

# Histogenesis of Repair Following Osseous Surgery

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by

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OSSEOUS SURGERY PROCEDURES are being performed on the vestibular bone over the tooth root by the profession for correction of alveolar bone deformities resulting from periodontal disease. Various mucoperiosteal flap operations are often used in conjunction with osseous surgery to gain access and visibility to the alveolar bone during the surgery. Osseous surgery correcting bone deformities can be classified as osteoectomy or osteoplasty. An osteoectomy in periodontal surgery indicates the bone immediately adjacent to the periodontal ligament is removed; an osteoplasty is performed if any of the remaining bone other than bone immediately adjacent to the tooth is removed. The two types may be utilized separately or together.

## REVIEW OF THE LITERATURE

Previous histological studies in animals and humans of healing process after elevation and replacement or replacement of different mucosal flap procedures have been reported in the literature. Kohler and Ramfjord<sup>1</sup> elevated and replaced gingival mucoperiosteal flaps over 15 human teeth and indicated the flap reattached to the underlying bone and tooth surfaces. Although some resorption occurred to the alveolar process in the early postoperative specimens, bone apposition was evident in the later specimens and as the result the bone crest was insignificantly lowered. Other human histological investigations<sup>2,3</sup> of the healing of mucoperiosteal flaps reported similar findings. Animal studies by others<sup>4,5</sup> presented findings similar to the findings in human investigations. In a clinical study involving humans Donnerfeld et al<sup>7</sup> recorded an average 0.63 mm of crestal bone loss over the radicular area. A mucoperiosteal flap was elevated and, although the flap was sutured in a

more apical position, the buccal alveolar bone over the tooth root was completely covered by the flap. Felts and McKenzie,<sup>8</sup> performing surgical techniques with and without osseous surgery and by clinical measurements of bone levels at approximately three months postoperatively, concluded slightly more bone loss occurred if osseous surgery was performed.

A split thickness flap technique<sup>9</sup> in dogs demonstrated complete restoration of the alveolar crest even though osteoclastic resorption occurred on the periosteal bone surface early in the postsurgical time periods. In the split thickness flap operation, the flap consisting of gingival epithelium and a superficial layer of lamina propria was formed by a sharp dissection through the lamina propria of the gingiva. The deep portion of the lamina propria and the periosteum over the bone was left intact. The split thickness flap was elevated and then replaced and sutured in its original position covering the retained lamina propria. A limited human study of the split thickness flap healing verified animal findings.<sup>2</sup>

In an animal histological study,<sup>4</sup> the periosteal or buccal alveolar bone surface was reduced with a rotary diamond stone. A mucoperiosteal flap operation was performed to gain access to bone. The authors indicated that on the 28 postoperative day and after osteoblastic repair to the bone, the alveolar crest was reduced and was located from 0.0 to 1.7 mm more apically than when a mucoperiosteal procedure alone was performed.

Matherson<sup>10</sup> in a microscopic and macroscopic study of the alveolar process following different surgical procedures, namely, a gingivectomy, mucoperiosteal flap surgery and osteoplasty with osteoectomy on three monkeys indicated no additional change of contour or loss of bone at the six-months postoperative sacrifice period.

Human histological investigations following osseous surgery have been limited to a study<sup>3</sup> around two teeth. A mucoperiosteal flap procedure and an osteoplasty, using a rotating steel bur, was performed over one tooth root and the results indicated an additional 0.3 mm crestal bone loss after 84 days of healing. Over another tooth root a mucoperiosteal flap and an osteoectomy procedure removing 2.0 mm of alveolar crest with a chisel revealed a 0.25 mm loss of crestal bone during the healing processes. The crestal bone loss in both osseous procedures was considered negligible. Histological interpretations of bone changes over the two teeth were presented at the two long term healing periods.

A recent clinical study<sup>11</sup> utilized standardized photographic techniques<sup>12</sup> for measuring alveolar bone height of the vestibular bone over tooth roots and for determining crestal loss after repair following osteoplastic and osteoectomy procedures. Measurements were made

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over 34 teeth from 20 patients with postoperative healing intervals ranging from 14 to 545 days. The average reduction in height of alveolar crest after healing was an insignificant 0.54 mm.

Histological animal and human investigations employing two other types of surgical techniques indicate some changes to the radicular alveolar process, the bone over the tooth root. Postsurgically either marginal alveolar bone<sup>13-15</sup> or the connective tissue<sup>15, 16</sup> of marginal gingiva over the bone remained exposed until healing occurred. The findings in the alveolar bone exposure procedure indicated considerable permanent loss of bone crest height had occurred in the radicular area with little or no permanent alteration of bone height in the interradicular area. Less radicular crestal bone was lost if some periosteal or gingival connective tissue was retained over the bone at the time of surgery.

Other investigations indicate little if any crestal bone loss occurs after healing in mucoperiosteal flap procedures alone, that is without osseous surgery.<sup>1, 6, 16, 17</sup> Animal and human studies reveal a difference of findings from relatively no loss to some loss of crestal bone following healing after osseous surgery. Only two animal short term histological studies<sup>4, 10</sup> utilizing seven animals investigated the effect of periodontal osseous surgery, and only one microscopic study<sup>3</sup> around two teeth presented an interpretation on healing of bone at three months following osseous surgery in humans. However, recently an animal study on monkeys<sup>18</sup> of two and one-half months duration investigated the healing of a combined mucoperiosteal flap operation and a minor osseous surgery procedure. This was a microscopic study that also incorporated an audioradiographic technique in its experimental design. An unmeasured limited osteoplasty was performed by a chisel on 1.0 mm of a slightly reduced alveolar bone crest. Almost complete bone repair occurred after 72 days. This study seemed to add to the confusion in the literature and did not simulate the usual clinical osseous surgery procedures.

This histological investigation is a long term study from 0 hour to 18 months with definite time periods to consider the detailed sequence of the histogenesis of repair in humans of tissues covering the tooth root, a most critical area, following a mucoperiosteal flap operation and an osseous surgery procedure, one of the most utilized type of surgical intervention used in periodontics in the past decade. The osseous surgery consisted of a combined osteoplastic and osteoectomy procedure. In addition, since this is a correlative investigation to the quantitative study of repair of the alveolar bone<sup>11</sup> previously reported, similar tooth reference points were placed at the time of the experimental surgery and microscopic measurements made either to affirm or negate the findings of the quantitative study.<sup>11</sup>

## MATERIALS AND METHODS

Twenty-three teeth with the periodontium from 23 individuals scheduled for full mouth extractions were used for this investigation. The individuals' ages varied from 35 to 68 years. The gingiva and the preiodontium around all teeth exhibited disease ranging from a slight gingivitis to a moderate periodontitis with a sulcus depth from 1.0 to 5.0 mm (Table 1). The teeth and periodontium selected for investigation included 5 maxillary central incisors, 9 maxillary lateral incisors, 4 maxillary cuspids, 1 maxillary first bicuspid, 3 maxillary second bicuspid and 1 mandibular cuspid.

A rotary diamond disc (4.4 mm in diameter and 1.0 mm thick) was used to place a reference notch  $N_1$  (Fig. 1 and 2) across the mesial distal width of the tooth crown outlining the marginal gingiva over the tooth. An internal, beveled incision, originating at the gingival margin and two vertical incisions were utilized to aid in the elevation of a gingival mucoperiosteal flap to expose the underlying alveolar process (Fig. 3). The thickness of the alveolar process was now estimated and classified by the operator as thin, medium or thick.

The diamond disc rotating under running water was used to place a horizontal groove (Fig. 2A) in the exposed vestibular bone approximately 5.0 mm apical to the alveolar bone margin. Vertical groove (B) and two cross grooves (C) were made as indicated on Figure 2. The horizontal and vertical grooves served as boundaries for bone reduction (Table 3). All grooves were made as constant in depth as possible in order to serve as uniform guides for an even removal of the vestibular surface bone to a consistent depth. This surface reduction, an osteoplasty, was carried out to the

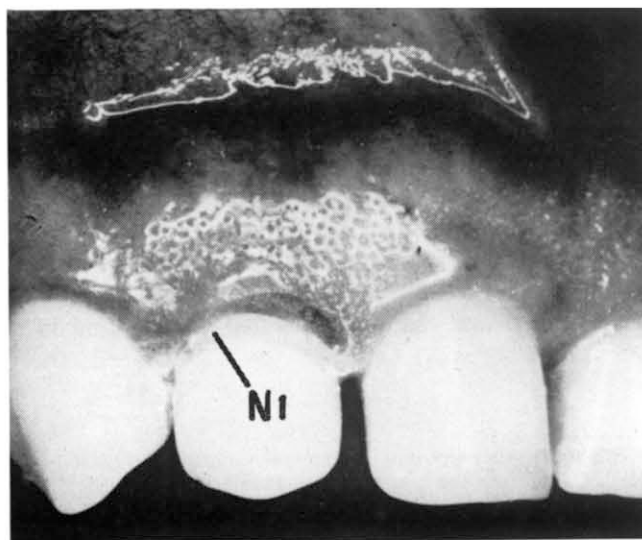


FIGURE 1. Preoperative clinical photograph of the experimental site, the right maxillary lateral incisor. Notch ( $N_1$ ) placed in crown marking the level of the marginal gingiva prior to the formation of a mucoperiosteal flap.

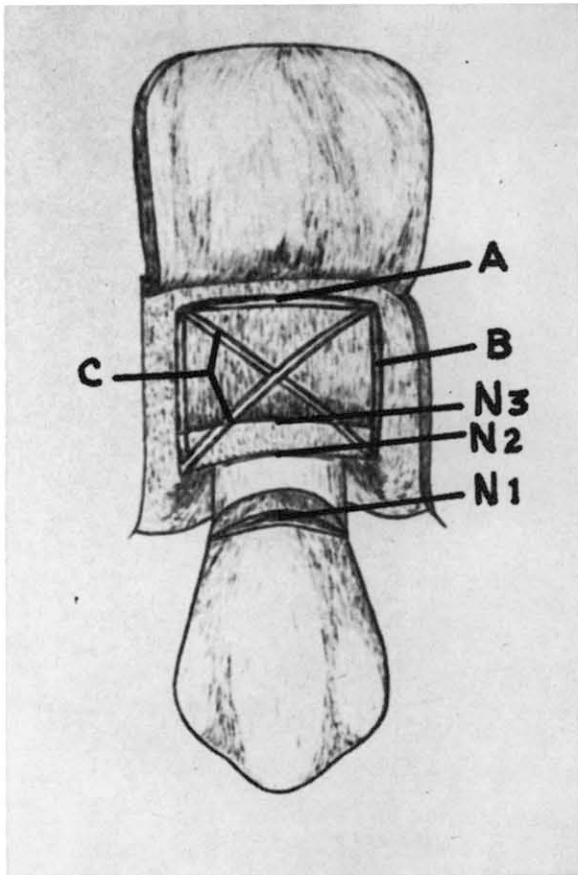


FIGURE 2. Diagram exhibiting grooves placed in bone to serve as guides for even surface removal of vestibular bone. Reference notches in right maxillary cuspid tooth are evident.

- A—Horizontal groove.
- B—Vertical grooves.
- C—Cross grooves.
- N<sub>1</sub>—Crown notch—location of presurgical marginal gingiva.
- N<sub>2</sub>—Scapel root notch prior to removal of 1.0 mm alveolar crest bone.
- N<sub>3</sub>—Scapel root notch after removal of 1.0 mm alveolar crest bone.

TABLE 1  
Vestibular Sulcus Depth as Measured by the Periodontal Pocket Measurer Prior to Surgery

Maximum	Minimum	Average
5.0 mm	1.00 mm	2.5 mm

TABLE 2  
Surgical Crestal Bone Reduction from N<sub>2</sub> to N<sub>3</sub> (Osteoectomy)\*

Maximum	Minimum	Average
1.6 mm	0.2 mm**	0.73 mm

\*Measured by standardized photographic technique at time of surgical intervention.

\*\*One area presented extremely thin vestibular plate and necessitated a minimal osteoplasty and osteoectomy.

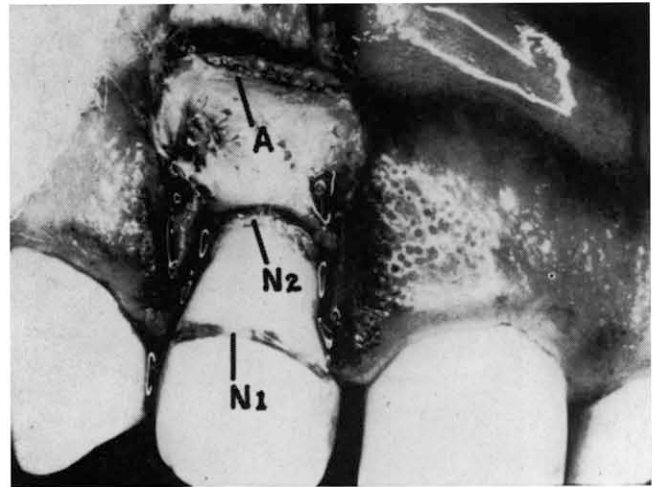


FIGURE 3. Same experimental site as Figure 1. Mucoperiosteal flap elevated exposing 5.0 mm of vestibular alveolar bone.

- N<sub>1</sub>—Crown notch.
- N<sub>2</sub>—Scapel root notch.
- A—Apical horizontal bone groove.

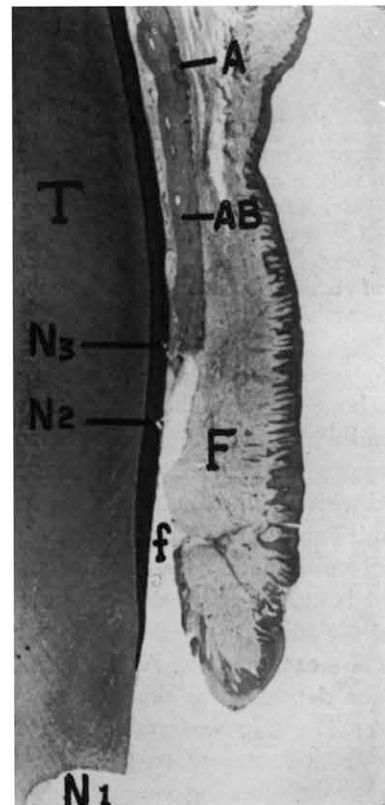


FIGURE 4. Photomicrograph of a zero hour specimen—maxillary area (X10). Hematoxylin and eosin stain. Replaced mucoperiosteal flap (F) covering reduced bone (AB) and tooth (T). Crown notch (N<sub>1</sub>) locating the marginal gingiva on tooth prior to forming a mucoperiosteal flap. (N<sub>2</sub>) is a root notch placed in tooth by scapel before removing 1.0 mm of alveolar bone crest. The lowered bone crest level is evident by root notch N<sub>3</sub>. Remains of apical horizontal groove (A) and an artifact (f) are evident.

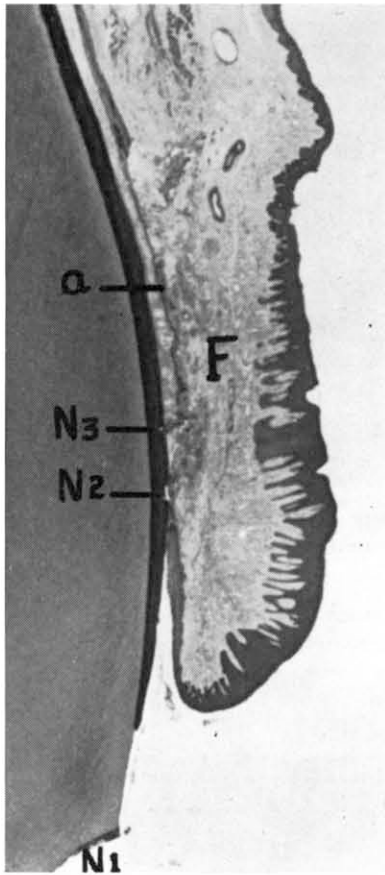


FIGURE 5. Photomicrograph of a one-week postoperative specimen—maxillary area (X10). Hematoxylin and eosin stain. Reduced alveolar bone (a) classified thin prior to reduction, is covered by mucoperiosteal flap (F). Flap also covers both root notches ( $N_2$  and  $N_3$ ). Young connective tissue between flap and reduced bone surface and tooth root is not evident at this magnification.

depth of the bone grooves under running water with a small diamond wheel. In addition, an osteoectomy, the removal of approximately 1.0 mm of crestal bone, was accomplished with a surgical chisel using hand pressure (Table 2 and Table 3). The tooth root was notched with a scalpel knife blade before and after the removal of the crestal bone. These notches represented in Figure 2 by line  $N_2$  and  $N_3$  served as points of references for histological measurements. A very minimal removal of root surface deposits was done using a scaler with light pressure. The flap was replaced and sutured to cover the bone and one or two millimeters of tooth root. A periodontal dressing was placed over the surgery. Both the dressing and the sutures were removed at one week. No specific changes were made in the individual's home care procedures. Specimens of the tooth and its vestibular periodontium were taken with a modified technique used by Kohler and Ramfjord<sup>1</sup> at time periods of 0 hour to 545 days after experimental surgery. Practically no arch deformity remained at the experimental sites after healing.

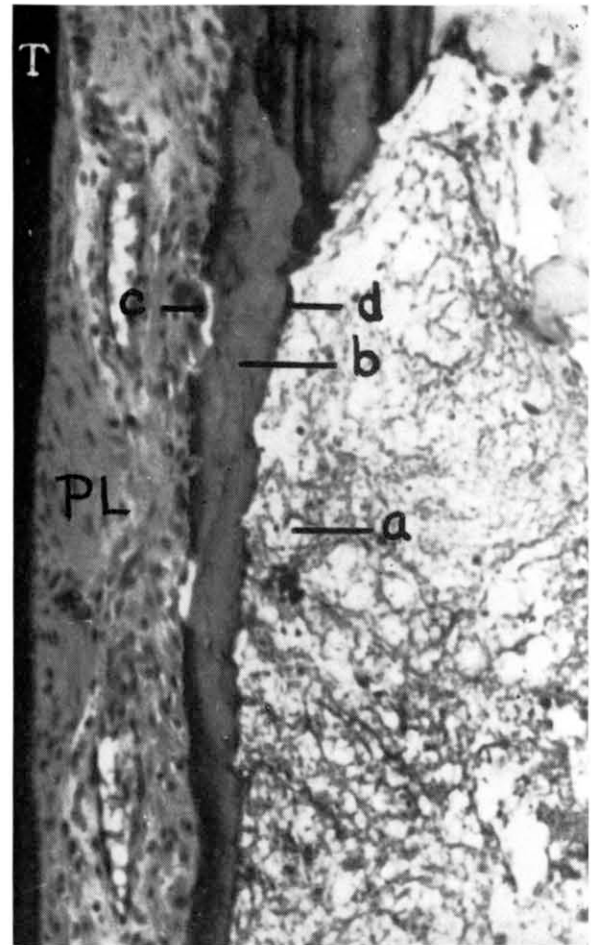


FIGURE 6. Photomicrograph of a one-week postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X160) of Figure 5 in the most apical area of the reduced bone. Young connective (a) tissue covers the reduced bone surfaces (b). Osteoclasts (c) and Howship's lacunae (d) are present along the periodontal surface of the alveolar bone. No inflammation is evident in the periodontal ligament (PL). Tooth (T).

The specimens were fixed in formalin and decalcified. After decalcification the specimen was divided into two specimens in a vestibular oral direction at the center of the tooth and along its long axis. The specimens were embedded and blocked in celloidin and sectioning of the block at ten microns was performed beginning at the divided surface of the specimen. Every fifth section was stained in hematoxylin and eosin and examined by light microscopy. A calibrated disc micrometer was used for microscopic measurements from reference notches  $N_1$ ,  $N_2$  and  $N_3$  (Fig. 2).

#### MICROSCOPIC FINDINGS

It was evident after microscopic examination of the postsurgical slides of early specimens that the vestibular bone evidently varied in thickness before surgery and could be classified as to appearance as either, thin, medium or thick. This corresponded to the operating sur-

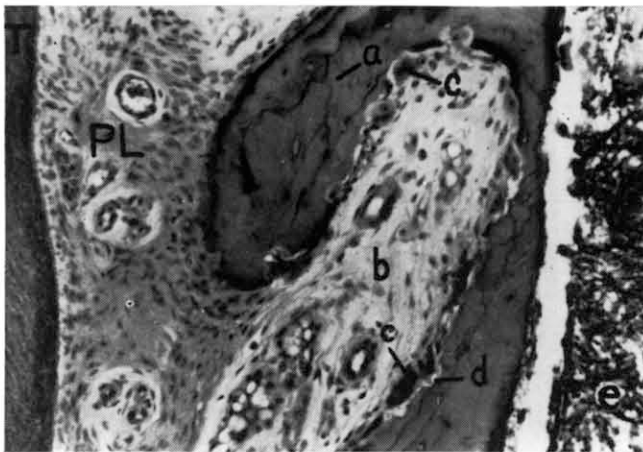


FIGURE 7. Photomicrograph of another one-week postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (original magnification X320) of reduced alveolar bone (a) that contained marrow space (b) facing the periodontal ligament (PL). Osteoclasts (c) and Howship's lacunae (d) are evident on marrow surfaces of the reduced alveolar bone but none on reduced periosteal surface of the bone that is covered by young connective tissue (e). Tooth (T).

geon's classification of bone thickness prior to surgery. Six specimens were rated as thin, 6 as thick and 11 as medium by the surgeon. It was evident that according to the histological healing processes the postoperative specimens could be arranged into ten groups. Either two or three specimens were represented in every group. The ten groups and number of individual specimens are listed in the following chart:

Group I	Unoperated control	2 specimens
Group II	0 hour	2 specimens
Group III	1 week	3 specimens
Group IV	2 weeks	2 specimens
Group V	3 weeks	2 specimens
Group VI	1 month	3 specimens (4, 4 and 5 weeks)
Group VII	2 months	3 specimens (6, 7 and 8 weeks)
Group VIII	3 months	2 specimens (90 and 98 days)
Group IX	6 months	2 specimens (5 and 6 months)
Group X	1 - or + year	2 specimens (9 + 18 months)

The unoperated controls and the 0 hour specimens were considered the controls in this histological study.

*Group II—0 Hour*

The mucoperiosteal flap was adapted to the reduced vestibular bone surface and to the tooth root on the zero hour microscopic specimens (Fig. 4). The gingival margin of the mucoperiosteal flap was at or near the crown notch (N<sub>1</sub>) that marked the original position of

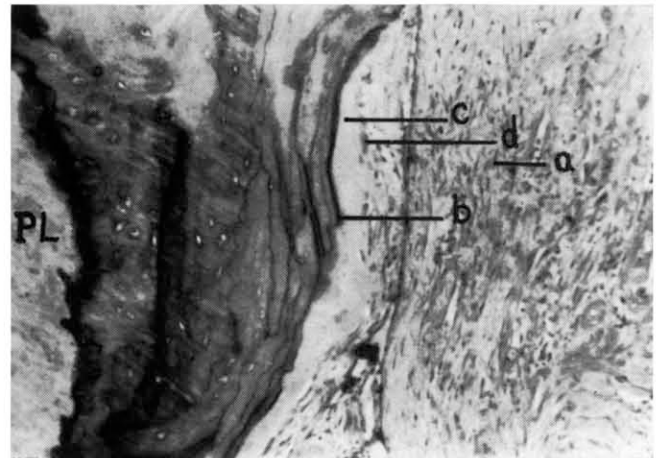


FIGURE 8. Photomicrograph of a two-week postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (original magnification X320) of elongated fibroblasts, collagen and some collagen fiber formation (a) in the young connective tissue covering the reduced periosteal bone surface (b). Periosteal bone formation in the crestal area of this particular specimen is indicated by the presence of an osteoid seam (c) and osteoblasts (d).

the marginal gingiva prior to elevation of the flap. The more coronal root notch N<sub>2</sub> Figure 4 marks the pre-operative bone level immediately prior to the osteotomy procedure, and the more apically located notch N<sub>3</sub> Figure 4 marks the level of the alveolar crest after the osteotomy. The reduced or cut vestibular bone surface was evident by its rough surface and basophilic staining.

*Group III—1 Week*

In the one-week postoperative specimens a zone of proliferating young connective tissue was evident between the surgically reduced vestibular bone surface and the wound surface of the replaced mucoperiosteal flap (Fig. 5 and 6). Also, at the more coronal experimental site, above the alveolar bone crest, young connective tissue was present beneath the replaced flap and the tooth surface it covered. This young connective tissue consisted mainly of new capillaries, large fibroblasts and mesenchymal cells that proliferated from the connective tissue of the mucoperiosteal flap and the cut

TABLE 3  
Bone Reduction as Determined by  
Microscopic Measurements

	Maximum	Minimum	Average
Coronal Apical Distance of Bone Surface Reduction (Osteoplasty) Measured from N <sub>3</sub> to A from 0 Hr. to Three-Week Group Specimens	6.1 mm	3.4 mm	5.1 mm
Amount of Alveolar Crest Reduction by Surgical Technique (Osteotomy) Measured from N <sub>2</sub> to N <sub>3</sub>	1.6 mm	0.3 mm	1.08 mm

periodontal ligament and had invaded the fibrin clot that previously sealed the flap to the bone and tooth during the first few days following osseous surgery. Remnants of fibrin clot and bone debris were present in the young connective tissue. An inflammatory process was evident around the blood vessels near the wound surface of the replaced flap and in the loose connective tissue in the most coronal region of the periodontal ligament. The epithelium of the marginal gingiva of the flap that was replaced on the tooth root did not display any apical proliferation along the root.

The absence of osteocytes in the bone immediately beneath the surgically reduced vestibular bone surface indicated some bone necrosis had occurred as the result of the experimental procedure. Osteoclasts and Howship's lacunae (Fig. 6) revealed that bone resorption was occurring along the the periodontal surface of the reduced alveolar bone over the tooth root if the bone was thin. No marrow spaces were evident in this thin plate of bone. The periodontal tissues beneath the reduced plate of bone appeared to be similar to the control specimens, except for a slight inflammatory process around vessels. Specimens with a thick or heavy plate of bone usually exhibited marrow spaces and osteoclasia was occurring on the bone surfaces of the marrow spaces (Fig. 7). Also, an inflammatory process was evident in the marrow spaces. These latter specimens exhibited little or no osteoclasia activity on the periodontal bone surface. Regardless of the thickness of the bone, except in several isolated areas of a few specimens, no osteoclastic activity was present on the reduced periosteal bone surface although young connective reparative tissue now covered the surface. The isolated resorption seemed to be mediated by cells with a single nucleus that were present in the young connective tissue.

Bone apposition was not present in the seven-day postoperative specimens except to a minor extent on bone surfaces of a few marrow spaces and this occurred adjacent to areas of osteoclastic activity.

#### Group IV—2 Weeks

The two-week postoperative specimens showed the formation of collagen (Fig. 8) and some collagen fibers in the young connective tissue that was present over the reduced bone surface and the tooth root. At this time the vascularity and inflammatory cell concentration decreased in the young connective tissue, while elongated fibroblasts were more evident and were arranged in the direction parallel to the bone surface and tooth root. Foreign body giant cells were present near any remnants of tooth, bone or fibrin clot debris remaining behind in the young connective tissue as the result of the surgery. The inflammatory process in the connective tissue of the

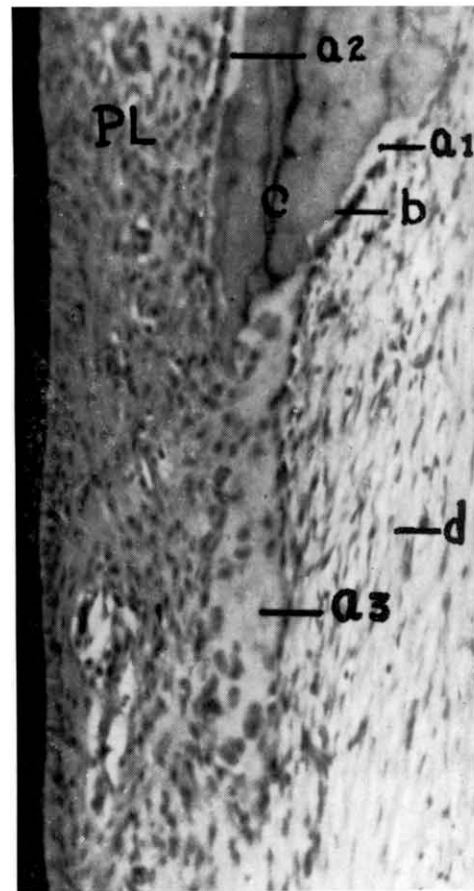


FIGURE 9. Photomicrograph of a one-month postoperative specimen—maxillary area. High power magnification (X160). Hematoxylin and eosin stain. The apposition of bone at the crest is obvious by the presence of osteoid tissue and overlying osteoblasts on periosteal surface ( $a_1$ ), periodontal surface ( $a_2$ ) and above the reduced crest ( $a_3$ ). Reversal line ( $b$ ) is present on the periosteal surface of the alveolar bone ( $c$ ). Collagen fibers ( $d$ ) are evident in the maturing young connective tissue over the periosteal bone surface ( $a_3$ ).

flap, periodontal ligament and in marrow spaces decreased when compared to the one-week postoperative specimens. The inflammatory cells at two weeks after surgery were polymorphonuclear leukocytes and lymphocytes with a predominance of lymphocytes. The epithelium on the tooth displayed evidence of proliferating in an apical direction along the tooth root.

A few osteoclasts were present only on the periosteal surface of the reduced bone, however no osteoclasia was evident along the periodontal surface of the bone or on bone surfaces outlining the marrow spaces of the thick specimens. Bone formation was evident in isolated areas on all these surfaces.

#### Group V—3 Weeks and Group VI—1 Month

The three-week and one-month postoperative specimens exhibited similar histological characteristics. The inflammatory process in the connective tissue continued to subside and by one month the only inflammation

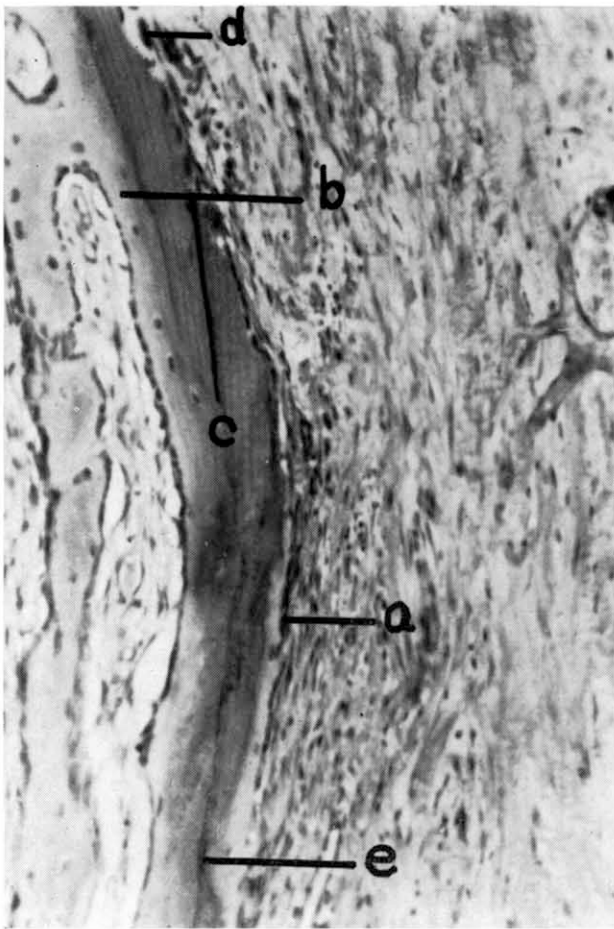


FIGURE 10. Photomicrograph of another one-month post-operative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X160) of bone formation on the periosteal bone surface (a) and on marrow bone surfaces (b) of the alveolar bone (c). Osteoclasts (d) indicate periosteal modeling resorption was occurring at the most apical area of the reduced bone. Reversal line (e) present indicates reduced thickness of bone due to osteoclastic resorption.

present was due to a reaction to deposits on tooth surfaces near the margin of the flap. Although a few collagen fibers were present in the 14-day postoperative specimens, at this time the amount of collagen fiber formation continued to increase in the maturing healing connective tissue (Fig. 9). The collagen fibers and elongated fibroblasts were parallel to the bone and tooth surface (Fig. 9). A few isolated collagen fiber bundles were now evident in the reparative connective tissue. The epithelium of the epithelial attachment continued to proliferate in an apical direction along the tooth root.

Osteoblastic activity reached its peak one month after surgery. A wide osteoid seam was evident on the periosteal bone surface, this was the reduced vestibular bone surface (Fig. 6-11), and also at the alveolar bone crest (Fig. 9). Bone surfaces of the marrow spaces in thick specimens (Fig. 10) and periodontal bone surfaces in all specimens also presented this osteoid seam (Fig. 9

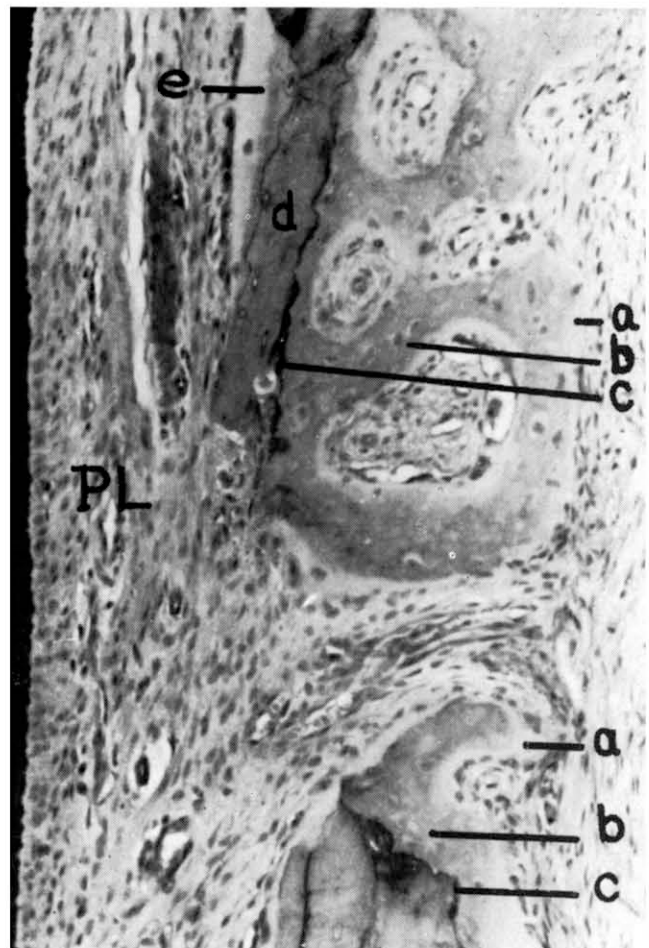


FIGURE 11. Photomicrograph of another one-month post-operative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X160) of maximum periosteal bone formation is apparent by the presence of osteoid tissue (a) covering immature bone trabeculae (b). The immature bone is apposed on a periosteal reversal line (c) present in vestibular alveolar bone (d). Osteoid tissue is seen on the periodontal surface (e) of the bone.

and 11). A scalloped reversal line near the periosteal bone surface in the three-week postoperative specimen signifies the end of periosteal resorption that had occurred between the two and three-week postoperative time period (Figs. 9-11). Periosteal and crestal bone apposition occurred on the periosteal and crestal reversal line (Figs. 9 and 10). At one month osteoid tissue was present on immature bone (Fig. 11) which indicated that the osteoid tissue apposed between three weeks and one month had calcified and formed immature bone. The apposition of bone on the reduced bone crest indicated the bone repair was in a coronal direction. Bone repair at the alveolar crest was an attempt to increase the coronal height of the crest reduced by the osteotomy procedure as well as the osteoclastic resorption that occurred between the two-week and three-week postoperative period. Slight periosteal osteoclastic activity was evident in areas apical to the bone surgery and to a minor degree at the alveolar crest when inflam-

mation occurred in this area at three weeks after surgery; however, osteoclastic activity was almost complete at the one-month postoperative time period.

#### Group VII—2 Months

The inflammatory process present at the earlier time periods was minimal to nonexistent in the two-month postoperative specimens at the experimental site, unless a plaque or deposit was present on the tooth root that was exposed to the oral cavity. Some collagen fiber bundle formation was present in the reparative connective tissue covering the alveolar bone (Fig. 12) and tooth root. The collagen fiber bundles (Fig. 12) were interspersed between collagen fibers. For the first time some fiber bundles immediately adjacent to the periosteal surface were embedded into the recently formed immature type of bone and into newly formed osteoid tissue (Fig. 12).

Osteoblastic activity continued, but considering all the specimens it was at a somewhat slower rate on the periosteal, periodontal and bone surfaces of marrow spaces. Only a small amount of bone was being added to the alveolar crest at this time. The marrow tissues in marrow spaces now had an appearance similar to marrow tissues of the controls (Fig. 12).

Formation of cementum in the reduced bone level notch ( $N_3$ ) was present. This was the first cementum formed on an area of the root that was exposed during the surgical procedure (Fig. 13).

#### Group VIII—3 Months

The predominate finding in the three-month postoperative specimens was the presence of collagen fiber bundles in the connective tissue repair area. The majority of the fibers (Fig. 14) and fiber bundles at this time were parallel to the tooth root and the vestibular surface of the alveolar bone. In some specimens a few fiber bundles were interspersed among and at right angles to the parallel arranged new fiber bundles.

Slight bone formation was present at the crest and considerably more on the periodontal surfaces and bone surfaces outlining the marrow spaces, but had almost completely subsided along the periosteal surface (Fig. 15). As in the two-month specimens, evidence of chronology of repair of the vestibular alveolar bone was apparent by the presence of immature bone on the periosteal bone surface (Fig. 15). Some modeling resorption was evident on the periosteal bone surface at the most apical area of surgical bone reduction (Fig. 15).

Some microscopic specimens of the three-month postoperative period displayed evidence of root resorption in the coronal area that had occurred at earlier postoperative time periods.

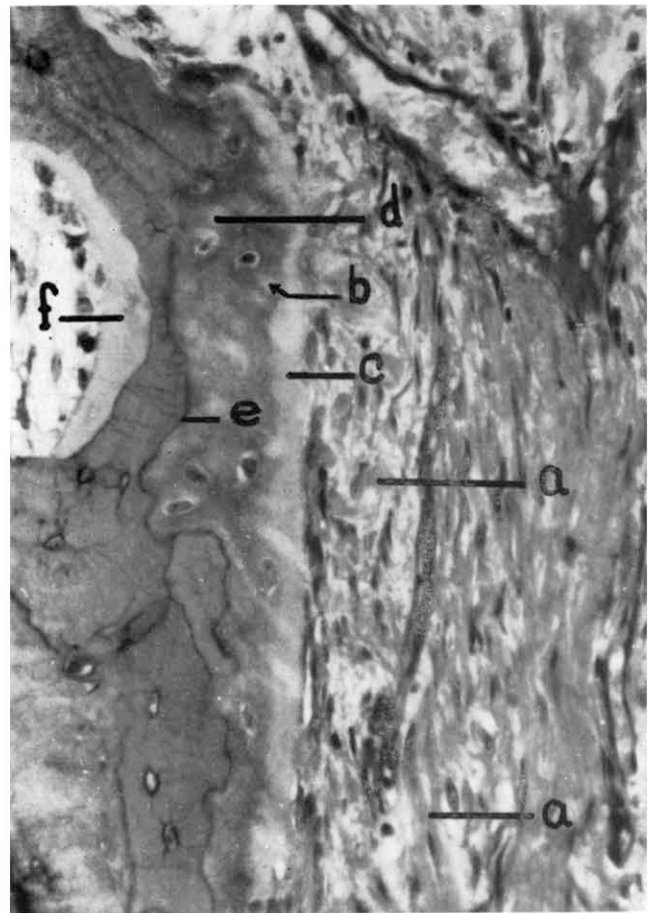


FIGURE 12. Photomicrograph of two-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X320) of collagen fiber bundles (a) over the periosteal bone surface. Entrance of fiber bundles (b) into periosteal osteoid tissue (c) and underlying immature bone (d). Reversal resorption line (e) is evident. Osteoid tissue on a marrow surface (f) is evident in this specimen.

#### Group IX—6 Months

For the first time, in the fifth and sixth-month postoperative specimens, the collagen fiber bundles in the repaired connective tissue inserted into root notch ( $N_3$ ) and the root coronal to this notch. A layer of cementoid being apposed on the tooth provided for the attachment of an angular insertion for the apically directed fibers that were formed in the reparative tissue (Fig. 16). The cementoid on the root surface above notch  $N_3$  was evident for the first time in specimens of the fifth-month postsurgical period (Fig. 16), however cementoid was seen in notch  $N_3$  at the three-month postsurgical period.

It is significant that the six-month postoperative microscopic specimens displayed slight or no bone apposition at the bone crest or periosteal bone surface (Fig. 17). At this same postoperative time, bundle bone was evident on the periodontal side of the vestibular bone (Fig. 17). The slight osteoblastic activity was evident on



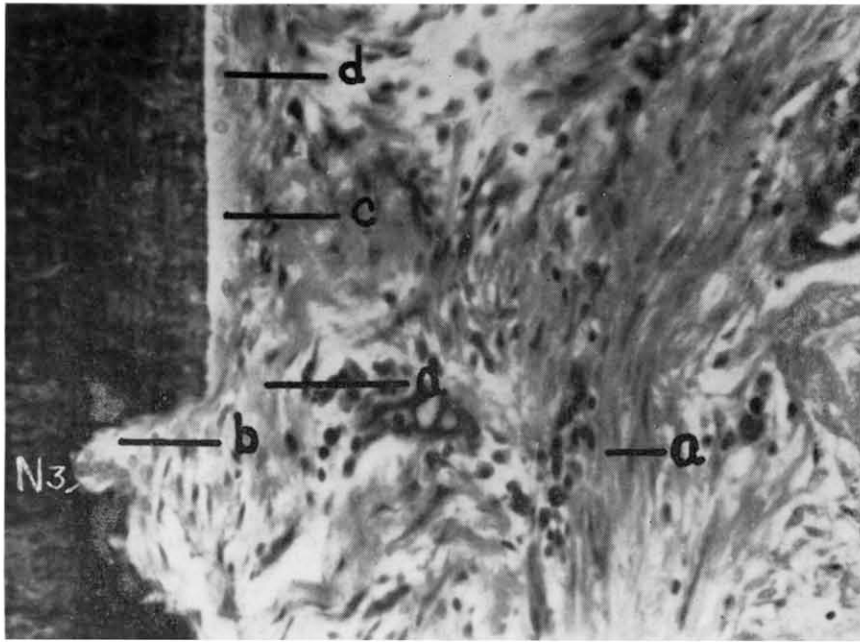


FIGURE 13. *Photomicrograph of two-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X320). Fibers (a) and fiber bundles were parallel to the tooth surface (T). Cementoid (b) being apposed in notch  $N_3$  and below notch  $N_3$  (c) at this time period. Cementoblasts (d) are present over the cementoid tissue.*

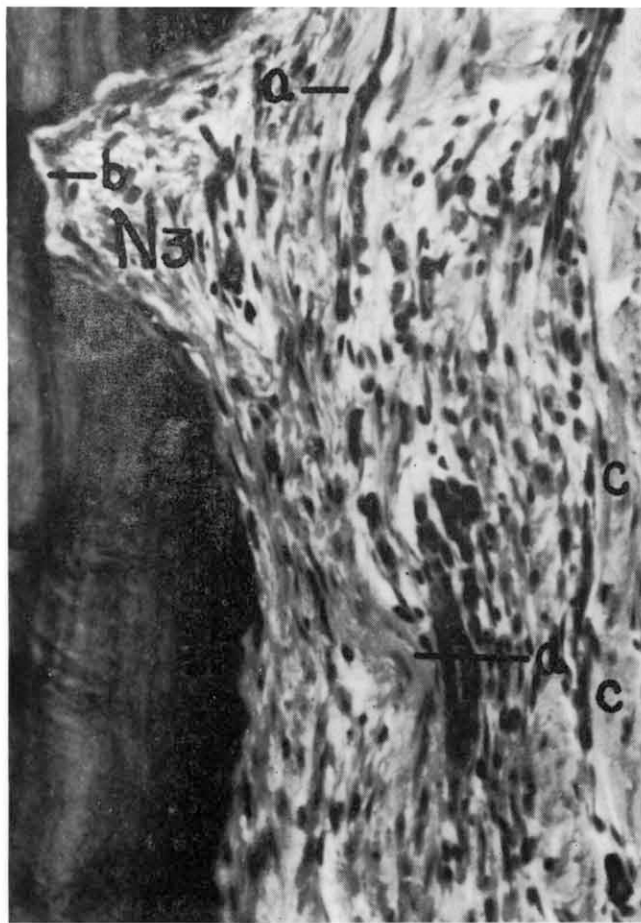


FIGURE 14. *Photomicrograph of a three-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X320). Collagen fibers (a) in the reparative tissue are parallel to the tooth root and definite cementoid tissue (b) is present in notch  $N_3$ . Collagen fiber bundles of the repositioned mucoperiosteal flap (c) covers the parallel arranged collagen fibers (a) of the reparative tissue.*

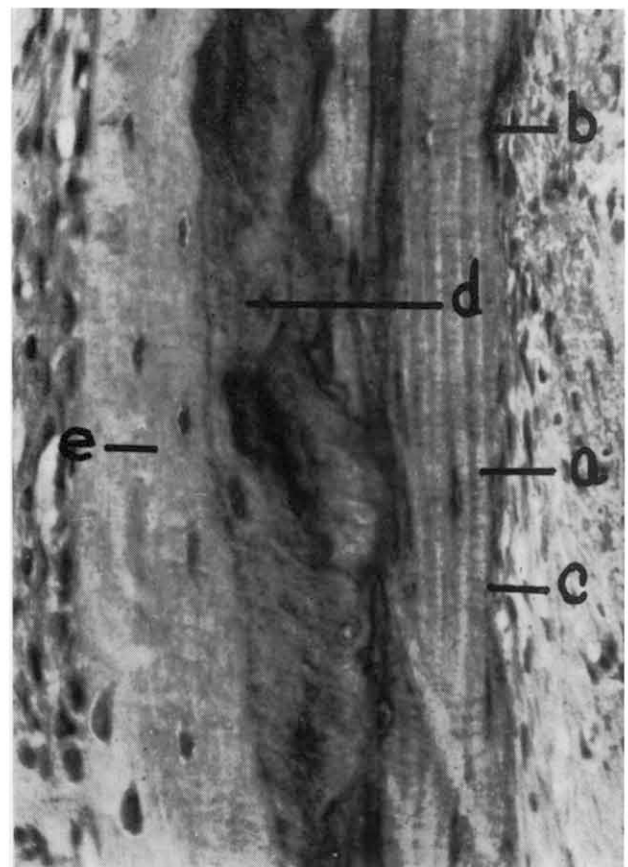


FIGURE 15. *Photomicrograph of another three-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X320). Regular layers of immature bone (a) are evident on the periosteal bone surface. Modeling resorption is occurring on the periosteal bone surface in the most apical experimental site (b). Little bone apposition is present on the periosteal bone surface (c). The remaining preoperative bundle bone (d) and recently formed immature bone (e) on periodontal bone surface is evident.*

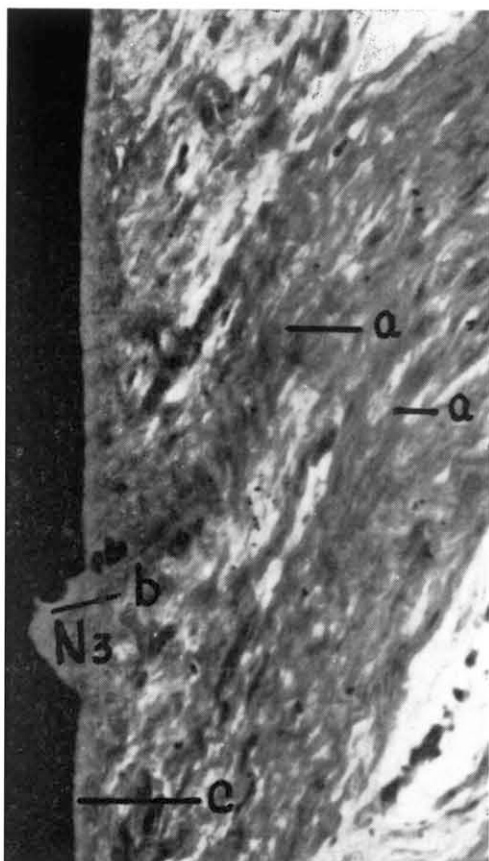


FIGURE 16. Photomicrograph of the five-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X320) displaying the angular insertion from an apical direction of collagen fiber bundles (a) into cementoid tissue (b) of the root notch  $N_3$  and into cementoid (c) coronal to notch  $N_3$ .

the periodontal bone surface or at the alveolar crest at the 6 months and was present in all postoperative specimens through 18 months may be the result of a compensatory reaction to the physiological occlusal migration of the tooth. The six-month postoperative specimens revealed a definitive periosteum over the entire periosteal bone surface, although the three-month microscopic specimens displayed a new periosteum in several areas over the recently repaired apposed periosteal bone surface.

#### Group X—1 - or + Year

The collagen fiber bundles of the gingiva of the nine-month postoperative specimen above the alveolar bone crest were arranged and inserted at right angles into the tooth root. The epithelium of the epithelial attachment was proliferating along the root. An inflammatory condition was present on the connective tissue of the gingiva and a bacterial plaque was evident on the clinical crown of the tooth. A slight amount or no bone was being apposed on the alveolar crest and periosteal surface in the 9 and 18-month postoperative specimens (Fig. 18). The 9 and 18-month postoperative specimens pre-

sented a lack of the immature type of bone on the periosteal side of the vestibular alveolar bone and at the alveolar bone crest that was apparent in the one, two, three and five-month postoperative specimens as well as the intermediate type of bone that was present in the six-month postoperative specimen at the same site (Fig. 17). Mature bone in the form of haversian systems and periosteal lamellae were present on the periosteal side of the vestibular alveolar bone and at the alveolar crest (Fig. 18). Beneath the mature bone structures a prominent reversal line locates the extent of repair or modeling resorption that occurred between the two-week and 18-month postoperative periods (Fig. 18). Some interstitial lamellae and bundle bone of the preoperative vestibular bone remaining behind was evident in this section (Fig. 18). No bone apposition or resorption was occurring at this time and cementum formation was minimal.

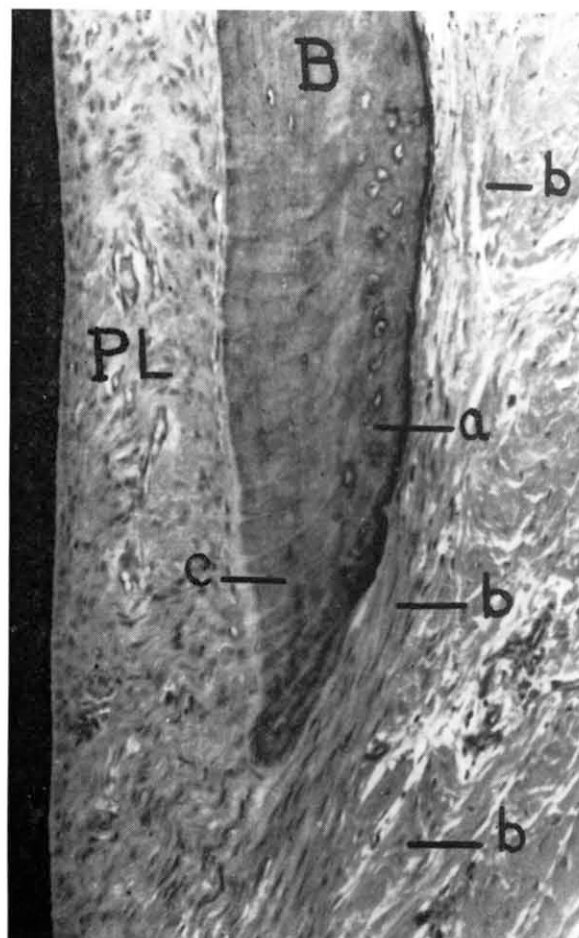


FIGURE 17. Photomicrograph of the six-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X160). Intermediate type of bone (a) present on the periosteal site of the alveolar bone (b) has replaced immature bone and osteoid tissue evident in earlier specimens. Collagen fiber bundles (b) are present in the area of connective tissue repair. Bundle bone is present on the periodontal surface of vestibular bone (c).

Microscopic measurements were made on the sections to determine the amount of crestal bone loss as the result of osteoclastic action in the earlier postoperative periods and the amount of bone apposition to the alveolar crest due to osteoblastic activity in the later postoperative periods. Root notch  $N_3$ , the point to which the bone crest was lowered during surgery, was the most important point of reference for microscopic measurements in all specimens for determining bone loss and bone apposition at the alveolar crest. Bone crest loss as the result of osteoclastic resorption was measured from  $N_3$  to the resorbed alveolar bone crest in the one and two-week postoperative specimens and to the crestal reversal line in the bone in the later postoperative specimens which exhibited bone apposition on this line. Likewise, bone crest apposition can be determined at these later time periods—the three-week and the remaining through five postoperative time groups—by measuring from  $N_3$  in the tooth root to the alveolar

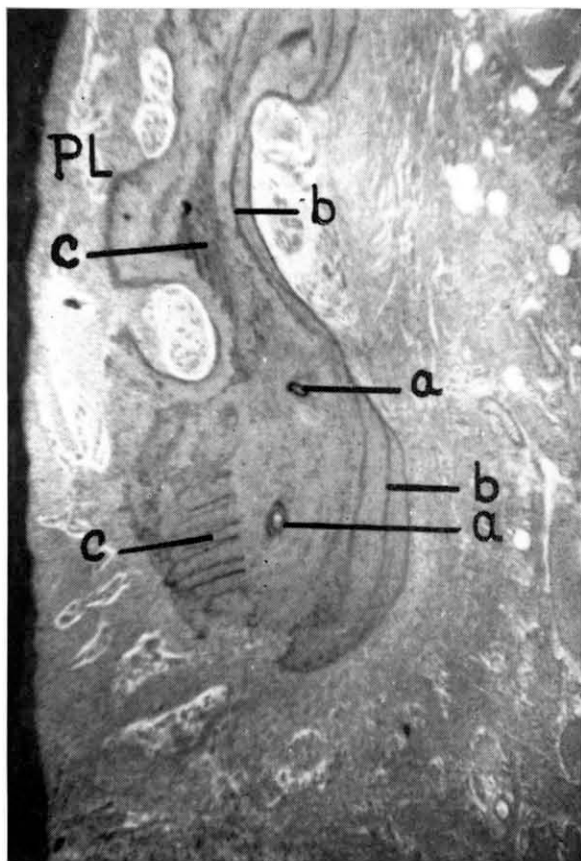


FIGURE 18. Photomicrograph of the 18-month postoperative specimen—maxillary area. Hematoxylin and eosin stain. High power magnification (X160). Haversian systems (a) or lamellae (b) of mature type of bone has replaced the immature and intermediate types of bone in the periosteal area of bone as was evident in earlier specimens. Interstitial bone and bundle bone (c) remaining after bone reduction and following the resorption process is apparent beneath Haversian systems and lamellae. Little bone or no bone apposition occurring at this postoperative time period.

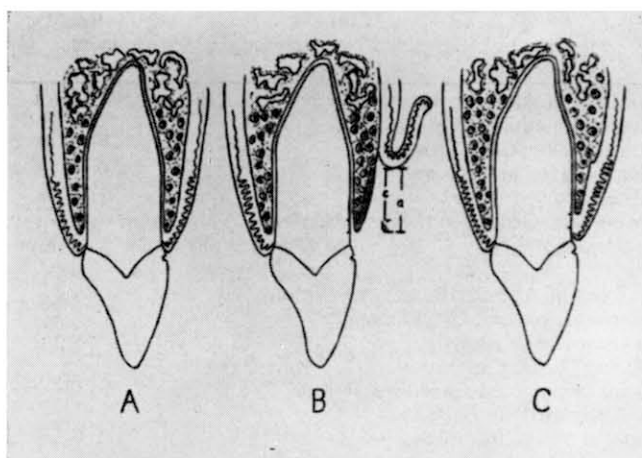


FIGURE 19. Diagrammatic illustration of vestibular-oral sections of a maxillary tooth presenting the surgical procedures utilized in this investigation. The preoperative specimen is illustrated in section A. Note the crown notch  $N_1$  marks the preoperative level of the marginal gingiva. In section B the mucoperiosteal flap has been elevated and 5.0 mm of vestibular alveolar bone (a) is exposed. In an osteoplastic procedure 5.0 mm of periosteal surface bone has been removed (distance a) and then an osteoectomy procedure 1.0 mm of crestal bone was removed (distance b). The osteoectomy procedure lowered the crestal bone from the root notch  $N_2$  and the vestibular bone remaining exposed is reduced to approximately 4.0 mm (distance c from root notch  $N_3$ ). The cross hatching of the bone indicates the bone removed by the osteoplasty and osteoectomy procedures. Section C illustrates the section of a 0 hr specimen after repositioning the mucoperiosteal flap so as to cover 2.0–3.0 mm of root surface and root notches  $N_2$  and  $N_3$ . Note the reduced vestibular bone in C.

crest and to the reversal line and calculating the difference between the two measurements. In these specimens the presence of immature and intermediate types of bone as well as the reversal line aided in determining crestal bone loss and apposition. Also, crestal bone apposition in the later time periods could be measured by measuring the amount of immature and intermediate types of bone present at the alveolar crest. Other reference structures used to determine both crestal bone loss and apposition were microscopic remnants of interstitial mature bone or bundle bone present in the initial preoperative bone that remained after resorption in the earlier postoperative periods.

The amount of crestal bone in the one and two-week postoperative time groups varied from maximum 4.47 mm to a minimum of 0.14 mm (Table 4) with the greatest loss in the specimens classified as thin and the least in the thicker specimens with and without marrow spaces. The average bone loss was 1.2 mm. The measured amount of bone apposition or repair at the alveolar crest from the three-week postoperative period through the end of the experiment varied from 1.15 mm to 0.14 mm, with the average being 0.4 mm (Table 4). The difference between the average crestal bone loss

TABLE 4  
Findings as Determined by Microscopic Measurements

Amount of Alveolar Bone Crest Reduction by Osteoclastic Resorption During Healing Measured N <sub>3</sub> to Alveolar Crest (One to Two-Week Groups in the Specimens)	<i>Maximum</i> 4.47 mm	<i>Minimum</i> 0.14 mm	<i>Average</i> 1.2 mm
Amount of Alveolar Bone Repair at Alveolar Crest Due to Osteoblastic Activity During Healing. Measurements of Apposed Immature and Intermediate Bone Plus Osteoid Tissue from Resorption or Reversal Line from Three Weeks to One and One-Half Years (Five Specimen Groups)	<i>Maximum</i> 1.15 mm	<i>Minimum</i> 0.14 mm	<i>Average</i> 0.4 mm
Calculated Loss or Reduction of Crestal Bone from Above Measurements			<i>Average</i> 0.8 mm

and apposition would be of 0.8 mm (Table 4). Thus, this difference of 0.8 mm represents the average reduction of bone crest as the result of the surgery and healing. This calculated average reduction of the bone crest from N<sub>3</sub> in the tooth root to the alveolar crest in sections was made from three weeks through 18 months postoperatively. The measurements indicated that the average lowering of the alveolar crest was 0.8 mm with a maximum of 3.1 mm and a minimum of 0.11 mm (Table 5).

#### DISCUSSION

Microscopic healing studies of mucoperiosteal flaps replaced at their original position have been done on animals<sup>6, 17, 19, 20</sup> and humans.<sup>1, 14</sup> The repair following osseous surgery that utilized a mucoperiosteal flap for facilitating the performance of osseous surgery had been studied in animals<sup>4, 10, 18</sup> and in one human study.<sup>3</sup>

The only human histological study by Friedman et al<sup>3</sup> demonstrated the appearance of healed tissue after two and one-half months on two specimens following removal of an unmeasured amount of vestibular bone. A recent similar animal study by Caffesse et al<sup>18</sup> presented the healing processes from 0 hour to two and one-half months. This human investigation presented some different microscopic findings from the above two studies. The variations in the findings were based partly on the removal of a measurable amount of osseous tissue and also on the length of this investigation that extended from 0 hour to 18 months.

The bone resorption present on the periodontal surface of one-week postoperative specimens that were rated as thin was not evident in the other human study.<sup>3</sup>

This lack of resorption may be due to presence of a heavy or thick plate of vestibular bone being present at the site of surgery. Close examination of their photomicrographs,<sup>3</sup> especially in reference to the location of the periosteal reversal line after two and one-half months postoperatively, indicated that the preoperative bone was thick and contained marrow spaces. Resorption occurred on the bone surface of the marrow spaces. This possibility was confirmed by microscopic findings in early time periods of this human investigation.

In the animal study,<sup>18</sup> osteoclastic activity was evident between five and nine days and occurred at the alveolar crest and extended apically 2.0 to 3.0 mm along the periosteal surface of the alveolar bone with only a slight amount present on the periodontal surface of bone. In this human investigation bone resorption on the periosteal surface occurred between two and three weeks following surgery, which was approximately one week after the beginning of periodontal bone surface resorption and resorption on bone surfaces of any existing marrow spaces and haversian canals. The periosteal surface resorption seemed to be a delayed process due to grinding on the bone if this investigation is compared to studies on healing of mucoperiosteal flap procedures.<sup>1, 5, 6, 17</sup> In the animal study by Caffesse et al,<sup>18</sup> first some alveolar crest bone and then periosteal bone 1.0 mm apically to the crest was removed with a hand chisel after elevation of a 4.0 to 5.0 mm mucoperiosteal flap. The loss of crestal bone after resorption or at the 21-day postoperative time period was 1.0 mm, while in this investigation at the same time period the crestal loss of bone varied from 0.14 mm to 4.47 mm and averaged 1.2 mm (Table 4). The difference in results may be that in this investigation 5.1 mm (Table 3) of periosteal bone surface was reduced by grinding with a diamond instrument which may be a more traumatic procedure.

After two and one-half months postsurgically, the end of the animal study,<sup>18</sup> osteoblastic activity completely restored the crestal bone loss during the earlier osteoclastic activity. This was statistically analyzed by the means of 30 measurements from each group of animals. Measurements were made from the cemento-enamel junction to the alveolar bone crest and it appeared as though the surgically reduced bone crest completely regenerated. In the present human study, osteoblastic activity resulted in an average of 0.4 mm addition to the alveolar bone crest, leaving an average repair deficit of 0.8 mm (Table 4), since the average bone loss due to osteoclastic activity was 1.2 mm. Friedman et al<sup>3</sup> reported a deficit of repair in two human specimens after two and one-half months of only 0.3 mm and 0.25 mm following an osteoectomy and osteoplasty respectively. The dissimilarity may be the result of the surgery being performed on bone of the spongy or cancellous type.

The marrow spaces provide an excellent surface of young connective tissue necessary for repair. This human investigation indicated that cancellous bone was not present in any of the specimens classified as thin and not present in some of the medium bone specimens. A similar explanation of the difference of bone repair between this human investigation and the animal study<sup>18</sup> could be the possibility of a more standardized thickness of bone and a lack of thin bone specimens in animals when compared to the experimental human specimens. Our microscopic measurements indicated that the least osteoblastic repair was evident in the thin alveolar bone specimens and, to a lesser extent, in some of the medium specimens. The osseous surgery in this investigation was probably more traumatic because of the greater quantity of bone removed during the osseous procedure and larger bone surface subjected to the grinding by diamond instruments. Osseous surgery performed with chisels in animals<sup>18</sup> was limited to one millimeter of crestal osteoplasty following very limited osteoectomy. This was about one-fifth the extent of the osseous surgery performed in this study. Peak bone repair occurred in animals and in the present human investigation between three and four weeks postoperatively. In all three studies osteoblastic activity was minimal after two and one-half to three months following surgery.

Another interesting finding was the variation in the rapidity of development of a new periosteum. In animals<sup>18</sup> a new periosteum began to differentiate at 14 days and was somewhat matured at two and one-half months, the end of the study. This could be compared to the periosteal repair in this human investigation in that at three months mature periosteum similar to the control was evident in isolated areas at the experimental site, and at six months postsurgically was present over the entire operated periosteal bone surface. In animals the development of periosteum was based partly on labeling by autoradiographic techniques, whereas this human investigation only mature periosteum of the controls was the criterion for evaluation. However with additional microscopic inspection of the specimens of this investigation and by utilizing for the criteria of an immature periosteum, first a very definite inner two or three-cell cambrium layer of large osteoblasts and, secondly, a deficiency of a dense arrangement of fibers in the outer fibrous layer, confirmed the presence of an immature periosteum at similar early time periods, that is, at time of bone formation. Although one difference was noted in this investigation, the periosteum never attached to necrotic bone.

Of great importance is the comparison of this microscopic study to the human correlative clinical study<sup>11</sup> with an identical surgical procedure and performed by

the same investigators. Both studies extended for 18 months, with the clinical study determining the crestal bone loss or gain by comparing the height of the alveolar bone crest after it had been reduced by surgery and then again reopening the experimental site at specific time intervals of healing. A standardized photographic technique<sup>12</sup> was used to gauge the results. The average loss of crestal bone in the clinical study was 0.54 mm compared to the average of 0.8 mm in the microscopic investigation. The difference could be explained on the basis that the disc micrometer measurements on histologic sections may be more accurate than those made with a Boley gauge on prints that were enlarged two times. The microscopic measurements indicated that bone loss in 14 specimens varied from 0.11 mm to 3.1 mm (Table 5) after three weeks to one and a half years, the total osteoblastic repair period, while the clinical study revealed no loss in some specimens at the same time periods. It was significant that 14 specimens were measured by the calibrated disc micrometer in this investigation, whereas 34 specimens were measured by the photographic techniques in the clinical study. A difference in the percentages of the preoperative classi-

TABLE 5  
Measurements to Substantiate Calculated  
Reduction of Bone Crest

Amount of Crestal Bone Loss or Reduction by Measurement from N <sub>3</sub> to Alveolar Crest in Specimen Groups from Three Weeks to 18 Months	<i>Maximum</i>	<i>Minimum</i>	<i>Average</i>
		3.1 mm	0.11 mm

fication of vestibular alveolar bone thickness in all measured specimens of the two studies was evident. In the clinical study 18 percent of the specimens were thin compared to 29 percent in the microscopic study; 46 percent were classified as medium in the clinical study compared to 50 percent in the microscopic study; 36 percent of the specimens presented thick alveolar bone in the clinical study and only 21 percent in the microscopic study. A greater percent of thin specimens and a lesser percent of thick specimens may account for more loss of crestal bone in this microscopic study as compared to the correlative clinical study.<sup>11</sup> Microscopic evidence indicated more bone loss and less bone repair occurred in the thin alveolar bone specimens and the reverse was true in the thick alveolar bone specimens.

The microscopic healing process above the alveolar crest and to the tooth surface has been investigated by many.<sup>1, 2, 5, 6, 9, 10, 13, 17, 18, 21, 22</sup> In this histological study, first a clot was present between the replaced flap and the bone and tooth, later to be invaded by young connective tissue followed by collagen and collagen fiber formation. Functional orientation of collagen fibers occurred five

months postsurgically in humans, although in animals<sup>18</sup> it was evident after two and one-half months. Complete maturation of new collagen fiber bundles in the new connective tissue over the root and over the bone did not occur until six months after surgery in humans.

Early loss of cementoblasts along the root in the area of surgery was noted in both the human and animal<sup>18</sup> investigations. New cementum formation on the root at surgical sites seems to vary from study to study. Seemingly, this variation was regulated by the amount of inflammation present in the gingival connective tissue during the experiment.

The epithelium of the gingival margin attaches to the tooth about one week after surgery. No apical migration of the epithelial attachment was evident in animal studies,<sup>17, 18</sup> but it did occur in all specimens of this study except one. Debris on the tooth surface and resulting connective tissue inflammatory response was probably the causative factor.

Parakeratosis of the crevicular epithelium was seen in the two-week specimen in one animal study<sup>18</sup> and was evident only in the 18 months postoperative human specimen of this investigation.

#### SUMMARY

This was a detailed histological investigation of the origin, rate and extent of repair of human periodontal tissues following the removal of a measured amount of vestibular osseous tissue over tooth roots after elevating a mucoperiosteal flap to gain access for the osseous procedure.

The findings indicated that the epithelial attachment to the tooth root was longer in all specimens beginning at the two-week postoperative period than was evident in the control specimens. An inflammatory process in the marginal gingiva caused by deposits accumulating postsurgically on the tooth root may have been the reason for the apical proliferation of the epithelial attachment.

Bone necrosis occurred in the bone immediately beneath the reduced periosteal bone surface. Resorption first occurred on the periodontal surface if the vestibular plate of bone over the tooth root was thin and began on bone surfaces facing marrow spaces and haversian systems if the bone plate was thick. The resorption on the surgically reduced periosteal bone surface was delayed and occurred between two and three-weeks postoperatively.

Bone repair by osteoblastic activity at the experimental site reached its peak between the third and fourth week after surgery. The first uncalcified osteoid tissue formed on the reduced periosteal bone surface at

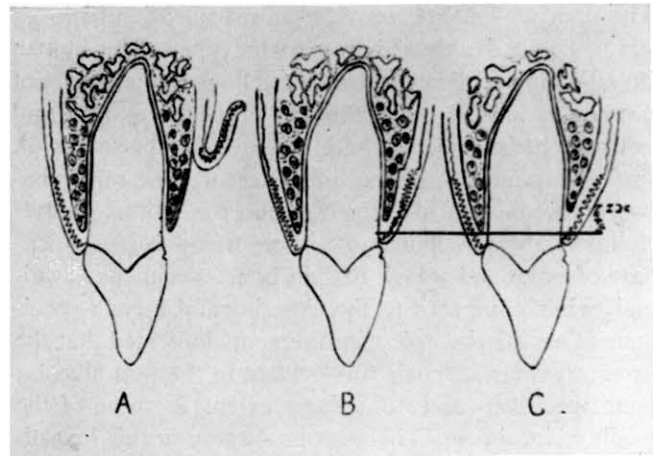


FIGURE 20. Diagrammatic illustration of vestibular-oral sections of maxillary teeth presenting the surgical procedures, and the results of histogenesis of repair of alveolar bone as related to the location of the alveolar crest. The A specimen represents the elevation of the mucoperiosteal flap. Also the osteoplastic periosteal bone surface reduction of 0.5 to 1.0 mm and osteoectomy that apically lowers 1.0 mm of the alveolar crest as presented by the shaded areas in the bone. Note the notches  $N_1$ ,  $N_2$  and  $N_3$  evident in all specimens. The B specimen presents the immediate postoperative diagram displaying the mucoperiosteal flap covering notches  $N_2$  and  $N_3$  and placed over 2 to 3 mm of root surface. The C specimen presents the alveolar bone in this experiment. The distance (a) represents the surgical lowering of 1.0 mm of the alveolar crest, while the distance (b) from  $N_3$  to the apical portion of shaded area represents the lowering of the alveolar crest as the result of osteoclastic resorption in the early postoperative time periods. This average measurement was 1.2 mm. The shaded area of the alveolar bone and measured by the distance (c) represents the amount of osteoblastic apposition of bone during the reparative stage of this study; the average loss of alveolar crest figured by the difference of the above two measurements would be 0.8 mm.

three weeks and calcified between the third and fourth week after surgery which became an immature type of bone at the alveolar crest and on the periosteal bone surface. At later postoperative time periods a minimal amount of bone formation occurred at these sites and continued to decrease throughout the experiment. Very little apposition was apparent after six months. Of interest was the replacement of immature bone by the intermediate type of bone at 6 months and its replacement by mature bone at 18 months after surgery. As the result of this experimental procedure and following healing the average loss of the alveolar crestal bone was 0.8 mm, although a few thin vestibular bone specimens exhibited a maximum of 3.1 mm loss of crestal bone. Maximum bone repair and almost complete anatomical restoration of the operated bone was achieved if the preoperative bone was the thick cancellous type with many marrow spaces.

Over the reduced bone surface a new periosteum was present in several areas at three months; however, a definitive periosteum as displayed in the unoperated

controls was not evident over the entire periosteal bone surface until six months postoperatively.

New collagen fiber bundles formed during healing were embedded in osteoid tissue on the operated periosteal bone surface at a few sites by the second month after surgery. In the area of the tooth root, the collagen fiber bundles were first parallel to the long axis of the root until the fifth and six-month postoperative time periods when they angled from an apical direction into and were attached to the root. A layer of cementoid being apposed for the first time on the root provided for the angular attachment of the collagen fiber bundles to the tooth. However, cementoid first formed in root notch ( $N_3$ ) at the three-month postoperative time period and below  $N_3$  at two months after surgery.

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