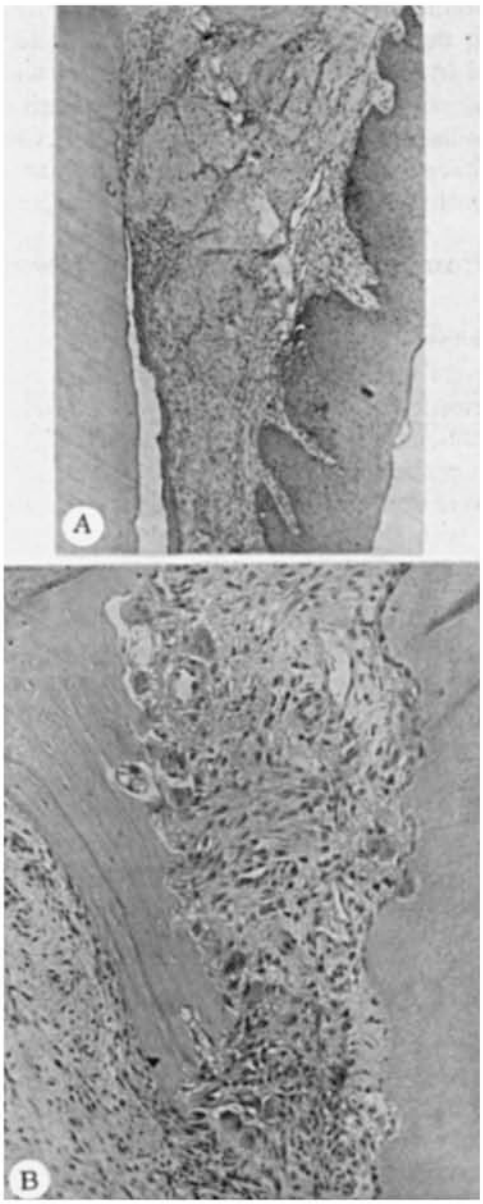

FIGURE 8. *(A) Buccal flap 9 days after surgery. Epithelial lining of gingival crevice chronic inflammation. Original magnification X77. (B) Periodontal tissues under poorly adapted buccal flap 9 days after surgery. Osteoclastic and cementoclastic activity. Granulation tissue replacing periodontal membrane. Original magnification X208.*

Seventy-two Days After Flap Surgery

Both the buccal and lingual flaps have healed (Fig. 12) and there is a well defined new epithelial attachment to cementum both on the buccal and the lingual aspect of the teeth. Total crevicular depth including epithelial attachment is about 1 mm. Some chronic inflammation is seen associated with the bottom of the gingival crevice. The mild gingival inflammation does not extend to the bottom of the new epithelial attachment. Small amounts of calculus are present on the lingual surface

of the teeth. There are several layers of newly formed bone on the tip of the alveolar crests. There is pronounced hyperkeratinization of the gingiva with parakeratosis of the free gingival margin. Gingival fibers have assumed a functional arrangement, but they still do not have the mature collagenous appearance seen in the control specimens. The periosteum appears normal.

HISTOMETRICAL VARIATIONS IN ATTACHMENT AND BONE LEVELS

The impact of the flap surgery on the attachment and bone levels was studied histometrically by comparing measurements of attachment and crest levels to the cementum-enamel junction from control, 21 days and 72 days postsurgical specimens. Only buccal measurements were used since they anatomically are most comparable both from the maxilla and the mandible. The means of 30 measurements from each group were compared (Table 1).

The loss of attachment as indicated in the mean difference between the control and measurements from the 21-day specimen was statistically significant: $P. < .05$ (Table 2).

It appeared that the alveolar chest also had been lowered as a result of the surgery when the measurements from the 21-day specimen were compared with the controls: $P. < .01$ (Table 2).

However, the measurements for attachment levels and bone loss (Table 2) from the 72-day specimen did not differ significantly from the controls.

When the measurements from the 21-day specimen were compared with measurements from the 72-day specimen, there was a statistically significant gain in the latter group for attachment, $P. < .05$, and for bone level $P. < .01$ (Table 2). It appears from these figures that an early loss of attachment and crestal bone 21 days after flap surgery was regained after 72 days of healing.

DISCUSSION

Previous reports of healing without significant loss of attachment or bone following reverse bevel surgery[8–11, 13] were confirmed by the present study.

However, a number of new observations were made on the various stages of healing and their relationship to flap positioning.

1. Flap Adaptation to Teeth and Bone

In order to have healing by first intention, the parts have to unite directly without the intervention of granulation tissues. Such healing was observed between the flap and the underlying tissues only in selected small areas with minimal surgical trauma and ideal flap adaptation. Healing by second intention (union by adhesion of granulating surfaces) was the common finding with a reasonably well adapted flap; however, between the tooth and the flap there was often an area of poor adap-

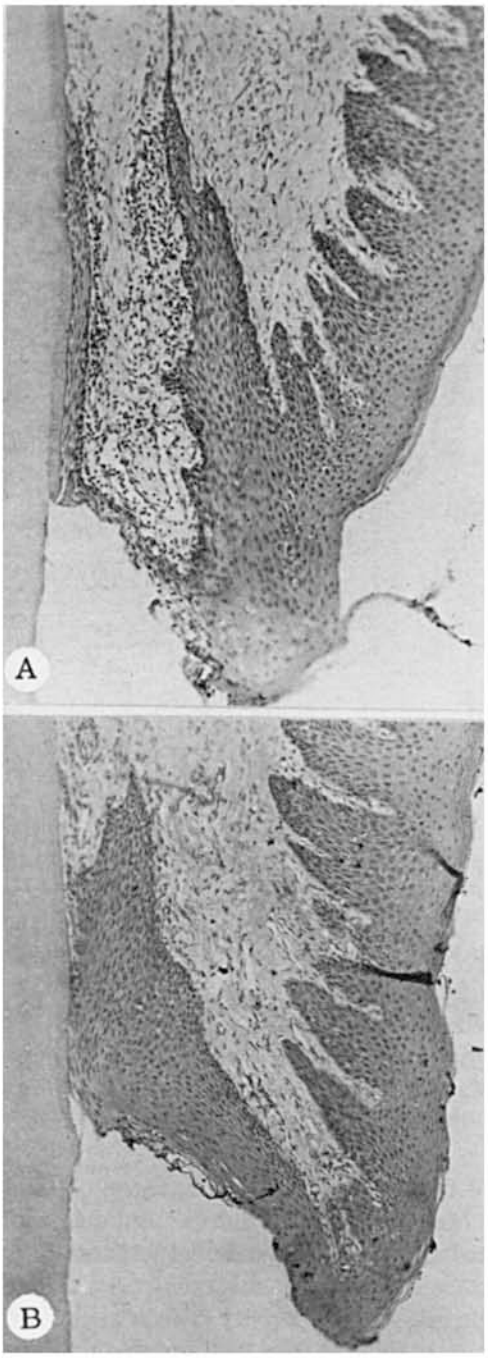

FIGURE 9. *(A) Buccal flap 14 days after surgery. Epithelial attachment to cementum. Epithelial ridge parallel to epithelial attachment and probably residual from epithelial proliferation at border of surgical flap. Original magnification X97. (B) Parakeratosis of crevicular lining 14 days after surgery. Deep downgrowth of epithelium which in part serves as epithelial attachment to cementum. Original magnification X103.*

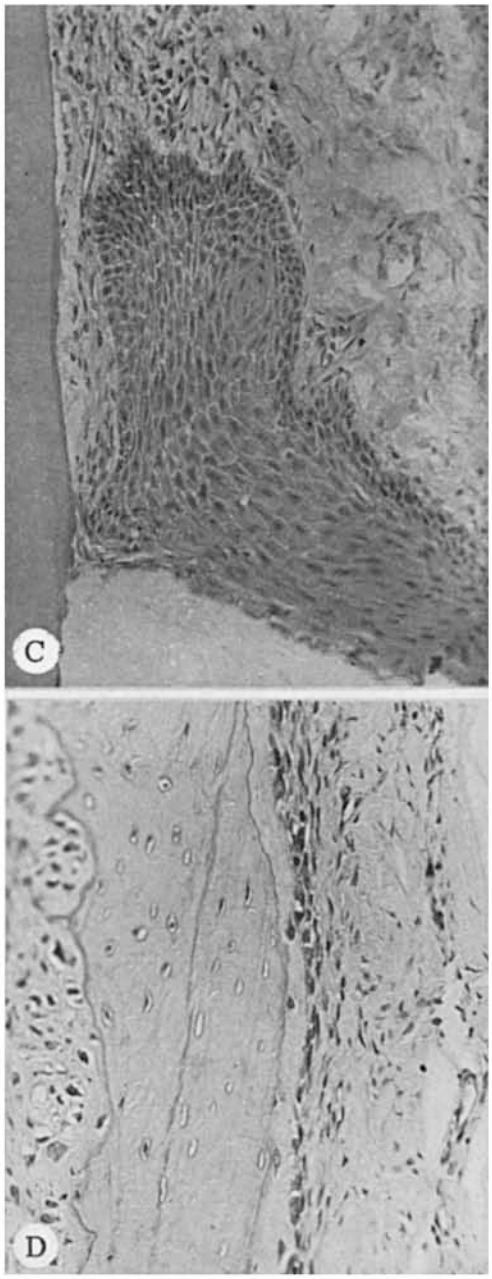

FIGURE 9C. *High magnification from same specimen as Fig. 9B. Epithelium extending apically to connective tissue attachment to cementum. Original magnification X224. (D) Buccal aspect of alveolar process 14 days after surgery. New bone formed on the surface of partially dead bone. Immature periosteum middle of picture. Original magnification X223.*

tation which had to heal by third intention (union by filling the void with granulation tissue).

An ideal tight flap adaptation to the tooth was followed by fast healing of all periodontal structures including the epithelial attachment. The inflammatory response was minimal, and did not extend beyond the immediate vicinity of the incision for the flap. Poor flap adaptation to the tooth not only lead to delayed healing by third intention but it also was followed by complications such as wound infection, necrosis, severe inflammation extending into the periodontal membrane, bone resorption, interference with cemento-genesis, and structural weakness of the regenerated free gingiva. The tissue reactions associated with a poorly adapted flap were essentially the same as has been described following the denudation of the alveolar process.[16, 17, 20]

Spaces filled with blood between the flap and the bone which were associated with poor adaptation also led to delayed healing, more inflammation and more bone resorption than occurred with close flap adaptation.

2. Epithelial Regeneration

The surgical separation of the flap had a "stunning" effect upon the premitotic activity of the epithelial cells (expressed as diminished tritiated thymidine labeling). Less than normal labeling was already observed one hour after the surgery, but a marked decrease (almost to total absence of labeling of the flaps) occurred in the 12- and 24-hour postsurgical specimens. This "stunning" or depressant effect was more marked in thin buccal flaps than in thick palatal flaps. The pattern and time sequence of labeling in the regenerating epithelium from the margin of the flap also was entirely different than the pattern and time sequence which has been reported after gingivectomy.[21] While the premitotic activity of the epithelium after gingivectomy reached its peak 24 hours after the surgery,[21] there was no labeling and no indication of epithelial migration at the surgical border of the buccal flaps at that interval of time. On the palatal aspect where the flaps were thicker there was an increase in number of labeled cells 0.5 to 1 mm palatally to the wound margin, but no labeling close to the wound. Even after 2 days, minimal labeling was observed, although some epithelial cells had migrated for a short distance over the gingival margin of the flap. Considerable labeling of angioblasts and fibroblasts indicated growth of granulation tissue from the periodontal membrane. Thus, after flap surgery the connective tissue regeneration starts earlier than the epithelial regrowth. The reverse sequence of premitotic labeling is seen after gingivectomy.[21]

After 3 days the labeling toward the border of the flaps was still minimal, but there was a marked increase in epithelial labeling 1 to 2 mm away from the incision. There was some epithelial migration over the borders of the flaps and granulation tissue had formed where the flaps did not make contact with the teeth. The epithelium approached the bone surface at the borders of the flaps while rapidly proliferating granulation tissue from the periodontal membrane had grown up between the flaps and the teeth. Thus the migrating epithelium partially

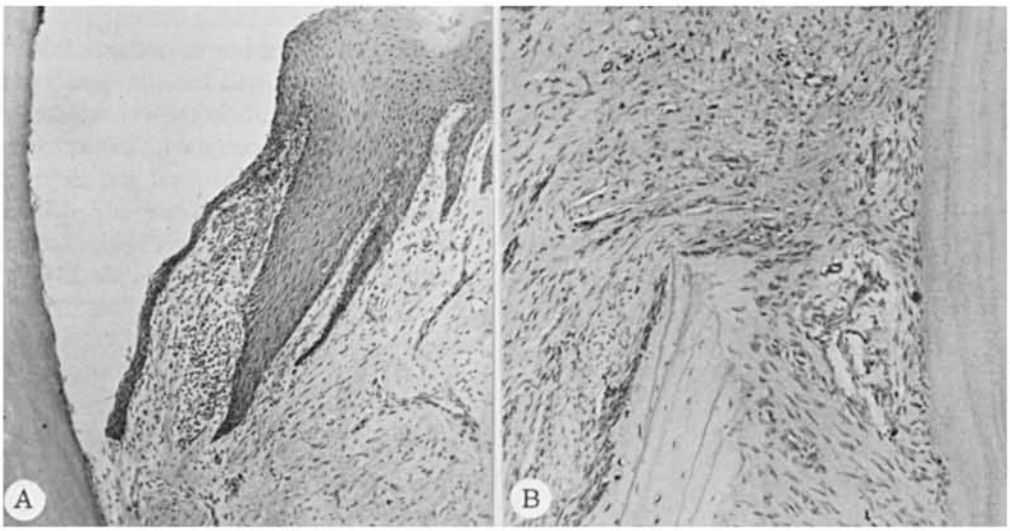

FIGURE 10. *(A) Buccal flap 21 days after surgery. Epithelial lining of gingival crevice and parallel projection of epithelium similar to Fig. 9A. Original magnification X127. (B) Alveolar crest from same specimen as (A). Regeneration of periodontal fibers. Periosteum still immature on buccal aspect of alveolar process. Original magnification X139.*

separated the flaps from the granulation tissue and the teeth.

The epithelial regeneration reached its peak as expressed in labeling 5 days after flap surgery (this occurred 1 to 2 days after gingivectomy).[21]

Four patterns of epithelial regeneration were found,

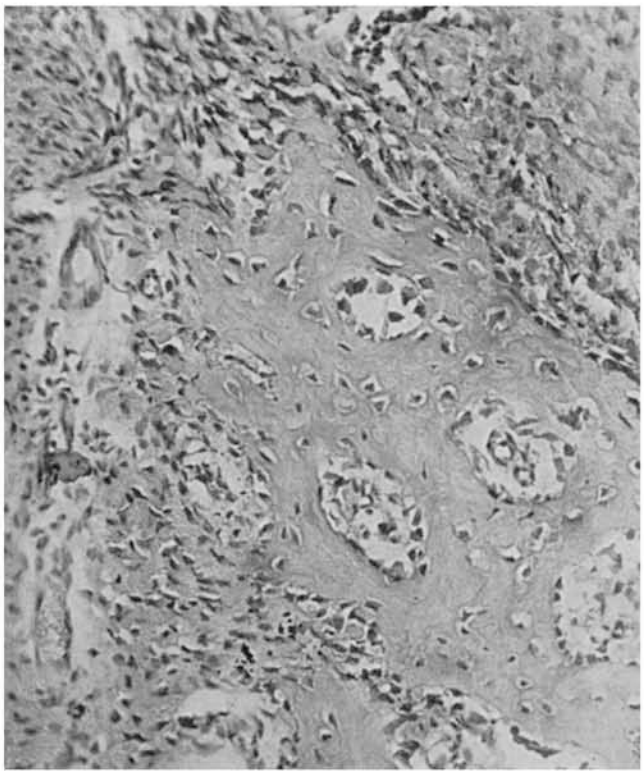

FIGURE 11. *New bone formation on buccal alveolar crest 35 days after surgery. Immature periosteum. Original magnification X190.*

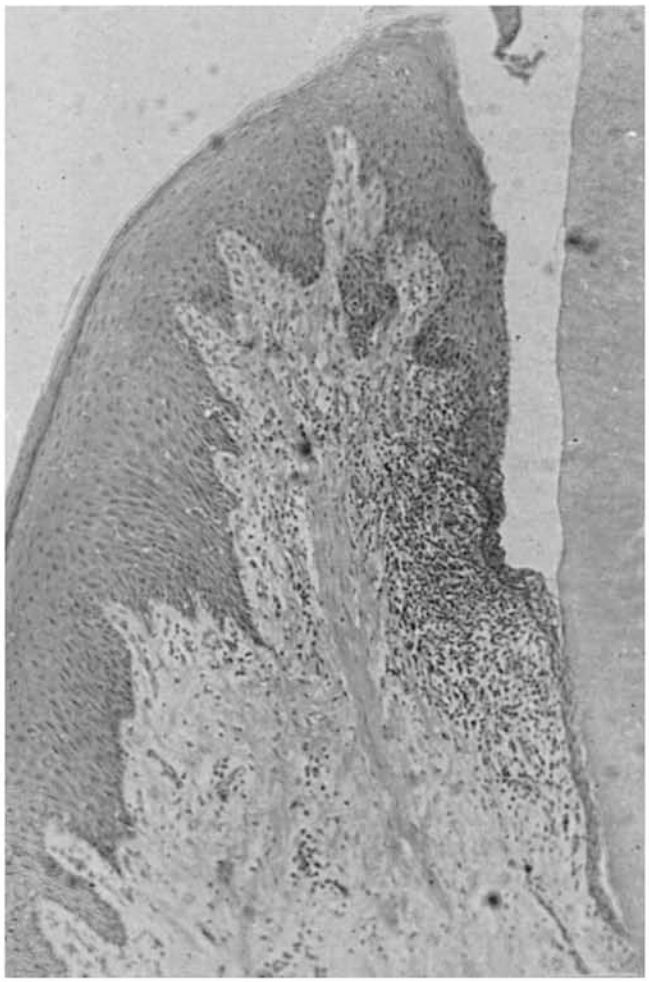

FIGURE 12. *Buccal flap 72 days after surgery. Chronic gingivitis with epithelial attachment on cementum. Original magnification X83.*

TABLE 1
Mean Distances Between C-E Junction and Epithelial Attachment and Between C-E Junction and Alveolar Crest

	*CEJ/Ep. Att.**		*CEJ/Alveolar Crest**	
	$\bar{X}$	*S.D.*	$\bar{X}$	*S.D.*
Control	.616	.37	1.276	.48
21 days	1.569	.46	2.224	.13
72 days	.789	.38	1.658	.28

*In millimeters.

dependent on the relationship between the flap and its surroundings.

A. If the flap was closely adapted to the tooth, the regenerating epithelium formed a new sucular lining and epithelial attachment in 2 to 3 weeks. However, this regenerating epithelium did not initially form an epithelial attachment or seal, since it regenerated under a thin layer of necrotic debris on the flap surface.

B. If the flap was well adapted to untraumatized underlying tissues but stopped slightly short of making contact with the tooth, the epithelium migrated over the granulation tissue which connected the flap with the underlying tissues and filled the space between the flap and the tooth. This pattern of epithelial regeneration appeared to be similar to healing following gingivectomy.[21]

C. A third and more unfavorable pattern of healing occurred when the flap did not contact the tooth and additional disturbing factors were present such as: (1) poor flap adaptation; (2) severe trauma to the flap and the underlying tissues; (3) bone exposure; (4) severe inflammation as a result of infection. In such cases a partially separating ridge of epithelium developed between the flap and the regenerated gingival tissues. Eventually a new epithelial attachment developed in these cases from proliferating epithelial strands interspersed in the regenerating granulation tissue and from epithelium proliferating from the border of the flap over the surface of the granulation tissue. The connective tissue between the epithelial ridge associated with the flap and the epithelial attachment was highly inflamed. It appeared that regenerated gingiva which included such epithelial ridges did not provide adequate gingival tone for the firm adaptation which is characteristic of a physiologic epithelial seal to the tooth. Although it must be assumed that these epithelial ridges gradually undergo involution, they represent a disturbed pattern of healing which can be avoided by careful surgical technique.

TABLE 2
Analysis of Between Group Differences

				S.D.	0.46		0.13	
				$\bar{X}$	1.569		2.224	
					21 days			
$\bar{X}$	S.D.				CEJ/E.Att.		CEJ/Bone	
0.616	0.37	CONTROL	CEJ / E. Att.	7	2.59 / 0.59	2.36	3.14	
1.276	0.48		CEJ / Bone					3.38 / 1.24
					CEJ/E.Att.		CEJ/Bone	
					72 days			
				$\bar{X}$	0.789		1.658	
				S.D.	0.38		0.28	

D. In some instances the surface gingival epithelium apparently had been folded under the margin of the flap against the tooth surface as a result of the pull and "deep bite" of the suture. The keratinized gingival epithelium did not initially function as a sealing epithelial attachment. However, there were too few specimens of this type to assess the role of such folded margins.

3. *Bone Reaction*

As indicated by previous investigators, the reaction of the bone to the surgery depended to a great extent on the presence or absence of a covering flap, and to a lesser extent on whether or not some bone had been surgically removed.[10, 13, 16, 22, 23] Untraumatized bone covered by well adapted flaps showed only mild delayed and very superficial bone resorption as part of the late (2 to 3 weeks) stage of healing. Bone that had been traumatized by chisels or files developed some empty lacunae immediately, indicating necrosis for a short distance under the surface, even when covered with a well adapted flap. The superficial necrosis was followed by slight resorption which started about 5 days after the surgery. Under a well fitting flap this resorption lasted only a few days, was superficial and underwent prompt repair. Similar limited resorption and fast repair occurred in areas in which a blood coagulum separated the overlying flap from the bone.

The most severe bone reaction occurred at the alveolar crest when the flaps were repositioned apically from the necks of the teeth, exposing bone. In such areas inflammation and bone resorption extended 1 to 2 mm into the periodontal membrane and the healing was very much delayed. It appeared that an opening of the periodontal membrane at the alveolar crest does not constitute an effective barrier against penetration of irritants into the periodontal membrane. An overlying epithelial or thick connective tissue covering is needed to avoid spread of inflammation into the periodontal membrane and subsequent bone resorption. Although the resorption was initiated as early as 5 days after the surgery, the most severe bone resorption was seen 9 days postoperatively and resorption was still present 35 days after the surgery. Resorption on the periodontal membrane side of the alveolar bone has been described by some investigators[9] but not found by others.[15] Apparently such resorption depends on whether or not the flap and residual tissue give sufficient protection to the underlying periodontal membrane. However, both the histologic observations and the histometric measurements confirmed previous reports[9, 10, 13] which indicated that the resorbed bone has a tendency to regenerate back to the preoperative level within 10 weeks.

4. Connective Tissue Healing

Protrusion of granulation tissue from the periodontal membrane in the early state of healing has been described by several investigators.[16, 17] According to the tritiated thymidine labeling in the present study, connective tissue proliferation in this area reached a peak 2 to 3 days after the surgery. The periosteal healing reuniting the flap with the bone however is a much slower process. When there were remains of periosteum on the bone and a good flap adaptation, numerous labeled cells appeared at the junction line between the flap and the residual periosteum in 3 to 5 days; but where the periosteum had been completely stripped from the bone or traumatic necrosis had occurred, no labeled cells were seen at the surgical border of the flap for at least 3 days. Osteoclasts developed and removed some of the surface of the bone, thus permitting the periosteum to be reattached to the bone. This osteoclastic stage of periosteal repair took place 7 to 9 days after the surgery. After 9 days there was a definite band of young connective tissue joining the flaps to the bone and to the underlying connective tissue. New bone formation and periosteal reattachment sometimes took place on the surface of dead bone, but such areas underwent subsequent remodeling resorption which was still going on 72 days after the surgery. The maturation of the periosteum and the regenerated gingival tissues depended a great deal on the degree of flap adaptation and was not entirely finished in the 72-day specimens. However, with ideal flap adaptation healing appeared to be fairly complete after 21 days.

Although not specifically investigated in this study, it appeared that a supraperiosteal flap would have resulted in much faster and less complicated healing than the healing which occurred following mucoperiosteal separation. This assumption is based on the observation of fast healing in areas where the periosteum had been left on the bone; also, in part, on Pfeifer's observations.[16]

Infection, inflammation and irregular epithelial regeneration interfered with the functional maturation of the connective tissues.

5. Cementoblastic Activity

In previous papers,[19, 24] it has been indicated that the cementoblasts are sensitive to metabolic changes in the periodontal tissues as they disappeared or showed an irregular arrangement in some instances for a distance of 0.5 to 1 mm under the flap incision as early as 1 hour after the surgery.

In the 3-day specimen the cementoblasts were absent as far as 2 to 3 mm from the incision and they did not reappear until the healing was fairly complete. The variations in cementoblastic activity seemed to depend on flap adaptation, surgical trauma and inflammation.

A few labeled cementoblasts were seen, mainly in the bi and trifurcation areas and at the apex areas of all the sections.

SUMMARY AND CONCLUSIONS

1. Healing following reverse bevel periodontal flap surgery may be by first, second or third intention; depending mainly on the state of flap adaptation to teeth and bone.

2. Close flap adaptation to the teeth and complete coverage of interproximal, as well as buccal and lingual bone, leads to a much faster and more uncomplicated healing than occurs following apical repositioning of the flap.

3. As a result of a "stunning" effect from the surgery, epithelial regeneration from the border of a flap is delayed 2 to 4 days.

4. Flap separation on top of the periosteum (split flap) will heal faster than a mucoperiosteal flap.

5. Transient lowering of the attachment level and bone resorption at the alveolar crest 3 to 4 weeks following flap surgery tend to heal back to the presurgical level within 10 weeks after the surgery.

6. Various disturbed patterns of healing and their possible clinical significance have been discussed.

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Announcements

ALBERT EINSTEIN COLLEGE OF MEDICINE

Albert Einstein College of Medicine announces the following courses:

Periodontics, PDP 69 (5 day participation course), Irving Yudkoff, D.D.S., and Marvin N. Okun, D.D.S., assisted by Joseph Puccio, D.D.S., and Zachary Dembo, D.D.S., Monday through Friday, November 4-8, 1968; $275.

Periodontics, DPD 66 (a ten session participation course), Marvin N. Okun, D.D.S. and Irving Yudkoff, D.D.S., assisted by Joseph Puccio, D.D.S. and Zachary Dembo, D.D.S., Wednesdays, February 19 through April 23, 1969; $325.

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For further information and application, write to: Dr. Irving Yudkoff, Director, Postgraduate Dental Program, Albert Einstein College of Medicine, Bronx, New York 10461.

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A course in Forensic Dentistry will be offered at the Armed Forces Institute of Pathology on October 7-11, 1968. It is the only course of its kind given to enable dentists to receive background information in a field that is quickly developing in the United States.

For additional information write: William G. Sprague, Colonel, USAF, DC, Chief, Dental and Oral Pathology Division, Washington, D.C. 20305.

DR. MARVIN M. ALDERMAN HONORED

Dr. Marvin M. Alderman, member of the American Academy of Periodontology, was the recipient of the 1968 University of Pennsylvania Alumni Award of Merit for outstanding service and devotion to the profession of dentistry, at the Annual Dental Alumni dinner held in Philadelphia, on Friday, May 17th.

A graduate of the University of Pennsylvania School of Dental Medicine in 1942, Dr. Alderman has been a part-time faculty member of that dental school's Department of Periodontics since 1962 which has entailed his flying to Philadelphia weekly, thus the nickname "the flying educator." He is a practicing periodontist in Syracuse. He has made a major study of the disorders of the Temporomandibular Joint and lectures widely on this subject.