Osseous Coagulum: A Histologic Study

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OSSEOUS COAGULUM: A HISTOLOGIC STUDY

by

LEON KEITH COVERLY

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science in Oral Biology

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BIOGRAPHY

Leon K. Coverly was born on May 22, 1938 in Highland Park, Michigan.

He was graduated from North Muskegon High School in North Muskegon, Michigan in May 1955. From 1955 to 1959, he attended Kalamazoo College in Kalamazoo, Michigan, and received the Bachelor of Arts degree, with a major in chemistry, in June of 1959.

In September of 1960, he began studies at the University of Michigan School of Dentistry, and received the Doctor of Dental Surgery degree in May 1964.

From July 1964 to July 1966, he served as a dental officer in the United States Army at Fort McArthur, San Pedro, California. Following his tenure of service, he conducted a private practice of dentistry in Ypsilanti, Michigan for four years.

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DEDICATION

To my wife, Carol, whose patience, understanding, faith, and encouragement have been of immeasurable value.
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To Dr. Anthony W. Gargiulo, under whose suggestion this study was undertaken, I wish to gratefully acknowledge his constant advice, supervision, and untiring assistance. It is by his example that the pursuit of excellence becomes a reality.

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CHAPTER I

INTRODUCTION

Osseous defects of alveolar and interdental bone frequently are found in patients requiring periodontal therapy. The successful management of such lesions is a problem of great magnitude. In general, the three basic therapeutic approaches have been osseous resection, new gingival attachment procedures through curettage, and bone grafting techniques.

The elimination of the osseous defects by the surgical removal and recontouring of the remaining osseous support of the teeth is well documented (1,2,3,4). These procedures, although of undeniable value, are limited by the size and topography of the defects. When large defects are present, the extent of removal and recontouring of bone necessary to eliminate such defects seriously reduces the support remaining for the involved teeth.

Obviously, the elimination of the periodontal osseous defects by apposition of new bone and cementum, and reestablishment of the periodontal ligament, would be more desirable and less harmful to the remaining supportive structures.
Goldman (5) recognized the periodontal osseous defect as a distinct entity which could be eliminated by thorough subgingival curettage. However, the presence of three bony walls is a prerequisite which must be met if regeneration of the destroyed periodontal structures is to be achieved (6). Partial restoration of the alveolar bone, periodontal ligament, and cementum may be obtained when two walls remain, and no osseous repair can be expected when only one wall is present (7).

In an attempt to facilitate the restoration of lost periodontal tissue and stimulate osteogenesis in osseous defects, a variety of mineralized materials has been grafted into such defects. These include boiled bovine bone powder (8,9), cementum and dentin particles (10), os purum (11), cartilage (12,13), homogeneous cancellous bone (9), heterogeneous cancellous bone (14), and autogenous bone (15,16,17,18,19).

A current interest in autogenous bone grafts is apparent (20, 21,22,23,24,25,26), and the value of the osseous coagulum as an induction graft material, as demonstrated clinically by Robinson (16) and histologically by Rivault (19), is most impressive. However, because of the unpredictable nature of surgical correction by the osseous coagulum technique and the disparity in the result to be expected based on the type of osseous defect treated, as reported in the literature, a histologic study of the comparative healing
phenomena in chronic two and three walled osseous defects corrected by the osseous coagulum technique was undertaken. The purpose of this was to investigate the repair phenomena of the corrected defects at the histologic level.
CHAPTER II

REVIEW OF THE LITERATURE

The first autogenous grafts employed in the treatment of periodontal lesions was reported by Hegedus in 1923 (27). Following the removal of a section of bone (with periosteum intact from the tibia) buccal and lingual mucoperiosteal flaps were reflected in the involved area of the periodontium. The roots of the teeth were carefully planed, the granulation tissue was removed, and the bone substance was "freshened" by the use of a chisel. The donor bone was then placed on the recipient bed, and the flaps were fixed at their original level. The author attributed his clinical successes, based on decreased tooth mobility and radiographic evidence, to the "bone-building and regenerating property of the periosteum."

Cross, in 1955 (14) and 1957 (28) published the first of his observations on the utilization of bone grafts. Although his work was not confined to autografts, and is therefore difficult to evaluate, he did report some success by radiographic and clinical determination, in the formation of new bone. He stated that for a graft to succeed, it is essential to have a clean, non-inflamed operative
field, minimal tooth mobility, and subsequent avoidance of postoperative infection. To facilitate such conditions, he advocated routine antibiotic coverage postsurgically. He was also the first to suggest grafting small fragments of bone to minimize the possibility of sequestration. Also, because he felt that the larger the contact surface between the graft and the host bone, the more rapid is the fixation replacement of the graft, he concluded that the three walled osseous defect provides the most suitable bed for a successful osseous graft (28).

In 1934, Beube and Silvers (8), because of their belief that the critical component of the graft material was the mineral salts, did the first of their work with heterogenous nonvital bone. Pulverized sheep bone was placed in surgically prepared defects in dog maxillae. Controls (contra-lateral side) were also obtained by leaving the untreated surgical defect empty. Histologic specimens demonstrated complete bone formation on the experimental side, and no bone formation on the control side. Although their experiments did not approximate periodontal defects, nor did they use autogenous bone, they did show that graft particles do indeed accelerate osteogenesis.

The following year, they successfully treated one out of five human periodontal defects by implanting boiled bovine bone powder into the osseous lesions (9). They attributed the success or failure of these grafts to the maintenance or loss of the blood clot. They
reasoned that the fibrin of the clot has a great affinity for calcium and therefore acts as a scaffold and binder for the powdered bone, which can then be reutilized by the cells involved in the repair of the osseous defect.

Subsequently, Beube (29) continued his experiments with heterogenous bone grafts of boiled bovine bone powder. In 1949, he studied the healing phenomena in surgically created osseous defects in dogs, and compared his results with controls in which no graft material had been placed. He determined that repair was fastest in the experimental defects. This study also provided a description of the healing sequence. It was noted that initially there is an acute inflammatory response which subsides and is followed by a proliferation of young fibroblasts and capillaries into the area. Then, after the granulation tissue is organized, osteoclastic resorption of the bone begins before osteoblastic activity occurs at the margins of the defect as well as occasionally in proximity to the graft fragments. Once again, he asserted that the availability of the mineral salts to the osteoblasts was the basis for the accelerated rate of repair.

In 1951, Linghorne and O'Connell (30) published their results on the repair of surgically created osseous periodontal defects in dogs in which they had implanted both autogenous cancellous and cortical bone, as well as fragments of dentin and cementum. By comparing, histologically, their findings with control defects in which no graft
had been placed, they were also able to demonstrate a significant increase in bone repair only in the grafted defects.

The same sequence of repair as that reported by Beube was noted. That is, following granulation tissue organization, the resorption of the grafted particles began before new trabeculae were formed from the margins of the defects. Osseous apposition was also detected at this time on the graft fragments. Thus, the grafts seemed to encourage osteogenesis by stimulating the growth of new trabeculae and by acting as islands of ossification.

They felt that the origin of the osteoblasts was probably the undifferentiated mesenchymal cells emanating from the bony margins of the wound. Because of the increased repair in the grafted defect, and because no essential difference was observed between the effect of grafts of bone and grafts of tooth structure, they concluded that it is the presence of resorbing calcified tissue that provides the stimulus for the differentiation of these cells. So, it appeared to Lingborne and O'Connell that the osteogenic effect of grafts is due less to their cellular content than to their calcified intercellular material.

However, Levander, in 1940, had grafted bone marrow only into the soft tissue of rabbits (31). New bone was formed, although morphological analysis of the tissue demonstrated that the grafted marrow cells had died. The author maintained that the bone marrow
stimulates bone formation through some inherent substance which induces the non-specific mesenchymal cells to differentiate into osteoblasts.

Further studies on the problem of bone induction by Goldhaber (32) in which homograft bone was implanted in the subcutaneous tissue of immunized mice, both within a millipore filter and free, offer interesting results. For, although new vital bone free of any homograft reaction was found on the host side of the filter, the control homograft bone implanted freely was uniformly found to be dead, surrounded by inflammatory cells, and showed no new bone formation. These findings suggest that the new vital bone found on the host side of the diffusion chambers was derived from host tissue in response to a diffusible osteogenic inductor coming from the homograft bone.

The realization that the graft material could provide more than just a matrix for bone formation, or serve primarily as a reservoir for mineral salts, stimulated much work on the induction powers of the graft (29). The origin of the osteoblasts became the focus of interest. Osteoblasts within the graft may come, of course, from pre-existing osteoblasts (33), from the endosteum which lines the marrow cavities (34), and from perivascular undifferentiated mesenchymal cells (35). These three sources could also act concomitantly (35).

Apparently, the graft material which seems most ideally suited is autogenous, hematopoietic bone marrow. Recent clinical reports on
hip marrow biopsy transplants by Schallhorn (17,36,37) and intraoral cancellous bone and marrow transplants by Rosenberg (18,38) are certainly promising.

Cushing (39), in his excellent review on the potential for induction of osteogenesis of autogenous red marrow grafts, stated that the great potential for this tissue to form bone appears to be due to the availability of source cells lining the vascular sinusoids which have the propensity to differentiate into osteoblasts. He added that this differentiation occurs as a result of an inductive signal seemingly initiated by products of necrosing marrow, and that similarly, necrosing bone will perform the same function.

Furthermore, observations by McLean and Urist (40) demonstrate that the proteins of the matrix of bone and dentin contain the precursor of the inducing substance. For, when bone or dentin is decalcified, lyophilized, and implanted in a muscle, a bone-induction system results. All the available interstitial spaces, and all the old vascular channels are repopulated with acute inflammatory cells from the circulating blood. The surfaces are later covered with ingrowing capillaries, fibrous connective tissue, and giant cells. The interaction of mesodermal cells and mesodermal derivatives during the process of resorption induces differentiation of preosteoblasts, osteoblasts, and new bone.

Therefore, since the source cells to which Cushing has referred
are the same perivascular undifferentiated mesenchymal cells found along blood vessels and capillaries in every tissue, it certainly is conceivable that the value of a graft may not be dependent on the survival of its own cell population. Its value may be its ability to release an inducting substance which will induce any reticular cell coming from the adjacent host tissue to differentiate into an active osteoblast.

In 1965, Nabers and O'Leary (15,41) introduced the concept of implanting autogenous bone chips removed during routine osteoplasty and osteoectomy procedures into osseous periodontal defects in an attempt to induce new bone formation. Eight clinical cases were reported, all of which demonstrated some osseous regeneration. Although their results were neither substantiated by surgical reentry procedures nor histologic evidence, they concluded that in all probability the graft material retains some of its vitality at the time of implantation, which may contribute to its value.

Robinson (16), in 1969, stimulated by the work of Nabers and O'Leary, published his clinical results in the osseous repair of periodontal defects in which autogenous cortical bone chips mixed with blood had been placed. His "osseous coagulum" technique was based on two premises. The first is that the smaller the particle size of the donor bone, the more certain are its resorption and replacement; and the second is that mineralized fragments can induce osteogenesis.
Because of the clinical results reported by Robinson (16), and because of the obvious desirability of using an intraoral source of donor material, Rivault (19), in 1969, undertook his histologic study of the healing phenomena in osseous periodontal defects corrected by the osseous coagulum procedure. Using the rhesus monkey as his experimental model, and by comparing the healing of experimental defects with control defects in which no coagulum had been placed, it was conclusively demonstrated that a more rapid osseous repair in the graft sites occurs. He reported that the small number of osteogenic cells within the cortical bone fragments probably do not maintain their viability. Rivault emphasized the appearance of osteogenesis on the grafted particles as well as from the osseous walls of the defect. It was also noted that the graft material undergoes necrosis before osteogenesis begins. The significance of using small (10 X 100 microns) bone chips was also elucidated, for, he found that large particles initiate localized inflammatory reactions which inhibit osteogenesis. Rivault concluded that, as osteogenic induction appeared to occur by contact with the graft material, the greatest number of bone particles should be introduced. However, this must be compatible with a density which would provide an acceptable matrix. That is, one which would not delay the ingress of an adequate amount of granulation tissue which provides the preosteoblastic undifferentiated mesenchymal cells.

By investigating the repair phenomena at the histologic level
in the two and three walled chronic periodontal osseous defects corrected by the osseous coagulum technique, our study should provide additional information in regard to the clinical application of the osseous coagulum graft.
CHAPTER III

MATERIALS AND METHODS

Four young adult female rhesus monkeys (Macaca mulatta mulatta) served as the experimental model in this study. They were in apparent good health from the inception to the conclusion of the experiment, and maintained the physical parameters recorded at their arrival.

Each of the animals possessed a full complement of teeth, and the periodontal health of each was remarkably similar. Although calculus formation was scanty, rather heavy deposits of plaque and debris were found. All presented with a slight marginal chronic gingivitis. The gingivae were firm in consistency and pink in color. The papillae, however, were slightly erythematous and boggy. Sulcus depth varies slightly. Buccal and interproximal sulci depths were between 1 and 2 millimeters (Figure 1). Radiographically and clinically, only minimal alveolar resorption was noted.

Each quadrant served as the site for both a two and a three walled surgically produced osseous defect which was either corrected by the osseous coagulum technique or by curettage (controls). When control defects were either prepared or corrected, this procedure was
accomplished immediately following the preparation or correction of the analogous graft defects in the antagonistic quadrant of the same animal.

Because all quadrants from each monkey were subjected to surgical procedures at varying time intervals, and because each quadrant was surgerized twice, a schedule was followed which would allow the maximum time interval (and also be compatible with a judicious program of animal maintenance) between surgical interventions in that particular animal. This was done to facilitate recovery between surgical experiences.

Specimens were obtained at 0, 1, 3, 5, 7, 10, 14, 21, 28, 60, 90, and 120 days postoperatively. All of these provided experimental defects in which the osseous coagulum graft had been placed. Control defects, which had been corrected by curettage, were obtained at 7, 21, 60, and 120 days.

One monkey, which weighed 3.6 kilograms, provided graft specimens of 5, 90, and 120 days postsurgically, as well as the 120 day control specimens. Another, which weighed 3.9 kilograms, provided graft specimens of 1, 10, and 60 days postsurgically, as well as the 60 day control specimens. Still another, which weighed 4.2 kilograms, provided graft specimens of 3, 14, and 21 days postsurgically, as the 21 day control specimens. And, finally, the remaining monkey, which also weighed 4.2 kilograms, provided graft specimens of 0, 7, and 28
days, as well as the 7 day control specimens.

Thirty minutes prior to the time of surgery, the monkey received an intramuscular injection of 4 mg. of Serrylan (Parke, Davis & Company, Detroit, Michigan) to facilitate a more favorable level of sedation. The monkey was then rendered unconscious by the intravenous administration of 90 mg. of Diabutal (Pfizer Laboratories, New York, New York). A state of general anesthesia was immediate. However, a patent vein was maintained and more anesthetic was utilized if the animal began to regain consciousness during the duration of the procedure.

At this time, the presurgical clinical observations were recorded, periapical radiographs obtained, and color slides procured (Figure 1). The body was then placed in a cradle on the operating table and the head was positioned in such a manner that respiration would not be obstructed and adequate accessibility would be afforded.

Throughout the course of the procedure, strict conditions of asepsis were maintained. After each member of the operating team had scrubbed and dressed with sterile gowns, masks, and gloves, the monkey was draped in the customary manner. Thus, only the oral cavity and immediately adjacent anatomical areas were exposed. The surgical field was then painted with a 1/1000 aqueous solution of zephirin chloride, and the surgical procedure undertaken.

A rubber bite block was placed between the maxillary and
mandibular arches of the contra-lateral side to further stabilize the field of operation as well as afford an aura of well-being to the surgical team. Calculus, plaque, and other tooth accumulated materials were then removed from the teeth of the quadrant to be surgerized by the use of Gracey curettes (Hu-Friedy Mfg. Co., Chicago, Illinois).

A. GENERAL PREPARATION

An intrasulcular incision to the alveolar crest, begun at the distal aspect of the first molar and carefully carried to the canine of the same quadrant was performed with a #15 Bard-Parker blade. Great care was exercised to avoid sacrificing the interdental papillae, as their maintenance is necessary if primary closure of the surgical wound is to be obtained. On occasion, a vertical relaxing incision was made at the labial aspect of the canine from the free gingival margin through the mucogingival junction. On occasion, the intrasulcular incision was extended mesially to the mesial aspect of the canine or the lateral incisor, with no relaxing incision being employed. The local anatomy, as well as the amount of access required for that particular procedure dictated this aspect of the initial incision.

A small periosteal elevator was then used to reflect a full thickness mucoperiosteal flap. Here again, great caution must be exercised, for although the attached gingiva is tenaciously fixed to the underlying structures, it appears to be more friable than that found
in humans. Thus, it is susceptible to inadvertent laceration, which should be avoided if variables in the healing sequence are to be minimized.

At this time, epithelial tags adherent to the inner surface of the flap were removed with small curved tissue scissors. Interproximal granulation tissue was eliminated, and the root surfaces thoroughly planed with Gracey curettes.

B. PREPARATION OF DEFECTS (Figure 2)

The sites of the defects to be prepared were then selected on the basis of the anatomy of the interproximal alveolar process. Whenever possible, the interproximal osseous septae mesial and distal to the second premolar were chosen for a number of reasons. These areas were easily observed, presented little or no access problems, provided a sufficient amount of interproximal bone in which the osseous defects could be prepared, and were furthermore protected to some degree post-operatively by adequate interproximal contacts between the adjacent teeth. Only on one occasion (60 day graft specimens) were these areas not utilized. Here, the roots of the first and second premolars were so closely approximated that an inadequate amount of interproximal bone was present. Therefore, the interproximal osseous structures mesial and distal to the first molar were selected as the experimental sites. Following the selection of the experimental sites, clinical
observations were again recorded and color slides taken of the area.

Two and three walled osseous defects were then created. The distal interproximal septum selected served as the site for the three walled osseous defect, and the mesial interproximal septum selected served as the site for the two walled defect. In this manner another degree of standardization in the experimental procedure was provided. Beginning at the alveolar crest immediately adjacent to the root surface of the mesial tooth (ie., second premolar, usually) a #4 round bur was used to penetrate 3 mm. apically. This depth was carefully obtained by frequent repeated measurement with a calibrated periodontal probe. The aperature of the defect was made as broad as possible without removing the interproximal bone adjacent to the mesial aspect of the root of the adjacent tooth (ie., first molar, usually). In this manner, a three walled osseous defect was created. Thus, the defect possessed buccal, distal, and lingual walls of bone, and a mesial wall of cementum, thereby fulfilling the criteria of the classification system.

The two walled defect was prepared in the same fashion. However, the buccal cortical plate adjacent to the defect was removed with the same rotary cutting instrument. Therefore, the two walled defect was characterized by lingual and mesial walls of bone, and a distal wall of cementum. In accordance with sound clinical procedures, all osseous removal was accompanied by frequent irrigation with isotonic saline.
This was done to minimize the damage incurred by the surrounding osseous tissue from the increased temperature concurrent with the use of the dental handpiece.

C. INTRODUCTION OF CHRONIC IRRITANT (Figures 3 and 4)

After again obtaining a record of the surgical area on photographic film, an irritant was introduced into each defect in the following manner. A wooden toothpick was cut at a length of approximately 8 mm., which would allow it to extend from the fundus of the surgically prepared defect through the coronal aperture into the oral cavity. It was imperative that the length be such that it could be readily removed by the operator and yet neither interfere with the existant occlusal relationships nor be readily accessible to the paws of the monkey. The toothpicks were then ligated to the intervening tooth with ligature wire (0.3 mm. in diameter), to afford fixation and insure their maintenance for the prescribed period of time. The wire was drawn taut from the lingual aspect, cut as short as possible, and closely adapted to the tooth to minimize postoperative irritation to the animal's tongue. The toothpicks not only provided a source of chronic irritation to the surgically prepared defects, but facilitated a portal of entry for bacteria and bacterial toxins. In this manner, lesions mimicking those so frequently encountered in periodontitis in man were to be obtained.

The mucoperiosteal flap was then reapposed at its presurgical
level. No attempt was made to reposition the flap either coronally or apically. However, an attempt was made to assure close adaptation of the flap to both the teeth and the alveolar process. The flap was then fixed by either interrupted interproximal, or suspensory sutures. Whenever vertical relaxing incisions had been made, the mesial and distal tissue was also approximated by suturing. 4-0 silk suture material was utilized throughout the experiment. Each knot was secured twice and the loose ends of the suture material cut very short. For, monkeys do have the ability to pull on the suture material and it was not considered desirable to encourage premature suture removal.

D. POSTOPERATIVE CARE

The oral cavity was then thoroughly irrigated and the clinical procedure evaluated. After determining that all was in order, a postoperative photograph and a periapical radiograph of the areas subjected to the surgical procedure were obtained. The animal was then given an intramuscular injection of 600,000 units of Lincocin (The Upjohn Company, Kalamazoo, Michigan), which is an antibiotic with a special affinity for bone, before returning it to its cage. A liquid diet was specified for the first 24 hours postsurgically, and a soft diet for the following 4 days, whereupon a regular diet could be followed.
E. REMOVAL OF IRRITANT (Figure 5)

Seven days following the creation of the osseous defects, the monkey was sedated with 4 mg. of Serrylan (I.M.) and the sutures, toothpicks, and ligature wire were removed. The clinical findings were noted, and a photograph and periapical radiograph obtained.

F. CORRECTION OF DEFECTS (Figures 6, 7, 8, 9, and 10)

Thirty days following the creation of the osseous defects, they were corrected either by the placement of osseous coagulum into the defects, or by curettage. Clinical observations were recorded, and pre- and postoperative radiographs and photographs, as well as photographs of the various stages of the procedure were, of course, obtained. The animal was prepared for surgery in the manner previously described. The same surgical considerations outlined for the preceding surgical intervention were closely adhered to throughout this surgical procedure. A full thickness mucoperiosteal flap was reflected, the root surfaces of the teeth thoroughly planed, and the granulation tissue in the defects as well as the surrounding areas meticulously removed by curettage.

When the two and three walled osseous defects were to be corrected by the placement of the osseous coagulum, an area within the same quadrant was chosen to provide the donor material for the graft. Although the problem of accessibility was certainly a consideration,
the area selected was as far removed as possible from the previously prepared osseous defects. The donor bone was most frequently obtained from the alveolar process or basal bone apical and anterior to the recipient sites of the maxilla or mandible.

During the process of procuring the graft particles, no aspirating device could be used. Hemorrhage was controlled by the use of gauze squares when necessary. Using a #6 round bur in a dental handpiece, small (approximately 10 X 100 microns) fragments of cortical bone were removed from the donor site. These chips were mixed with the monkey’s blood, collected with a curette, and placed on a dental mirror. This procedure was repeated until cortical bone fragments sufficient in quantity to fill the osseous defects had been secured.

The surgically prepared two and three walled defects were then again inspected to verify the complete removal of granulation tissue, and the cortical bone fragments mixed with blood were placed into the defects. The osseous coagulum was rather firmly packed into the defects with an amalgam plugger in a step-like fashion. Gauze squares were used periodically to remove excess blood and thus assure a complete fill. Although an attempt was made to fill the defects, no attempt was made to overfill the areas. Furthermore, caution was exercised to avoid too vigorous a condensation, for, in part, the graft material must function as a scaffold for the ingress of young fibroblasts and capillaries, important in the preliminary organization
of the repair of the defects.

In those quadrants which were to provide control specimens for histologic study, the same procedure was followed. However, the osseous defects were curetted of all granulation tissue, and the osseous coagulum was neither obtained nor placed into the lesions.

After the osseous lesions had been corrected by either the graft technique or by curettage, the mucoperiosteal flap was reapprised. Meticulous attention was exercised to assure the complete coverage of the grafted or curetted osseous lesions by the soft tissue. The flap was firmly fixed to place by interproximal interrupted sutures. Again, every consideration was made to insure securing the flap in a manner which would deter postoperative displacement by the monkey. A postoperative regimen as previously prescribed was followed.

Seven days following the surgical experience, the animal was sedated, and the sutures removed. Clinical findings were recorded, and a color transparency and periapical radiograph obtained. The monkey was then maintained until the next procedure, or until the time of sacrifice.

G. COLLECTION OF SPECIMENS

At the predetermined time of sacrifice (which provided specimens of 120, 90, 60, 28, 21, 14, 10, 7, 5, 3, 1, and 0 days), radiographs and photographs were again obtained. The animal was given an intravenous
injection of a lethal dose of Totaltox (Chicago Veterinary Supply, Chicago, Illinois). A section in which the experimental defects were located from each quadrant was then prepared for removal. The periphery of the area to be removed was delineated by incising through the soft tissue to the underlying bone from the buccal and lingual or palatal aspects. The block specimen extended from the distal of the first or second molar to the mesial of the canine, and extended as far apically as possible. The bone was cut free of the surrounding structures with an electric oscillating orthopedic saw under constant water irrigation. Each block specimen was then thoroughly washed with water, tagged as to its origin, and placed in 10% formalin.

H. PREPARATION FOR HISTOLOGIC EXAMINATION

Following adequate fixation, each specimen was decalcified in formic acid and sodium citrate (50:50). After complete decalcification, the blocks were trimmed, embedded in paraffin, sectioned at 6 microns in a transverse buccolingual plane, and stained with hematoxylin and eosin. From each experimental area all of the prepared slides were studied, and a representative histologic section was selected for detailed histologic analysis.
A. CLINICAL OBSERVATIONS

The gingival tissues of the animals were slightly inflamed at the initiation of the study (Figure 1). They presented with a slight marginal chronic gingivitis which was confined primarily to the papillae, and a sulcus depth of 1 to 2 millimeters. The underlying osseous structures were normal in configuration. One week following the surgical creation of the osseous defects and placement of the toothpicks, the gingivae were markedly inflamed, with concurrent pocket formation prevailing. The marginal gingivae were edematous and erythematous (Figure 5). Removal of the irritants at this time was accompanied by rather profuse hemorrhage. The inflammatory process began to subside, and the edema was progressively resolved. Thirty days postoperatively, at the time of correction of the lesions, a decreased amount of inflammation was present, however the areas in proximity to the prepared defects were still considerably inflamed. The gingival margin was rolled, and the interdental papillae usually bulbous in form, erythematous in color, boggy in consistency, and readily bled upon
slight provocation (Figure 6). Significantly, at this time the sulci in the areas of the prepared defects were consistently 5 millimeters in depth.

Furthermore, it was interesting to note the changes in the osseous topography adjacent to the surgically prepared lesions at the time of correction. Thirty days following the creation and introduction of the irritant into the defect, the result of marked additional bone resorption was consistently observed. The three walled defects at this time were considerably broader in all dimensions than they had been at the time of their preparation. Frequently a trough-like defect was seen which extended around the distobuccal aspect of the adjacent tooth. The buccal cortical plate was maintained, however. The two walled lesion at this time demonstrated a loss of adjacent buccal cortical plate mesially and distally. The defects were now typically V-shaped, with the apex of the V at the fundus of the lesion. Bone resorption was often quite extensive, and the contour of the two walled defects was markedly altered as a consequence of the previous placement of the toothpicks (Figure 7).

One week following the surgical correction of the defects, the healing process was proceeding normally. The gingival margins were rolled and the interdental papillae were erythematous and slightly boggy. However, the other areas were firm and pink (Figure 10). Throughout the duration of the experiment, the gingivae continued
to return to its previous state of health. The interdental papillae
coronal to the experimental sites never attained a contour as physio-
logically normal as previously exhibited. Even in the late stages of
the study these papillae were more bulbous than they had been pre-
surgically.

B. HISTOLOGIC OBSERVATIONS
1. INTRODUCTION

Epithelial changes throughout the experiment were minimal, and
the viability of the tissue was maintained. The changes which did
occur were predominantly inflammatory in character, and reflected the
response of the tissue to the surgical insult as well as epithelial
changes normally concomitant with chronic gingivitis.

Within the connective tissue in all specimens, the inflammatory
response was evident. Intercellular edema, vascular dilatation, and
inflammatory cell infiltration were constant observations in the mar-
ginal gingiva. The character of the response to the surgical procedure
varied with the amount of time from the surgical insult.

The alveolar bone and marrow tissue of all specimens presented a
similar density of trabeculae and marrow content. The histologic changes
relating to the repair process were most dramatic in close proximity
to the defect sites, and the contribution of the cellular elements to
the repair of the surgically corrected defects, and previous attempts
at repair of the created defects were noted.

Dramatic changes were seen at the histologic level in the defect sites during the healing process as the defects underwent repair. It was in these areas where the contribution of the bone fragments to the repair process could be readily observed.

2. TWO WALLED GRAFT

a. EPITHELIUM

0 Day

Normal keratinized stratified squamous epithelium was present.

1 Day

Normal keratinized stratified squamous epithelium with slight intercellular edema near the incision site was observed. A polymorphonuclear leukocytic infiltration was also present in this area.

3 Day

Normal keratinized stratified squamous epithelium which had migrated apically along the inner surface of the wound, here separating the flap from the underlying fibrinopurulent exudate was noted. This epithelium was characterized by intercellular edema as well as a polymorphonuclear leukocytic infiltration.
5 Day

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was present.

7 Day (Figure 11)

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was seen. There was present here a marked migration of the epithelium apically, which had separated the flap from the underlying fibrinopurulent exudate. The proliferating strand of epithelial cells was in contact with graft particles.

10, 14, 21, 28, 60, 90, and 120 Days

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was present. The epithelium was closely adapted to the surface of the tooth. In these specimens, the epithelium was intact and repaired, and presented a normal epithelial attachment.

b. CONNECTIVE TISSUE

0 Day

The full thickness mucoperiosteal flap was detached.
The tissue was essentially normal and exhibited minimal hyperemia and slight hemorrhage at the incision site. A few perivascular inflammatory cells were also present.

1 Day

The connective tissue demonstrated a frank fibrinopurulent exudate. There was marked interstitial edema, dilated blood vessels, and a great increase in the number of inflammatory cells present.

3 Day

Interstitial edema, vascular dilatation, loss of collagen, and a heavy monocytic inflammatory cell infiltrate were present. Elongated mesenchymal cells bordering the graft material were seen emanating from the tissue of the flap.

5 Day

The connective tissue was characterized by interstitial edema, loss of collagen, vascular dilatation, and a perivascular fibrinopurulent exudate.

7 Day

Interstitial edema, vascular dilatation, and a moderate perivascular cellular inflammatory infiltrate were present. The connective tissue of the flap was attached to the underlying osseous structure, and young capillaries
and mesenchymal cells were seen emanating from the connective tissue to the adjacent graft particles in the defect site.

**10 Day**

The crestal area of the connective tissue was the site of the greatest inflammatory response here and in subsequent specimens. Interstitial edema, vascular dilatation, and a cellular inflammatory infiltrate were evident.

**14 Day**

The inflammatory response had decreased in intensity. However, interstitial edema, vascular dilatation, and perivascular inflammatory cells were still readily observed in the lamina propria of the col region.

**21 Day (Figure 12)**

The flap was repaired and in intimate contact with the graft particles and underlying osseous structure. Well organized gingival fibers, particularly the circular group of the gingival ligament, were well demonstrated. A chronic inflammatory response was present in the lamina propria in proximity to the coronal aspect of the defect and the col area. It was characterized by interstitial edema, vascular dilatation, and a mixed inflammatory cell infiltrate of polymorphonuclear leukocytes, plasma cells, and lymphocytes.
28, 60, 90, and 120 Days (Figure 13)

The flap wound was repaired and the histologic observations were those of the typical inflammatory response found in chronic gingivitis. The lamina propria in the col area consistently demonstrated interstitial edema, vascular dilatation, loss of collagen, and a mixed inflammatory cell infiltrate of polymorphonuclear leukocytes, plasma cells, and lymphocytes.

c. ALVEOLAR BONE

0 Day

The marrow cavities in close proximity to the defect site showed evidence of repair from the initial injury of defect preparation. They were surrounded by basophilic reversal lines, indicating recent osteoblastic activity. These marrow spaces also demonstrated an increased vascularity and increased mesenchymal cell population as compared with normal marrow spaces.

1 Day (Figure 14)

The histologic picture was similar to that seen at 0 hours, with reversal lines and increased mesenchymal cellularity the most striking features. However, osteoclastic activity was occurring on the walls of the marrow cavities.
Adjacent to the lingual wall of the defect, a mature chronic inflammatory tissue was found. The adjacent alveolar bone was being remodeled, and osteoclastic and osteoblastic activity was in evidence.

3 Day (Figure 15)

In addition to the presence of reversal lines, an increased mesenchymal cell population, and osteoclastic activity, Howship's lacunae were seen in the osseous walls of the marrow spaces. An invasion of the graft site from the adjacent marrow spaces by mesenchymal cells and capillaries was also occurring.

5 and 7 Days (Figures 16 and 17)

The marrow cavities remained dynamic, and both osteoclastic and osteoblastic activity was observed on the osseous walls of the marrow spaces as remodeling and repair occurred. The wall of the defect now exhibited marked osteoclastic activity.

10 and 14 Days (Figure 18)

The marrow spaces still exhibited an increased mesenchymal cell population. The walls of the marrow spaces were characterized by new bone formation in which osteocytes had been trapped. Although the wall of the defect was still undergoing osteoclastic resorption, some osteoid
was being deposited in certain areas, and immature trabeculae of bone were seen at the base of the defect.

**21 Day** (Figures 19 and 20)

The adjacent marrow spaces were reduced in diameter because of the new bone formation. These spaces also presented a heavier mesenchymal cell population than normal. The defect wall was characterized by bone apposition. Precementum was also seen on the dentinal surface of the tooth.

**28 Day**

The histologic picture was similar to the previous section. The adjacent marrow spaces were still very cellular and the diameter of these marrow cavities continued to decrease because of the new bone formation. The defect wall showed increased osteoblastic activity, and many young trabeculae were seen projecting from the osseous wall.

**60, 90, and 120 Days**

The marrow spaces adjacent to the defect sites contained a slightly increased mesenchymal cell population, and osteoblastic activity along these walls continued. Immature trabeculae of bone projected from the walls of the defect.
d. **OSSEOUS COAGULUM GRAFT**

**0 Day (Figure 21)**

The osseous defect was filled with red blood cells and grafted bone particles. The osseous coagulum was intimately adapted to the surrounding bone, cementum, and periodontal ligament.

**1 Day (Figure 14)**

The clot-graft complex, the osseous coagulum, presented the same basic picture. However, the area had been invaded by many polymorphonuclear leukocytes which were rather randomly distributed throughout the defect site.

**3 Day (Figure 15)**

The grafted bone fragments were scattered throughout the fibrinopurulent clot, in which there was a very heavy inflammatory cell infiltrate present. Although there was no particular pattern to the graft site at this time, many of the inflammatory cells were in close proximity to the bone fragments. Hemolysis of the red blood cells was also quite pronounced.

**5 Day**

The grafted bone fragments were scattered throughout the fibrinopurulent clot. A decrease in the concentration of inflammatory cells was evident. However, the graft
particles were surrounded by inflammatory cells as well as an occasional osteoclast. Young mesenchymal cells and capillaries were seen streaming from the adjacent marrow spaces.

7 and 10 Days (Figures 22 and 23)

The graft site was characterized by a proliferation of mesenchymal tissue. Each bone fragment was completely enveloped in mesenchyme and young capillaries. Some osteoclastic activity could be seen on the graft particles. Most of the polymorphonuclear leukocytes were gone. However, a fibrinopurulent exudate was still in evidence at the coronal aspect of the defect.

14 Day

The grafted bone fragments were scattered throughout a very cellular well organized fibrous connective tissue matrix. Osteoclastic activity was occurring, and many of the fragments stained more basophilic than others, apparently indicating dissolution of these fragments. Some fragments demonstrated very early apposition of osteoid on their surfaces.

21 Day (Figure 24)

The bone fragments, all of which had assumed a more basophilic character, were scattered throughout the highly
cellular young connective tissue matrix. Each particle was completely enveloped by a dense cellular infiltrate. Osteoclastic activity continued. However, osteoid and new bone formation was seen in proximity to the fragments.

28 Day (Figures 25, 26, 27, and 28)

The graft site was filled with young trabeculae of bone in which were scattered more basophilic graft particles. The trabeculae were being actively remodeled. The marrow spaces possessed a high cell density, and the walls of these cavities were lined with cells. A well developed periosteum had formed on the buccal surface of the repairing graft.

60, 90, and 120 Days (Figures 29 and 30)

The graft sites demonstrated progressively decreased cellular activity. However, even at 120 days postsurgically, remodeling was still occurring and the graft particles could still be seen within the new trabeculae. A well developed fibrous marrow was present. The defect had been repaired.

3. TWO WALLED CONTROL

a. EPITHELIUM

7, 21, 60, and 120 Days

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear
leukocytic infiltration in the col area was present.

b. CONNECTIVE TISSUE

7, 21, 60, and 120 Days

Interstitial edema, vascular dilatation, and a moderate perivascular inflammatory cell infiltrate were present. The cellular infiltration, which consisted primarily of polymorphonuclear leukocytes, plasma cells, and lymphocytes, was most pronounced in the col areas, which were in close proximity to the defect sites.

c. ALVEOLAR BONE

7 Day

The marrow spaces adjacent to the defect site demonstrated evidence of repair from the initial injury of defect preparation. They were surrounded by basophilic reversal lines, indicating recent osteoblastic activity. These marrow spaces demonstrated an increased vascularity as well as an increased mesenchymal cell population when compared with normal marrow spaces. Active remodeling of the marrow spaces was occurring, with both osteoblastic and osteoclastic activity in evidence. An invasion of the defect site from the adjacent marrow spaces by slender mesenchymal cells and capillaries had also occurred. The defect wall exhibited osteoclastic activity. However, osteoblastic
activity was also seen, with the formation of osteoid in some areas.

21 Day

The marrow spaces adjacent to the defect site were reduced in diameter because of the recent new bone formation. These spaces also possessed a heavier mesenchymal cell population than normal. The defect wall was characterized by osteoid formation.

60 Day

The adjacent marrow spaces contained a slightly increased mesenchymal cell population. Some osteoblastic activity continued to occur, and immature trabeculae of bone had appeared at the wall of the defect.

120 Day

The marrow spaces adjacent to the defect site had a normal cellularity. Bone apposition had occurred on the defect walls.

d. DEFECT SITE

7 Day

The defect site was characterized by a proliferation of young fibroblasts and capillaries from the adjacent marrow spaces and overlying connective tissue. Few peri-vascular inflammatory cells were seen.
21 Day (Figure 31)

The defect site was filled with a mature fibrous connective tissue. A few spicules of young bone were seen near the mature bone.

60 and 120 Days (Figure 32)

The defect was almost filled with new bone in which there was a high density of osteocytes. This bone appeared to have arisen from the defect walls. A small crestal osseous defect, in which osteoblastic activity was still occurring, was filled with mature fibrous connective tissue. The defect had been repaired.

4. THREE WALLED GRAFT

a. EPITHELIUM

0 Day

Normal keratinized stratified squamous epithelium was present.

1 Day

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was observed.

3 Day

Normal keratinized stratified squamous epithelium which had migrated apically along the inner surface of the
wound, here separating the flap from the underlying fibrinopurulent exudate was noted. This epithelium was characterized by intercellular edema as well as a polymorphonuclear leukocytic infiltration.

5 Day

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was present.

7 Day

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the incision site was seen. A marked apical migration of the epithelium which had separated the flap from the underlying connective tissue was present. The proliferating strand of epithelial cells was in contact with graft particles.

10, 14, 21, 28, 60, 90, and 120 Days

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration in the col area was present. The ulceration had been bridged. In these specimens, the epithelium was intact and repaired, and presented a normal epithelial attachment.
b. CONNECTIVE TISSUE

0 Day

The full thickness mucoperiosteal flap was detached. The tissue was essentially normal and exhibited minimal hyperemia and slight hemorrhage at the incision site. A few perivascular inflammatory cells were also present.

1 Day

The connective tissue demonstrated a frank fibrinopurulent exudate. There was marked interstitial edema, dilated blood vessels, and an increase in the number of inflammatory cells present.

3 Day

Interstitial edema, vascular dilatation, loss of collagen, and a perivascular inflammatory cell response were present. The flap exhibited a connective tissue attachment to the underlying osseous structures in some areas.

5 Day

The connective tissue was characterized by interstitial edema, loss of collagen, vascular dilatation, and a perivascular fibrinopurulent exudate.

7 Day

Interstitial edema, vascular dilatation, and a moderate perivascular cellular inflammatory infiltrate were
present. The connective tissue flap was attached to the underlying osseous structure, and young capillaries and mesenchymal cells were seen emanating from the connective tissue to the adjacent graft particles in the defect site.

10 Day

The crestal area of the connective tissue was the site of the greatest inflammatory response here and in subsequent specimens. Interstitial edema, vascular dilatation, and a cellular inflammatory infiltrate were evident.

14 Day

The inflammatory response had decreased in intensity. However, interstitial edema, vascular dilatation, and a heavy perivascular inflammatory cell infiltrate were still seen in the lamina propria of the col region.

21 Day

The flap wound was repaired and in intimate contact with the graft particles and underlying osseous structure. A chronic inflammatory response was present in the lamina propria in proximity to the coronal aspect of the defect and the col area. It was characterized by interstitial edema, vascular dilatation, and a mixed inflammatory cell infiltrate of polymorphonuclear leukocytes, plasma cells, and lymphocytes.
28, 60, 90, and 120 Days

The flap wound was repaired and the histologic observations were those of the typical inflammatory response found in chronic gingivitis. The lamina propria in the col region consistently demonstrated interstitial edema, vascular dilatation, loss of collagen, and a mixed inflammatory cell infiltrate of polymorphonuclear leukocytes, plasma cells, and lymphocytes.

c. ALVEOLAR BONE

0 Day

The marrow cavities in close proximity to the defect site showed evidence of repair from the initial injury of defect preparation. They were surrounded by basophilic reversal lines, indicating recent osteoblastic activity. These marrow spaces also demonstrated an increased vascularity and increased mesenchymal cell population when compared with normal marrow spaces.

1 Day

The histologic picture was similar to that seen at 0 hours, with reversal lines and increased mesenchymal cellularity of the marrow spaces the most striking features.

3 Day

In addition to the presence of reversal lines and an
increased mesenchymal cell population, the marrow spaces were undergoing active remodeling. Osteoclastic and osteoblastic activity was occurring on the osseous walls of the marrow cavities. An invasion of the graft site from the adjacent marrow spaces by slender mesenchymal cells and capillaries was also occurring.

5 and 7 Days

The marrow spaces remained dynamic, and both osteoclastic and osteoblastic activity was observed on the osseous walls of the marrow cavities as remodeling and repair occurred. The walls of the defects exhibited osteoclastic activity. However, areas of immature trabeculae of bone were also seen.

10 and 14 Days (Figures 33 and 34)

The marrow spaces still exhibited an increased mesenchymal cell population. The osseous walls of the marrow spaces were characterized by new bone formation in which osteocytes had been trapped. Although the walls of the defects were still undergoing osteoclastic resorption, some areas of new trabeculae projecting from the defect walls were also observed.

21 Day

The marrow spaces adjacent to the defect were reduced
in diameter because of the new bone formation. These spaces also presented a heavier mesenchymal cell population than normal. The defect wall was characterized by bone apposition. Precementum was also seen on the cut dentinal surface of the tooth.

28 Day

The histologic picture was similar to the previous section. The adjacent marrow spaces were still very cellular and the diameters of these marrow cavities continued to decrease because of the new bone formation. The defect wall showed increased osteoblastic activity, and many young trabeculae of bone were seen projecting from the osseous walls.

60, 90, and 120 Days

The marrow spaces adjacent to the defect site contained a slightly increased mesenchymal cell population, and osteoblastic activity along these walls continued. Immature trabeculae of bone projected from the walls of the defects.

d. OSSEOUS COAGULUM GRAFT

0 Day

The osseous defect was filled with red blood cells and grafted bone particles. The osseous coagulum was
intimately adapted to the surrounding bone, cementum, and periodontal ligament.

1 Day

The clot-graft complex, the osseous coagulum, presented the same basic picture. However, the area had been invaded by many polymorphonuclear leukocytes which were rather randomly distributed throughout the defect site.

3 Day

The bone fragments were scattered throughout the fibrinopurulent clot, in which there was a very heavy inflammatory cell infiltrate present. Although there was no particular pattern to the graft site at this time, many of the inflammatory cells were in close proximity to the bone particles. Hemolysis of the red blood cells was also quite pronounced.

5 Day

The bone fragments were scattered throughout the fibrinopurulent clot. A decrease in the concentration of inflammatory cells was evident. However, the graft particles were surrounded by inflammatory cells as well as an occasional osteoclast. Young mesenchymal cells and capillaries were seen streaming from the adjacent marrow spaces.
7 and 10 Days

The graft site was characterized by a proliferation of mesenchymal tissue. Each bone fragment was completely enveloped in mesenchyme and young capillaries. Both osteoclastic and osteoblastic activity was occurring on graft particles. Few polymorphonuclear leukocytes were in evidence.

14 Day

The bone fragments were scattered throughout a very cellular well organized fibrous connective tissue matrix. Osteoclastic activity was occurring, and many of the fragments stained more basophilic than others, apparently indicating dissolution of these fragments. Many fragments of bone also demonstrated osteoblastic activity and osteoid formation on their surfaces.

21 Day

The bone fragments, all of which had assumed a more basophilic character, were scattered throughout the highly cellular young connective tissue matrix. Each particle was completely enveloped by a dense cellular infiltrate. Osteoclastic resorption of the fragments continued. However, osteoid and new bone formation was seen in proximity to the fragments. In addition, bone formation was seen to be
occurring with no immediate environmental relationship to the implant particles at all.

**28 Day**

The graft site was filled with young trabeculae of bone in which were scattered more basophilic graft particles. The trabeculae were being actively remodeled. The marrow spaces had a high cell density, and the walls of these cavities were lined with cells.

**60, 90, and 120 Days**

The graft sites demonstrated progressively decreased cellular activity. However, even at 120 days postsurgically, remodeling was still occurring, and the graft particles could still be seen within the new trabeculae. A well developed fibrous marrow was present. The defect had been repaired.

**5. THREE WALLED CONTROL**

a. **EPITHELIUM**

**7, 21, 60, and 120 Days**

Normal keratinized stratified squamous epithelium with slight intercellular edema and a polymorphonuclear leukocytic infiltration in the col area was present.
b. CONNECTIVE TISSUE

7, 21, 60, and 120 Days

Interstitial edema, vascular dilatation, and a moderate perivascular inflammatory cell infiltrate were present. The cellular infiltration, which consisted primarily of polymorphonuclear leukocytes, plasma cells, and lymphocytes, was most pronounced in the marginal gingiva, which was in close proximity to the defect sites.

c. ALVEOLAR BONE

7 Day

The marrow spaces adjacent to the defect site demonstrated evidence of repair from the initial injury of defect preparation. They were surrounded by basophilic reversal lines, indicating recent osteoblastic activity. These marrow spaces demonstrated an increased vascularity as well as an increased mesenchymal cell population when compared with normal marrow spaces. Active remodeling of the marrow spaces was occurring. Osteoclastic and osteoblastic activity was in evidence. An invasion of the defect site from the adjacent marrow spaces by slender mesenchymal cells and capillaries had also occurred. The defect wall exhibited osteoclastic activity. However, osteoblastic activity was also seen, with the formation of osteoid as
well as young trabeculae of bone in some areas.

21 Day

The adjacent marrow spaces were reduced in diameter because of the recent new bone formation. These spaces also possessed a heavier mesenchymal cell population than normal. The defect wall was characterized by the formation of osteoid and young trabeculae of bone.

60 Day

The adjacent marrow spaces contained a slightly increased mesenchymal cell population. Some osteoblastic activity continued to occur, and immature trabeculae of bone projected from the wall of the defect.

120 Day

The adjacent marrow spaces had a normal cellularity. Bone apposition had occurred on the defect walls.

d. DEFECT SITE

7 Day

The defect site was characterized by a proliferation of young fibroblasts and capillaries from the adjacent marrow spaces and overlying connective tissue. Few peri-vascular inflammatory cells were seen.

21 Day

The defect site was filled with a mature fibrous
connective tissue. A few trabeculae of young bone were seen projecting from the defect wall.

60 and 120 Days

The defect was almost filled with new bone, in which there was a high density of osteocytes. This bone had formed from the defect walls. A small crestal osseous defect, in which osteoblastic activity was still occurring, was filled with mature fibrous connective tissue. The defect had been repaired.
CHAPTER V

DISCUSSION

A. INTRODUCTION

The two and three walled chronic periodontal osseous defects corrected by the osseous coagulum technique and by curettage in the rhesus monkey in this study healed by regeneration. That is, the architecture and function of the lost tissue was completely renewed. The healing phenomena in both the two and three walled lesions was similar histologically and temporally.

It is significant that in the early stages of the healing process, those defects in which the osseous coagulum had been placed demonstrated a more advanced level of regeneration than did the controls, which had been corrected by curettage. Only after two months following the surgical correction did the control specimens demonstrate a comparable degree of regeneration.

These results do not at all belie the value of the osseous coagulum graft in the treatment of periodontal osseous defects in clinical practice. It is certainly conceivable that the earlier occurrence of osteogenesis in the grafted defects may inhibit the apical migration
of the epithelial attachment during the early stages of repair and thereby prevent the reformation of the periodontal pocket.

B. CREATION OF CHRONIC OSSEOUS DEFECTS

Chronic periodontitis can be successfully simulated in primates by the method employed in this study. Osseous defects can be created with a marked degree of similarity to one another and subsequently rendered into chronic lesions for healing studies.

This phenomenon was apparent clinically and histologically. Clinically, an obvious increase in size and alteration of the architecture of the defects was observed at the time of surgical correction. Resorption of the osseous tissue adjacent to the surgically created lesions was noted (Figures 2 and 7).

Histologic examination revealed reversal lines in the alveolar bone parallel to the periphery of the defects, increased mesenchymal cellularity of the marrow cavities in close proximity to the defects, and a rather mature vascular fibrous connective tissue component adjacent to the osseous walls of the defect in one specimen. The lamina propria was characterized by the presence of plasma cells, lymphocytes, intercellular edema, and an increased vascularity. These findings verify the clinical impression of chronicity.

Although other investigators have induced periodontitis in experimental animals (42,43), it is significant that chronic
three walled periodontal osseous defects could consistently be obtained by the method employed in this study. It is felt that the disease so produced can serve as an excellent experimental model in the study of surgical osseous therapy.

C. EPITHELIAL REGENERATION

The ability of the gingival epithelium to withstand trauma and to regenerate was well demonstrated. The viability of the epithelium was maintained throughout the experiment, and repair was achieved in one week. Mobilization of cells to colonize the surgical wound was achieved by two distinct processes. These were (1) the movement of the cells into the part, and (2) the provision of a number of cells sufficient for regeneration by local mitotic division.

The epithelial cells tended to move as a "sheet" with a free edge, beginning within 24 hours of the surgical procedure. This ability to move across a solid substrate is an inherent property of epithelium (44). When the cells are completely surrounded by like-cells, their movement is apparently inhibited. This phenomenon is known as contact inhibition (45). It arises from adhesion between the surfaces of like-cells or perhaps due to some special property of their microenvironment. When contact between like-cells is broken, an inevitable consequence of wounding, the cells at the free margin tend to move across the surface of the substrate into the space so created. The
cells continue to move until contact is made with other cells. If the colliding cells are alike, movement stops. Contact may be maintained, or the cells will move off in new directions. Cells never move over each other.

This process of regeneration, which accounts for the rapid healing of epithelial wounds, was also seen to be detrimental to the total regeneration of structure in some specimens. For, in those specimens in which the mucoperiosteal flap was not adequately adapted and fixed to the subjacent structures, the epithelial cells migrated apically along the inner surface of the wound, thereby retarding or inhibiting connective tissue reattachment of the flap to the underlying structures. This would be a contributing factor in the subsequent reformation of the periodontal pocket, as reported by Caffesse, Ramfjord, and Nasjleti (46).

D. CONNECTIVE TISSUE REGENERATION

The connective tissue of the flap was also the site of considerable cellular activity, although the tissue underwent minimal disorganization, and regeneration was rapid. The connective tissue showed evidence of inflammation, followed by repair, with resolution within three weeks.

Restoration of the integrity of the connective tissue involved the production of new fibroblasts, migration of these cells into the
wounded area, formation of new intercellular material, and remodeling. The young fibroblasts were seen to originate from the loose perivascular connective tissue, not from the dense fibrous connective tissue adjacent to the wound. In fact, as reported by other investigators (47), it seemed implausible to separate the proliferative response of the capillaries from that of the fibroblasts.

E. OSSEOUS TISSUE REGENERATION

The responses involved in the healing of the osseous defects were in many respects similar to those which effected healing of the soft connective tissue wounds. However, because of the specialized nature of bone, differences did exist. As in the connective tissue, wounding was followed by the formation of a blood clot, which was removed by macrophages and replaced by loose connective tissue. This sequence was followed by osseous regeneration due to the presence of osteogenic or potentially osteogenic cells.

The osteogenic cells have an inherent potential for producing bone, and are those of the endosteum, bone marrow, and of the cambium layer of the periosteum of young bones (48). It is the perivascular undifferentiated mesenchymal cell which has the capacity to be induced in some manner into becoming an osteogenic cell (49).

The concept that the osteogenic and potentially osteogenic cells responsible for the production of new bone in the osseous defects
have their main origin in the adjacent marrow spaces (50,51) was remarkably well demonstrated by the changes which occurred in the surrounding alveolar bone during the repair process. The marrow cavities adjacent to the osseous defects were characterized by an increased mesenchymal cell population when compared with those marrow spaces further removed from the lesions. As the need for additional cells to repair the defects developed, osteoclastic resorption was seen on the osseous walls of the marrow cavities. This was part of the accommodation process. Additional space was required to accommodate the greatly increased quantity of mesenchymal cells needed to facilitate repair. As the defects underwent repair, the space necessary for cellular accommodation was diminished, and osteoblastic activity occurred on the osseous walls of the marrow cavities. New bone was formed, resulting in a reduction of the diameter of the marrow cavities as a return to a more normal cell density of these spaces transpired.

Thus, although young mesenchymal cells and capillaries were seen to eminate from the periodontal ligament, and from the overlying connective tissue flap as the defects became organized by young connective tissue, the great majority of these cells had their main origin in the marrow cavities.

The manner in which these perivascular undifferentiated mesenchymal cells became involved in osteogenesis is of more than academic
interest. This study demonstrated that osteoclastic resorption of the osseous defect walls and grafted bone fragments plays a significant role in the subsequent osteogenesis by which the defect undergoes regeneration of normal tissue. It is the initial osteoclastic resorption which in some manner induces the undifferentiated mesenchymal cells to differentiate into osteoblasts, and thus facilitate osseous repair. The biologic mechanism by which this occurs may involve the release of the inductive substance(s) from mature bone by osteoclastic activity. Such a histochemical material may then be free to effect the differentiation of the undifferentiated mesenchymal cells into osteoblasts. This concept is well illustrated by the following synopsis of the osseous regeneration in the experimental defects in this study.

It was consistently observed that following the organization of the defect site by young granulation tissue, osteoclastic resorption occurred along the osseous defect walls before osteoblastic activity began. The 10 and 14 day specimens were characterized by both processes occurring simultaneously. Areas of resorption and osteoid formation along the defect walls were seen, as well as the growth of young trabeculae from the osseous walls into the defect sites.

The same sequence of events, that is osteoclastic resorption preceding osteoblastic activity, was also seen on the bone fragments that had been implanted into the defects. At 7 days postoperatively,
each bone particle was completely enveloped in mesenchyme and young capillaries. Each particle appeared to have its own periosteum, and an occasional osteoclast was found in close proximity to the grafted bone chips. At 14 days postoperatively, these fragments had assumed a more basophilic character, and osteoclastic activity was more pronounced. However, even at this time, some of the fragments showed evidence of osteoid deposition on their surfaces. New bone apposition continued from these multiple independent foci of ossification until a confluence of new bone was achieved between these centers and the defect walls at 28 days. This phenomenon was seen in both the two and three walled graft specimens, and is very similar to the involucrum formation occasionally seen in osteomyelitis, whereby fragments of necrotic devital bone devoid of osteocytes (sequestra), become surrounded by new viable bone (52).

Regeneration of the osseous structure in the control specimens was not as rapid, although eventually it was as complete. In these specimens, osteoblastic activity began at and near the defect walls following an initial phase of osteoclastic resorption, and continued until the osseous anatomy had been restored.

Thus, the inductive power of the grafted bone fragments was well demonstrated. The cortical bone fragments did not themselves proliferate and form new bone, but induced the undifferentiated mesenchymal cells of the host site to differentiate into osteoblasts and to
organize and form bone. It is entirely plausible that the histochemical substances of the matrix of bone contain the precursor of the inducing substance as suggested by McLean and Urist (40). This material may be released by the osteoclastic activity, and is thus free to effect the differentiation of the undifferentiated mesenchymal cells into osteoblasts.
CHAPTER VI

CONCLUSIONS

Chronic periodontitis can be successfully simulated in primates by the method employed in this study. Osseous defects can be created with a marked degree of similarity to one another and subsequently rendered into chronic lesions for healing-repair studies.

The chronic periodontal osseous defects corrected by the osseous coagulum technique and by curettage in the rhesus monkey in this study were repaired by the regeneration of the architecture of the lost tissue.

The use of the osseous coagulum in two and three walled periodontal osseous defects led to a more rapid osteogenesis in such defects as compared to correction by curettage alone. This rapid filling of the osseous defects may serve to inhibit the apical migration of the epithelial attachment during the early stages of repair, and thereby inhibit a subsequent recurrence of the defect.

Histologically and temporally, no readily apparent distinction could be made in the healing process between the two and three walled lesions.
CHAPTER VII

SUMMARY

A study of the healing phenomena of chronic two and three walled osseous periodontal defects which had been corrected by the osseous coagulation technique was undertaken.

Four adult rhesus monkeys served as the experimental model, and provided 32 specimens from 0 to 120 days postoperatively. Twenty-four of these were graft specimens, and 8 were control specimens in which the defects had been corrected by curettage.

Histologic sections were prepared from each specimen, a sequential description of the healing process was recorded, and the variations and similarities in the regeneration of the periodontium were discussed.

The simulation of chronic periodontitis in primates was accomplished by the method employed in this study.

The grafted bone fragments did induce osteogenesis and facilitated a more rapid osseous repair. Only after 60 days did the control specimens demonstrate a similar level of regeneration.

Healing in the two and three walled defects was similar in the rhesus monkey histologically and temporally.
CHAPTER VIII

ILLUSTRATIONS
Fig. 1.—Clinical illustration of the gingiva, preoperatively. Note that the slight marginal chronic gingivitis was confined primarily to the interdental papillae. Sulcus depth was less than 2 mm.
Fig. 2.—Clinical illustration of the surgically created two and three walled osseous defects adjacent to the mandibular left second premolar.
Fig. 3.--Clinical illustration of the placement and fixation of the toothpicks in relation to the prepared osseous defects.
Fig. 4.—Clinical illustration of the replacement and fixation of the full thickness mucoperiosteal flap. Note the toothpicks mesial and distal to the second premolar.
Fig. 5.—Clinical illustration 1 week postsurgically. Note the marked inflammation of the gingival tissue.
Fig. 6.--Clinical illustration of the gingiva 30 days subsequent to the creation of the osseous defects. Note the changes in the gingival tissues. The gingival margin was rolled, and the interdental papillae were bulbous in form, erythematous in color, and boggy in consistency. Significantly, at this time the sulci in the areas of the prepared defects were consistently 5 mm. in depth.
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X 100
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BIBLIOGRAPHY


This thesis, submitted by Leon K. Coverly, has been read and approved by three members of the faculty of the Department of Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 15, 1972
Date

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Signature of Advisor