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Free Gingival Grafts: A Histological Evaluation in Humans

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FREE GINGIVAL GRAFTS: A HISTOLOGICAL EVALUATION IN HUMANS

BY

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AUTOBIOGRAPHY

The author was born on July 16, 1939 in Fort Worth, Texas. A rather nomadic life makes the schools attended for primary education too numerous to mention here. His public education was completed in 1957 at Lubbock Senior High, Lubbock, Texas. Athletics were limited to football and boxing.

After completing one semester attending Texas Technological College, he volunteered for the draft. He was inducted into the Army and while finishing a European tour, decided on dentistry as his future course of study. Upon release from the service, he attended The University of Texas full time for one year. The remainder of his pre-dental work was done on a part time basis while attending The University of Houston and South Texas Junior College.

He successfully completed his dental education in June of 1967 receiving his Doctor of Dental Surgery from The

University of Texas Dental Branch. He was fortunate enough to be accepted into Loyola's graduate program the same year and received his Specialty Certificate in Periodontics in June of 1969.

ACKNOWLEDGEMENTS

The author extends his thanks and acknowledges that this thesis would not have been written except for the help of the following:

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Dr. Gustav Rapp whose concern for his graduate students transcents the usual and ordinary.

My father, brother and memory of my mother without whose moral support I might not have pursued any college education.

Special thanks to my wife for fulfilling the roles of mother, breadwinner and wife much longer than she expected: for

these she will be recompensed.

DEDICATION

This thesis is dedicated solely to Mr. Jay Gordon, principal of Lubbock Senior High 1954-1957, of whose insight, patience, discipline, and fairness I represent the ultimate in beneficiaries. Without his help I would have remained a High School Drop-out.

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INTRODUCTION

Free gingival grafts are relatively new to periodontal therapy. This is highlighted by the absence of any mention of this technique in the World Workshop in Periodontics, 1966.[17] The great vascularity of the oral mucosa enhances the free gingival grafts applicability for such gingival defects as 1)gingival clefts, 2) absence of attached gingiva, 3) vestibular extensions, 4) areas of this gingiva, 5) frenum problems, 6) as a simple adjunct to speed epithelialization of broad wounds.

The mechanism of graft "take" has been the subject of much speculation and valid research. Gargiulo and Arrocha,[5] Nabers,[11,12] Sullivan et al,[22,23] Oliver et al,[13] have researched the techniques used in successful grafting. Clinical data is plentiful in the literature; however, histological studies have shown the response only so far as the soft tissues are concerned. The purpose of this thesis is to correlate previous histologic findings with changes in the

underlying alveolar bone and periodontal ligament in the human.

LITERATURE REVIEW

The use of free gingival grafts in periodontal therapy is a direct outgrowth of medicine's widespread successful use of skin grafting. It is interesting to note that free grafts were first used in dentistry as adjunctive therapy in prosthetics and involved transplanting skin into the mouth.[6,14,15]

HISTORICAL DEVELOPMENT OF SKIN GRAFTS

According to Converse[1] the first clinical report of a successful free graft was made by Bunger in 1823. A piece of skin ten by six centimeters was taken from the lateral border of the thigh and applied to a nasal defect. The graft was successful in spite of one hour and thirty minutes delay from removal of graft to placement on the recipient bed. Around 1840 the first grafting operations were done in the United States by Warren.

Reverdin's astute observation of the "spreading epithelium"

from epithelial islands in the center of granulation tissue did much to stimulate the clinical use of skin grafts.[18] He advocated and used small pinch grafts to try to reproduce these naturally occuring islands. Although he tried to limit the depth of the epidermis, he admittedly went much deeper.[18]

Three years later, Oliver hypothesized that larger full thickness grafts of over eight square centimeters would yield better results. He first emphasized the need for immobilization of grafts, using a plaster bandage for this purpose.[2] As early as 1875, Wolfe emphasized the importance of removing all areolar tissue from the underside of a full thickness graft to insure first intention take.[27]

Thiersch was most quoted in the literature as the early advocate and researcher on "thin grafts" which were later to become known popularly as the split thickness grafts.[24] His contemporary, Wolfe, did much work on the use and techniques involved in successful "thick grafts".[27] Until the advent of the dermatome, these two types of graft were used predomi-

nantly.

The intermediate split thickness graft was first described by Blair and Brown in 1929. Padgett's introduction of the dermatome in 1939 made practical the use of split or intermediate thickness grafts.[2]

Converse and others[1, 2, 4, 19, 21] classify skin grafts as follows:

FULL THICKNESS GRAFTS

Since the full thickness graft includes part of the fatty layer, it must be either very narrow (as in the case of scalp grafts), or the fat must be meticulously trimmed away as it forms a barrier to the new capillaries as well as the initial "plasmic" circulation. Since the base of the hair follicle is implanted in the adipose tissue subcutaneously, full thickness grafts must be used in scalp grafting. Revascularization takes place at the margins rather than from the bed.[4]

According to Converse, failures of thick grants occur when placement is in a less vascular area. The graft depends on surface area versus volume of graft as it relates to the ability of the tissue fluid from the recipeint side for nourishment of the graft until new capillaries rebuild the blood supply.(2)

SPLIT THICKNESS OR INTERMEDIATE THICKNESS GRAFTS

When Padgett (1939) disected through the deeper layers of the dermis he started what was termed as the three-quarters thickness graft. This split thickness graft had special applicability after the introduction of the dermatome, as it allowed for a smoother subsurface and the uniform absence of fat.

THIN GRAFTS

In thin grafting techniques, the lower borders of the epithelial ridges are left as natural "islands" for the re-epitheliaization of the donor site. Until the dermatome, these grafts were extremely difficult to perform. Several calibrated knives were used before

the dermatome to try to approach uniformity of thickness of the donor.

According to Converse and others [1, 2, 3, 19, 21] principles necessary to insure successful take are as follows: 1) Graft fixation, (complete suturing varying with the type of graft), 2) Pressure dressing, (important due to critical period of plasmotic circulation and fixation of graft during the first five hours by a fibrin bond), 3) Immobilization, (the immediate area to keep from disrupting fibrin bond or (later) the early vascularization of the graft), 4) Complete hemostasis of recipient bed (to keep clots from forming gaps too wide for vascularization to bridge).

HISTORICAL DEVELOPMENT OF INTRAORAL GRAFTING

Gorney et al [6] first applied grafting techniques to aid in prosthetics; using thigh tissue to deepen a mandibular vestibule of an edentulous patient. Sophisticated graft technique was in its infancy in 1942 and this was reflected in the intricacy of their technique. Valauri [25] repeated

this operation and simplified the technique with similar success.

Propper [14] first advocated and performed free intraoral grafts utilizing oral mucosa as the donor tissue. He noted that when using the buccal mucosa, the full thickness grafts make superior donor tissue for ridge extension.

In the periodontal literature, the techniques for correction of gingival defects was developed first along the lines of the pedical graft. Grupe et al [8] first advocated the use of the pedical gingival graft and termed it the "lateral sliding flap". Others picked up the lateral sliding flap technique and refined it, but until Propper [14] pointed out the feasibility of the free graft, little was done to develop this therapeutic arm.

Nabers first used gingival tissue as the donor site in extending the vestibule. [11] His was the first use of a free graft in the dentulous patient. The technique used was vastly more streamlined than those advocated earlier. Instead of making elaborate facilities for immobilization, he used rubber dam to allow

movement with as little friction as possible transmitted to the graft. Later [12] he defines a good donor site as "one that requires removal of a volume of gingival tissue". Haggerty [9] used the free gingival graft to create a favorable environment for a full crown and stimulated interest in the free gingival graft by clearly stating the limitations of the lateral sliding flap.

Gargiulo and Arrocha [5] published the first definitive wound healing study of autogenous free gingival grafts. A synopsis of their histological findings with regard to healing may be found on page of the appendix.

Sullivan and Atkins [22, 23] took the principles laid down by Converse, Mays and other plastic surgeons and applied them directly to gingival grafting. The direct applicability of these principles will be taken up in the discussion of this paper. In a later study [23] the soft tissue response in the graft and recipient bed was compared to the histology of the surrounding gingiva. Since the biopsies of the grafts were timed rather late in the healing period, most of their

discussion on mechanisms of healing is conjecture. The third part of Sullivan et al's study[23] of gingival grafts dealt with the classification of gingival clefts and how the classification related to successful therapy via the free gingival graft.

Oliver et al (1968) reported histological confirmation of Sullivan's et al speculation on the mechanism of gingival healing and reconfirms findings of Gargiulo et al.[13]

Wound healing studies imply a relationship between extent and nature of the injury and healing control. Many researchers feel that healing is governed by cytolytic products of injured cells. The development of these theories and their applications in research are well documented by Tier et al.[28] It has been shown that like most biologic processes, there is a "threshold" which must be reached before cytolytic products act to effect rate and type of regeneration.

MATERIALS AND METHOD

The materials used in this study were as follows:

Bard-Parker #15 Blades	Gracey currettes
000 silk sutures	Orban periodontal dressing
700 fissure burrs	Cavitron with p-10 tips
Adaptec (Johnson & Johnson)	Xylocaine 2% with 1/100,000 epi
Iris scissors	2"x2" gauze pads
10% formalin	Specimen bottles
High speed handpiece	Normal sterile saline
Paraffin	Hemotoxylin and Eosin stain
Microtome	decalcifying agent
kodachrome II film	27 guage needles
Hu-Friedy #6 periosteal elevator	Assorted filters for photo-micrographs
Glass microscope slides	Cover slips
Ethanol for drying	Universal extraction forceps

All materials used in conjunction with any surgical procedure were sterilized and sterile surgical procedure was adhered to at all times.

Seven human patients who were scheduled for maxillary immediate dentures were selected for study. Due to the voluntary nature of participation in the study, total control over the position of the grafts and recipient sites was impossible. An attempt to control such variables as alveolar bone thickness was made by limiting both donor and recipient sites to the attached gingiva of the maxillary anterior teeth.

Donor and recipient sites were selected and one to two days prior to grafting scaled and debrided. All patients exhibited moderate to severe periodontal disease, hence no attempt was made to limit donor or recipient sites to clinical normals. All specimen material exhibited varying degrees of inflammation. During all surgical procedures the patients were anesthetized with 2% xylocaine containing 1/100,000 epinephrine.

The recipient sites were prepared in the following manner. A three by four millimeter area of attached gingiva was re-

moved from the labial surface of an anterior maxillary tooth using a Bard-Parker #15 scapel. Care was taken to center the recipient site over the thinnest part of the alveolar bone. The periosteum was left intact, but the connective tissue was thinned as much as possible with iris scissors. Digital pressure was applied using 2"x2" gauze pads to aid in hemostasis.

A graft was obtained from the previously prepared donor site by passing a scapel through the connective tissue parallel to the alveolar bone resulting in a split thickness graft. The thicker grafts were thinned on the inner surface with iris scissors. The grafts were transferred to the freshly prepared but no longer bleeding recipient bed. 000 silk sutures were used to hold the grafts in place by suturing the undisturbed gingiva at the edge of the recipient bed, looping the sutures over the graft and tying them in the form of an X (see Fig. 1). This method of suturing was chosen so no part of the graft-bed interphase would be disturbed by

sutures and in order to maintain the integrity of the eipthelial surface of the graft.

Adaptec* was used to cover the graft and digital pressure was applied for three minutes to allow for fibrin formation and to prevent extensive clot formation which might compromise graft "take". Orban periodontal dressing was then placed and the patient cautioned about hard foods, chewing, and other disturbances to the graft. Sutures were removed when the specimens were obtained or on the seventh post-graft day on those subjects designated for longer term specimens.

Modified block sections were acquired after the manner of Ramjford[16] as follows. Parallel vertical incisions were made bordering the lateral margins of the graft from the gingival margin into the mucous membrane below the mucogingival junction through the periosteum to the underlying bone. A horizontal incision connecting the two parallel incisions

*Johnson and Johnson

was made at the mucogingival junction. All three incisions were made with a Bard-Parker #15 scapel. (See Fig. 2A) Mucoperiosteal flaps were elevated and retracted away from the graft laterally and apically exposing the alveolar bone at the margins of the graft. With a sterile 700 fissure burr and high speed handpiece, cuts were made parallel to the borders of the graft, apically and laterally through the alveolar bone into the periodontal ligament space. Thus the alveolar bone underlying the graft was left affixed only to the tooth by periodontal ligament fibers. (See Fig. 2C) The tooth was then extracted by conventional means taking care not to dislodge the specimen and placed in formalin solution for fixation.

After remaining extractions and alveolectomies were performed, the defect was covered with soft tissue by approximating the borders of the attached gingiva and suturing with 000 silk sutures. (Fig. 2D) Where it was necessary to effect a full closure, relief incisions were made distal

to the cuspid areas. Immediate dentures were inserted and recovery was uneventful.

The specimens were prepared for histologic examination as follows. The specimens were washed of free blood for one minute and placed directly into 10% formalin solution for forty-eight hours. After fixation was complete the specimens were washed in running water for four hours. Decalcification was accomplished with formic acid and sodium citrate solution and the specimens were again washed for twenty-four hours in running water. Drying was accomplished by successive ethanol baths of 75%, 95% and 100% and then the specimens were placed in normal saline prior to paraffin infiltration. After being mounted in blocks of paraffin, the specimens were cut on a rotary microtome @ 6 micron thickness, mounted on glass slides, and stained with hemotoxylin and eosin for study.

FINDINGS

In the earliest available section (two days) the graft was clearly delineated from the host by large numbers of polymorphoneuclear leukocytes, some free red blood cells, and large dialated capillaries (Fig. 3,4,5). The graft interphase* showed an abundance of fibrin, and evidence of early capillary invasion of the graft (Fig. 4,5). The epithelium is thinner than normal and showed evidence of hydropic degeneration (Fig. 3,4,6). The buccal surface of the alveolar bone showed no evidence of osteoclastic activity (Fig. 3,4,5,7). The periosteum appeared to be edematous with some signs of inflammation (Fig. 5,7). As reported by Steffileno in dogs, the periodontal ligament side of the alveolar bone showed marked evelation of osteoclastic activity hereafter referred to as "rebound effect" (Fig. 3,4.7). The overall graft represented a "take" without question (Fig. 3,4).

*Area between the inner graft surfaces and outer recipient surfaces.

The four days specimen's graft-recipient interphase shows more organization with the loss of distinct delineation, and the appearance of an abundance of capillary sinusoids were present. Some fibrogenesis was in evidence, and the fibrin interphase of the two day specimen has disappeared completely (Fig. 8). The epithelium, although very still thin and flattened, appeared normal (Fig. 9). Some surface osteoclastic activity was present on the alveolar surface in this specimen (Fig. 10). Rebound osteoclastic activity was much more prominent than seen in the previous specimen. The periosteum showed no edema or inflammation at this time, however (Fig. 10).

At eight days the most prominent feature of the graft is that the capillary loops are prevalent throughout the graft, the interphase, and the recipient tissues areas, extending from the periosteum to the base of the epithelium (Fig. 11). The epithelium, although still much thinner than normal gingiva, shows a complete recovery from the hydropic

changes in evidence in earlier samples (Fig. 11). Rebound osteoclastic activity is still in evidence along the margin of the buccal bone (Fig. 14). The connective tissue fibers show orientation running almost exclusively parallel with the alveolar bone (Fig. 13).

The seventeen day specimen's graft interphase showed the remains of sinusoids or what might have been interpreted to be lymphatics, but capillaries were still numerous. The boundaries of the interphase are no longer discernable (Fig. 15). The epithelium is thicker but still lacks distinct regeneration of the rete pegs(Fig. 16). Some early evidence of mitosis was found in the basal cell layer of the epithelium (Fig. 17). Osteoclastic activity had returned to normal on the periodontal ligament side of the alveolar bone. (Fig. 18, 19, 20). The rapidity of the osteoblastic activity was demonstrated by an bundance of osteiod tissue without osseous deformity (Fig. 20). The connective tissue fibers had oriented themselves almost exclusively to the surface of the

alveolar bone in this specimen also (Fig. 15).

At twenty-three days connective tissue pegs are regenerating but the most prominent feature of the graft is the abundance of capillaries. The connective tissue fiber orientation continues parallel to the bone, thinness of the epithelium and the rapidly forming bone on the buccal aspect are the only features distinguishing this area from normal, unoperated area (Fig. 21, 22, 23).

The forty-nine day sample's epithelium showed complete maturation with the return of distinct ridges and pegging of the epithelium and connective tissue respectively (Fig. 24). The connective tissue fiber orientation parallel to the alveolar bone was still present (Fig. 25, 26). The periodontal ligament appeared normal as did the alveolar bone (Fig. 26).

The six month specimen displayed epithelium, connective tissue, and denseness of vascularity indistinguishable from

non-grafted gingiva (Fig. 27). The only residual evidence of grafting might be construed to be the orientation of the connective tissue fibers parallel to the labial surface of the alveolar bone (Fig. 28).

DISCUSSION

It is evident that graft adaptation and "take" is initiated within forty-eight hours, and total repair is accomplished by the eighth day. On the fourth day, capillary bridging is accomplished and the graft no longer depends on the "plasmotic" circulation mentioned by Gargiulo, et al.(5) Other researchers have shown the mechanism of the graft "take" of gingiva is the same as that of skin. These findings are supported by this study.

In this study all of the patients had moderate to severe periodontal involvement. None of the graft tissues were completely normal at the time of grafting. Clinically all showed signs of inflammation and yet there was a take of the graft with an eventual new locus of attached gingiva. While a meticulous approach is to be applauded in any surgical periodontal procedure it is felt that some researchers

have overcomplicated the techniques to be used and qualifications for suitable donor tissue to the point of discouraging use of what is found to be a relatively simple, almost totally predictable, adjunct to routine periodontal surgery.

In fact, it may ultimately prove beneficial to use slightly inflamed tissue as the donors since mobilization of defense mechanisms (i.e. local production of antibodies) would be improbably in the graft during the initial period when it is totally dependent on "plasmotic" circulation. Wittwer et al demonstrated the presence of antibodies in inflamed gingiva and the presence of antibodies should act beneficially during the degenerative phase of the graft.(26)

The epithelium of the graft undergoes immediate degenerative changes which may be attributed to ischemia. It is not attributible in any way to graft rejection because: 1) of its immediate onset, (rejection is delayed) and 2) its obvious reversibility. The earliest specimen exhibiting mitotic

figures in the basal layer of the epithelium was at seventeen days. The increase in epithelial thickness from eight to seventeen days would indicate mitosis was initiated at a much earlier date. Further study is indicated to ascertain how long the graft epithelium can survive without mitosis.

More important than the rapid epithelialization of the wound surface, is the marked decrease in surface osteoclastic activity and the early initiation of extensive osteoblastic activity apparently thickening the labial alveolar plate. The alignment of the cells along the labial surface of the alveolar bone in the eight days specimen is interpreted to be the initiation of osteogenesis. Not only is the earlier initiation of osteogenesis evident, but the rapidity with which the bone is laid down is attested to by the abundance of osteoid in the seventeen day specimen. This may indicate that the graft may act positively on the bone as an initiator of osteogenesis.

The epithelial degenerative changes and accompanying loosening of the connective tissue may be necessary for the survival of the graft. By changing from a "gel" to a "sol" state and the loosening of the connective tissue fibers prepare the graft for the invasion of capillaries from the recipient bed.

The clinical cicatrization and maintenance of graft form after histologic incorporation by the surrounding tissue was mentioned in the literature. The basis of this clinical scarring may be the connective tissue fiber orientation. The connective tissue fibers in the specimens uniformly showed post-graft parallel orientation to the alveolar bone. It is interesting to note that this parallel orientation was found throughout graft and recipient connective tissue. Whether this fiber orientation is due to the rapidity of maturation of the connective tissue or from the inflammatory response to healing is speculative. It does supply some insight into why surgical flap procedures may

breakdown however, as we know that random fiber orientation would not allow for lines of cleavage as we see post-surgically.

This study was undertaken primarily to ascertain the effects of free gingival grafts on the underlying bone. To understand the beneficial effects of gingival grafts on bone we must first recognize that the graft acts as a truly biologic dressing protecting the underlying tissues from the deleterious effects of standard periodontal dressings. Since "take" of the graft is dependent on a fibrin binding it obviously seals off the wound surface from the oral environment, as well as possibly carrying antibodies as an additional barrier to bacterial infection.

The trauma of surgery undoubtedly shocks the buccal plate of bone and retards osteoclastic activity. The surgical trauma is not sufficient to stop immediate osteoclastic activity on the periodontal ligament side, and on the contrary acts to induce and accentuate osteoclastic activity. This

"rebound effect" is seen on periosteal retention procedures also, but without the early initiation and magnitude of osteogenesis seen under free grafts.

Advocates of split thickness, apically repositioned flaps praise less dramatic reversible deleterious effects of this technique on the alveolar bone. The major difference in free grafting is the lack of nutrient supply to the tissue placed over the periosteium and underlying bone. In apically repositioned split thickness flaps and pedical grafts of any sort, the independent nutrient supply of the "graft" is maintained. ("Take" is essentially the same as in free grafts, dependent on fibrin binding.) Since the blood supply is maintained, the degenerative changes are minimal with less possibility for the development of cytolytic influences. Perhaps the basis for the increased magnitude of osteogenesis under free grafts is due to some specific cytolytic product of the connective tissue produced in the degenerative phase of the graft. This seems to be the most logical explanation,

especially in the light of work demonstrating autoregulation of growth by tissue breakdown products. Further study along these lines is certainly indicated.

SUMMARY

Seven human block sections were obtained and studied microscopically to ascertain the effects of free gingival grafts on the underlying bone. The most significant finding was early initiation of osteogenesis giving more emphasis to what was once thought of as an adjunct to routine periodontal therapy.

CONCLUSION

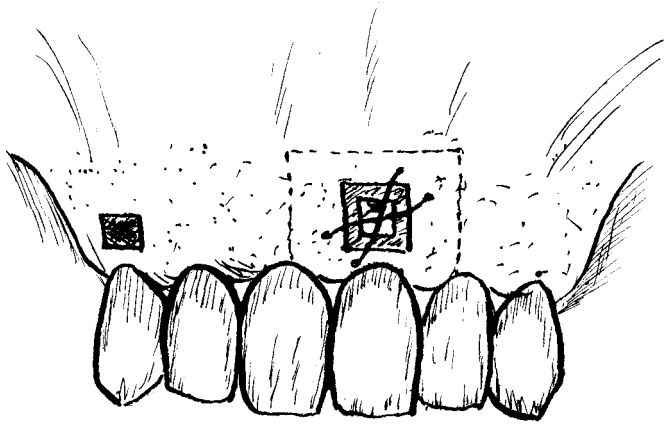
Autogenous free gingival grafts have advantages over certain procedures used more commonly. Lack of buccal resorption, rapid onset of osteogenesis, and the volume of bone production make free grafts especially applicable in areas where the buccal plate of cortical bone may be fused with the cortical bone of the periodontal ligament side making the area especially susceptible to fenestration or dehiscence formation. We see less osseous trauma in the early phases also.

The strict requirements for successful "take" in skin grafting need not be transposed rigidly to free gingival grafts. This research showed this to be true at least as far as the use of inflamed donor tissue is concerned.

Because of the dependability of take, availability of graft

material, and the advantage of earlier osteogenesis, the autogenous free gingival graft should become more commonly used in periodontal surgical techniques.

Fig. 1.



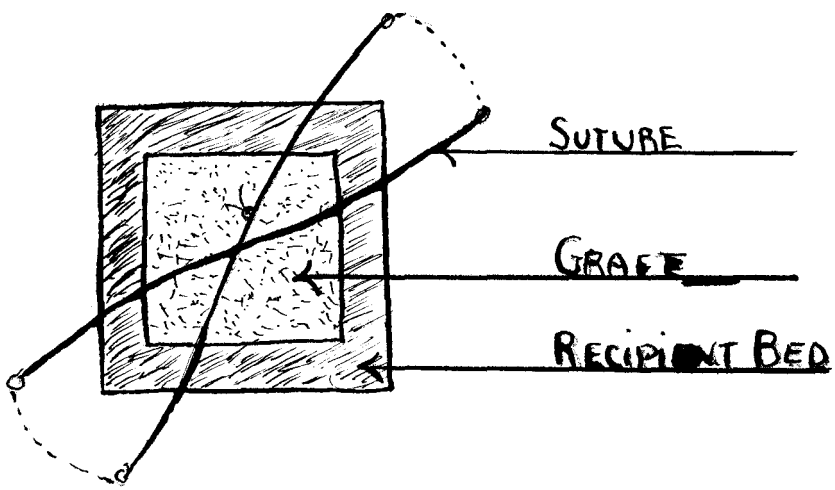
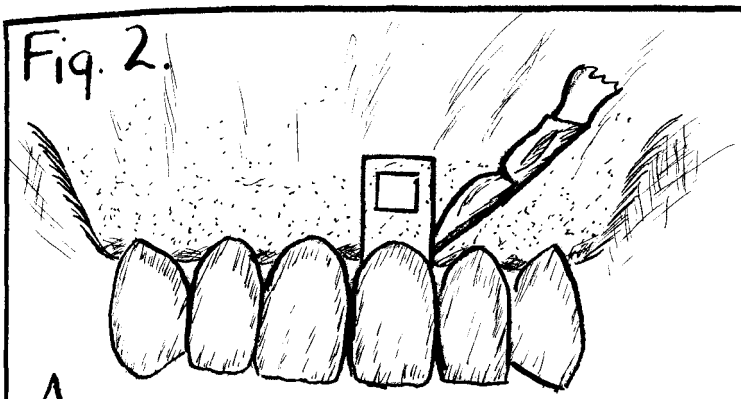
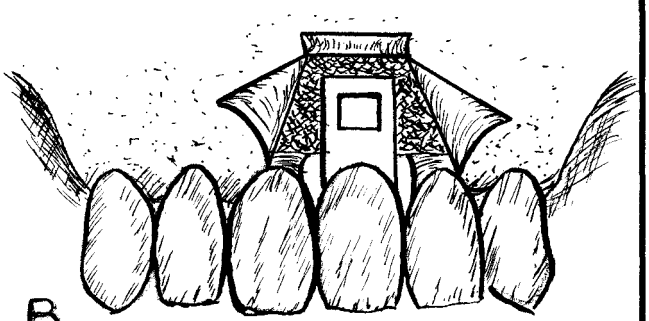


Fig. 2.

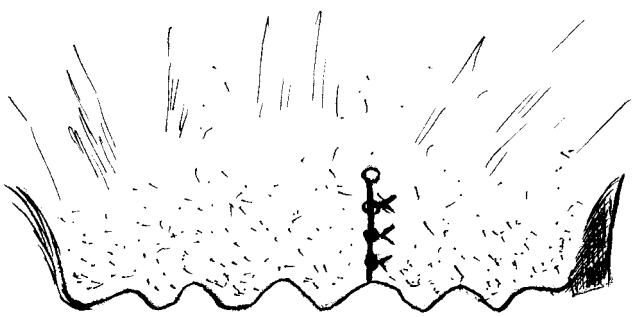


A.



B.

C.



D.

FIGURE NO. 3

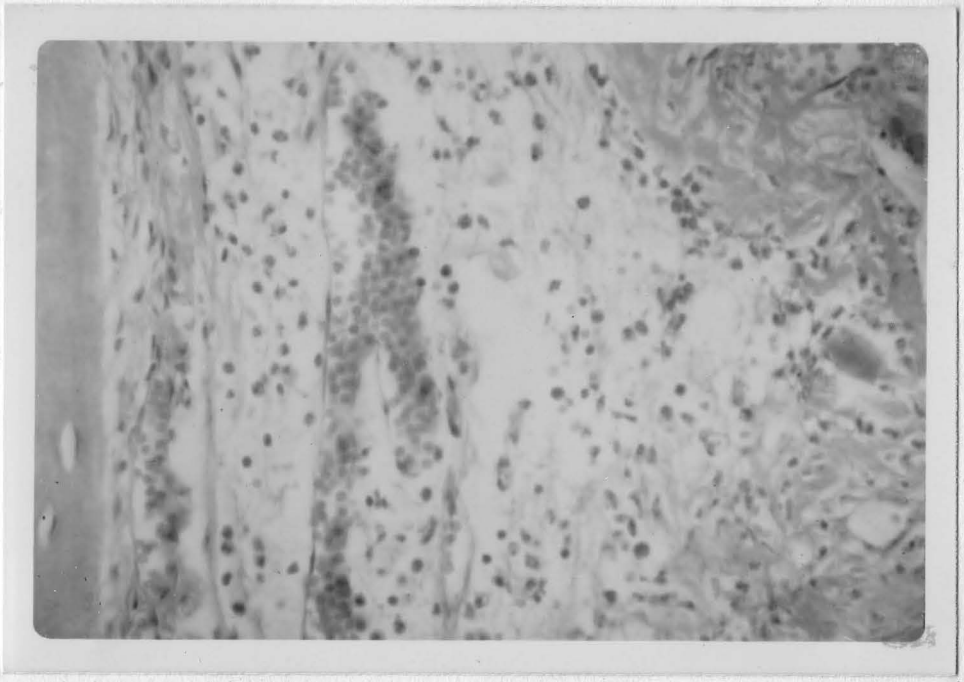
40X: Two Day Sample

Survey section of two graft showing interphase clearly delineated with inflammatory cells, large dilated capillaries and free erythrocytes. Note the absence of surface osteoclastic activity on labial side and marked osteoclastic activity on the periodontal ligament side, i.e. "rebound effect".

FIGURE NO. 4

100X: Two Day Sample

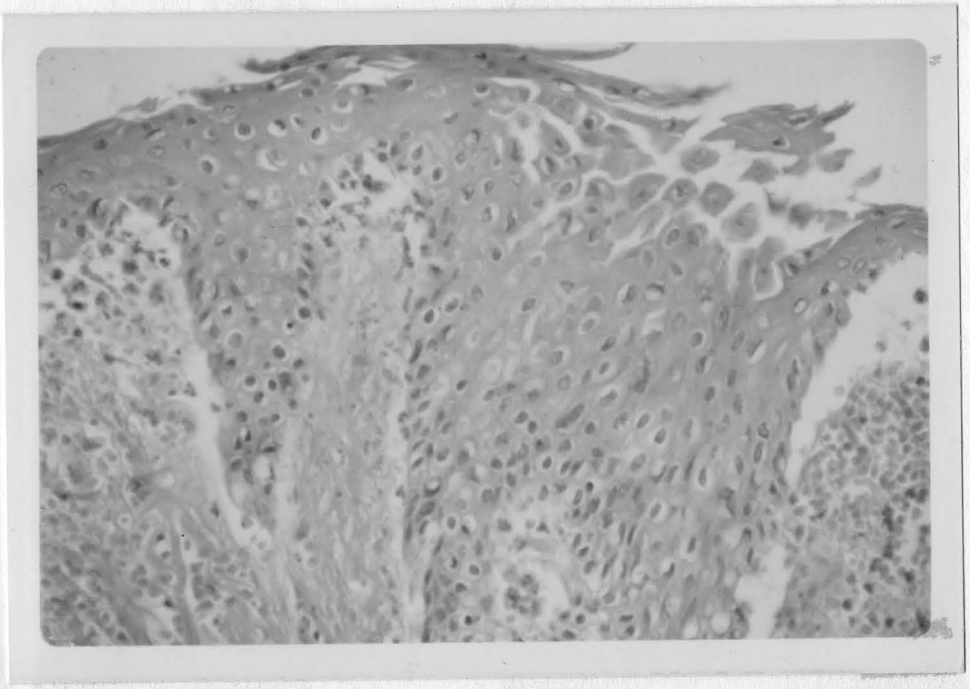
FIGURE NO. 5



250X: Two Day Specimen

High power of interphase area showing fibrin and early capillary dilatation and invasion of the graft. Note the edematous periosteum.

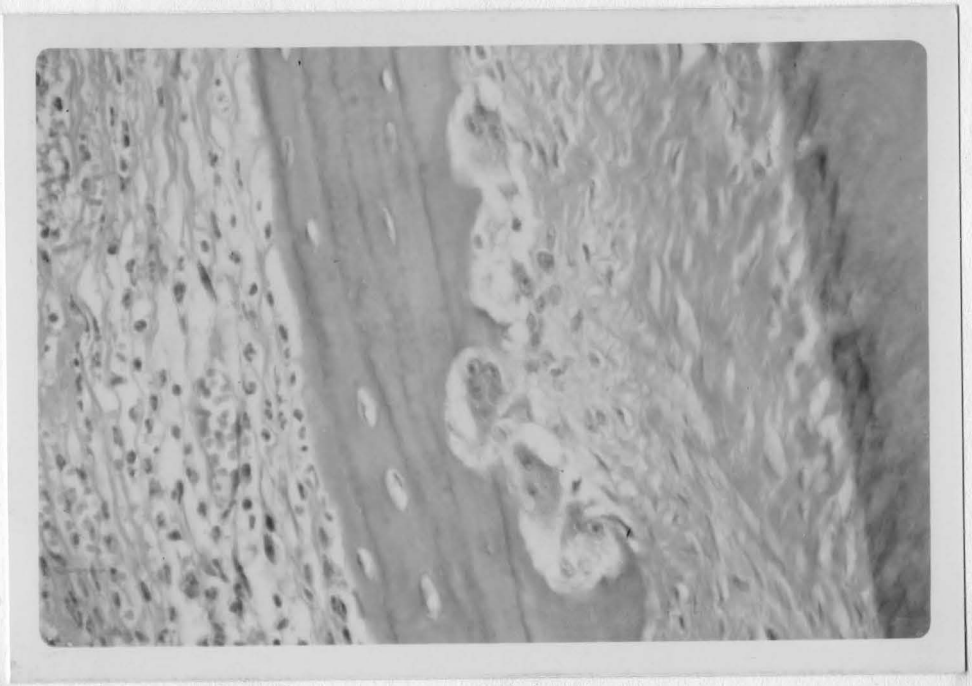
FIGURE NO. 6



100X: Two Day Specimen

Graft epithelium showing "hydropic like" degenerative changes.

FIGURE NO. 7



250X: Two Day Specimen

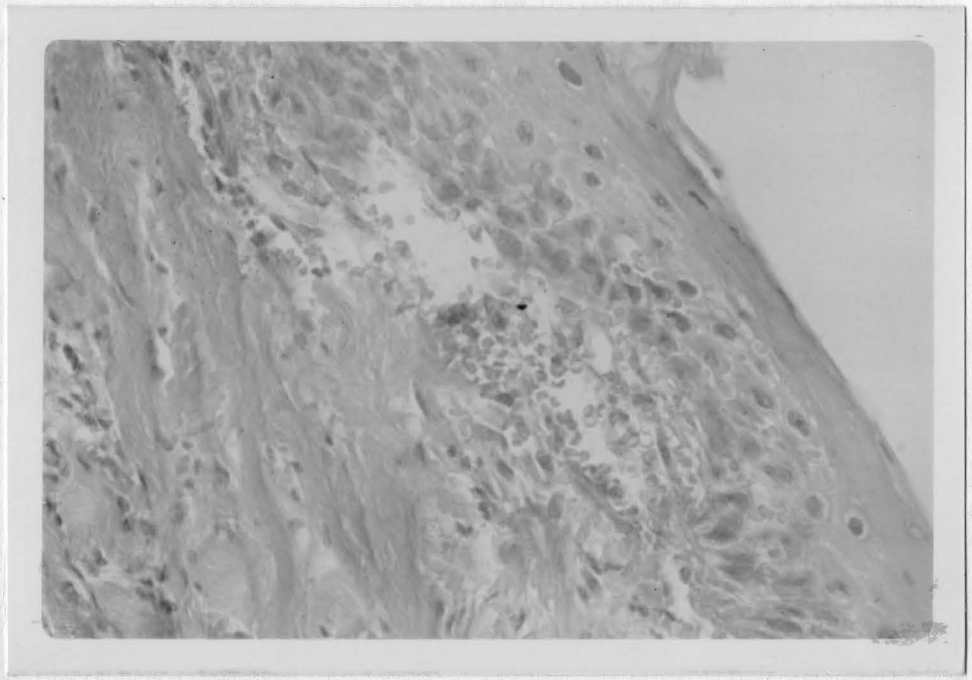
High power of edematous periosteum and osteoclastic rebound activity.

FIGURE NO. 8

100X: Four Day Specimen

Four day graft showing thinned epithelium and abundance of capillary loops extending into the graft. Note the absence of fibrin in the area.

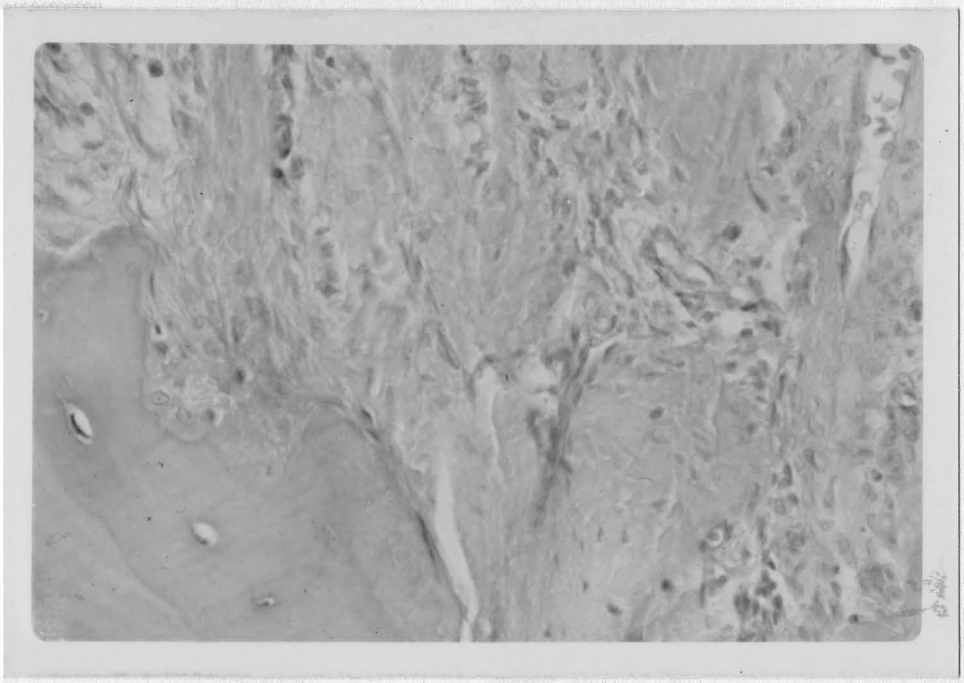
FIGURE NO. 9



250X: Four Day Specimen

Atrophic Papillary Projections in Epithelium (but showing less degenerative changes than the two days specimen).

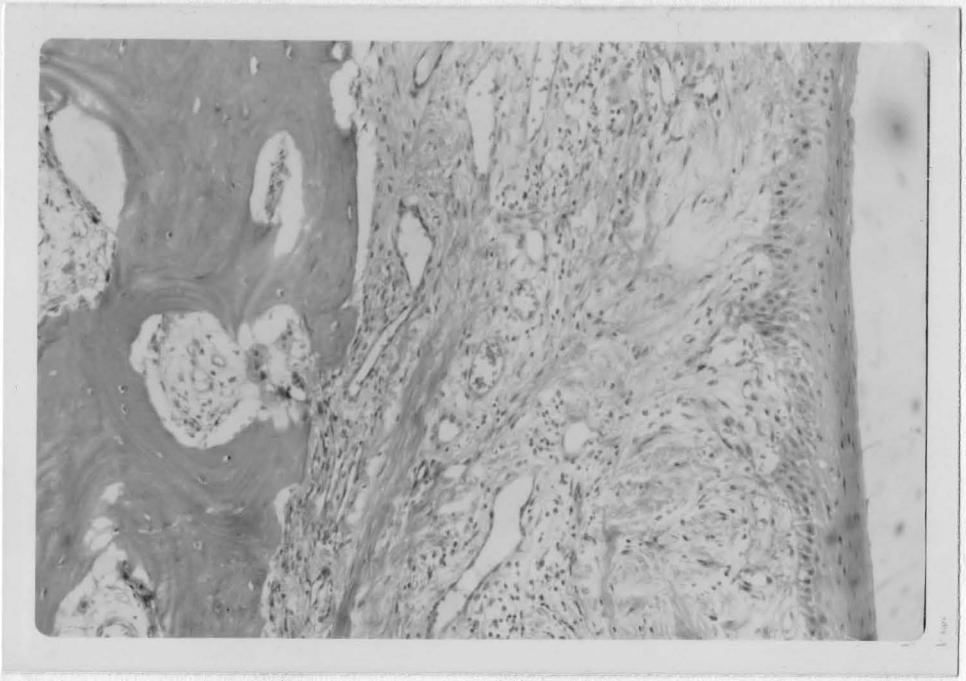
FIGURE NO. 10



250X: Four Day Specimen

Four day specimen showing mild surface osteoclastic activity and resolution of edema in periosteum.

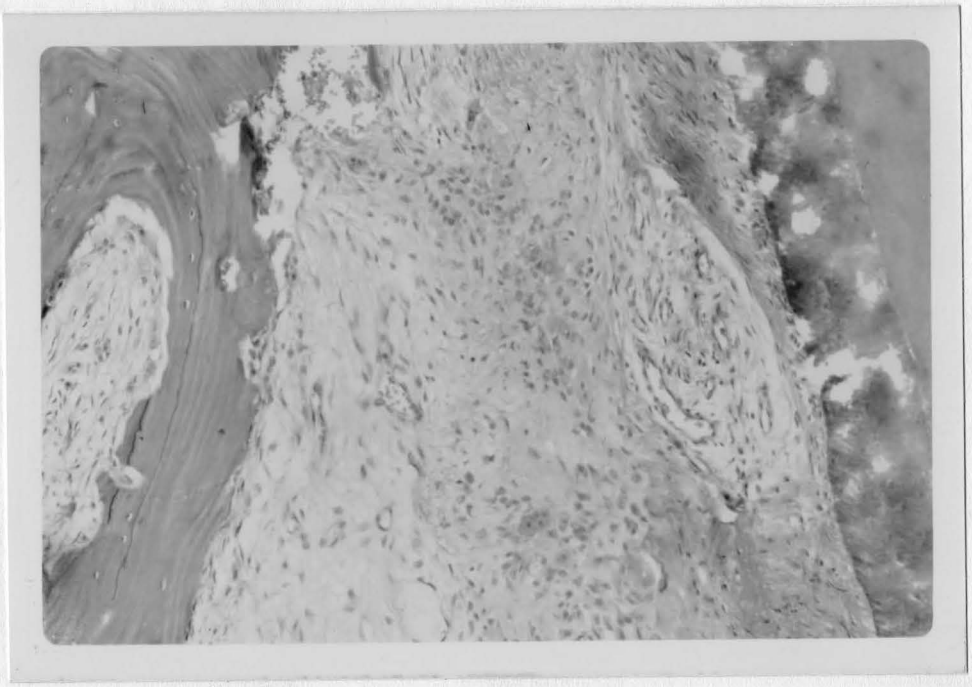
FIGURE NO. 11



100X: Eight Day Specimen

Eight day sample showing an abundance of capillary loops extending from recipient bed to base of epithelium.

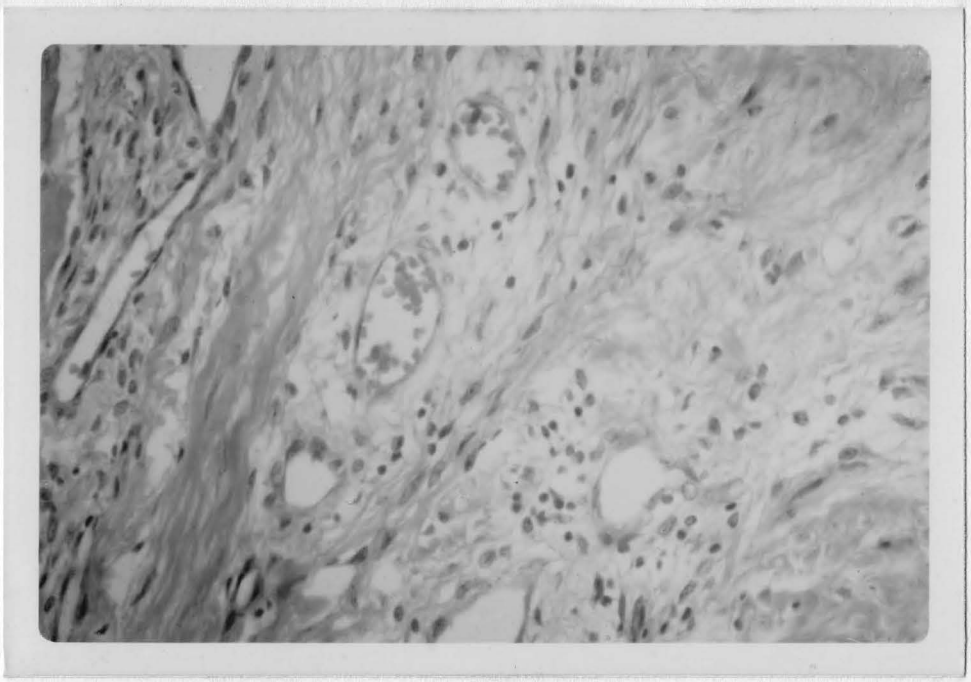
FIGURE NO. 12



100X: Eight Day Specimen

Eight day periodontal ligament space showing less rebound activity and almost normal appearing periodontal ligament.

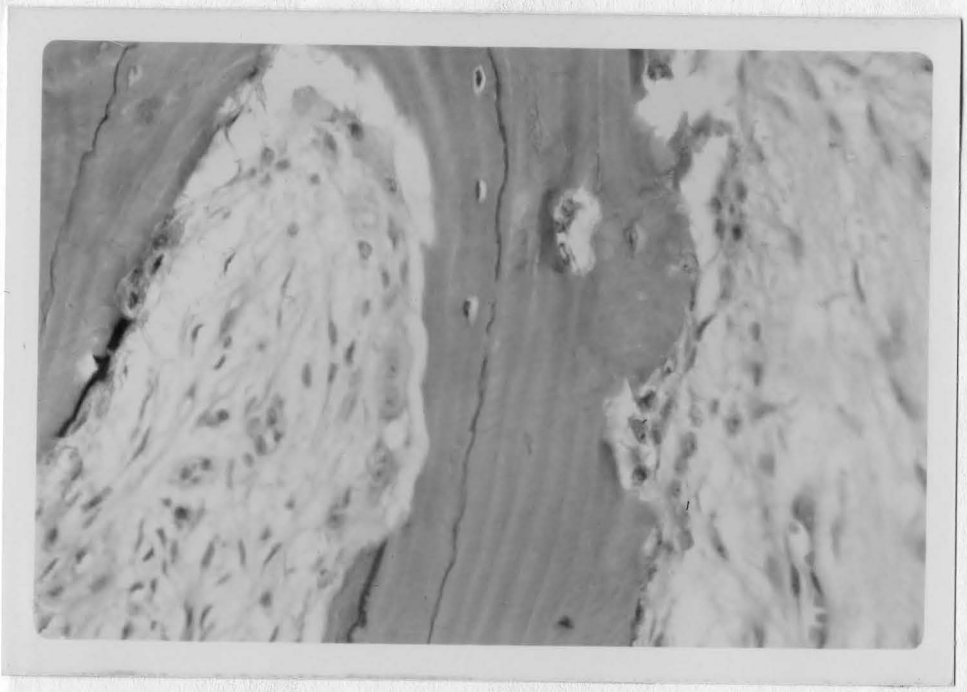
FIGURE NO. 13



250X: Eight Day Specimen - Interphase Area

Note the maturing connective tissue and abundance of capillaries. The connective tissue fibers seem to be aligning themselves parallel to the alveolar bone as they mature.

FIGURE NO. 14



250X: Eight Day Specimen - Alveolar Crest

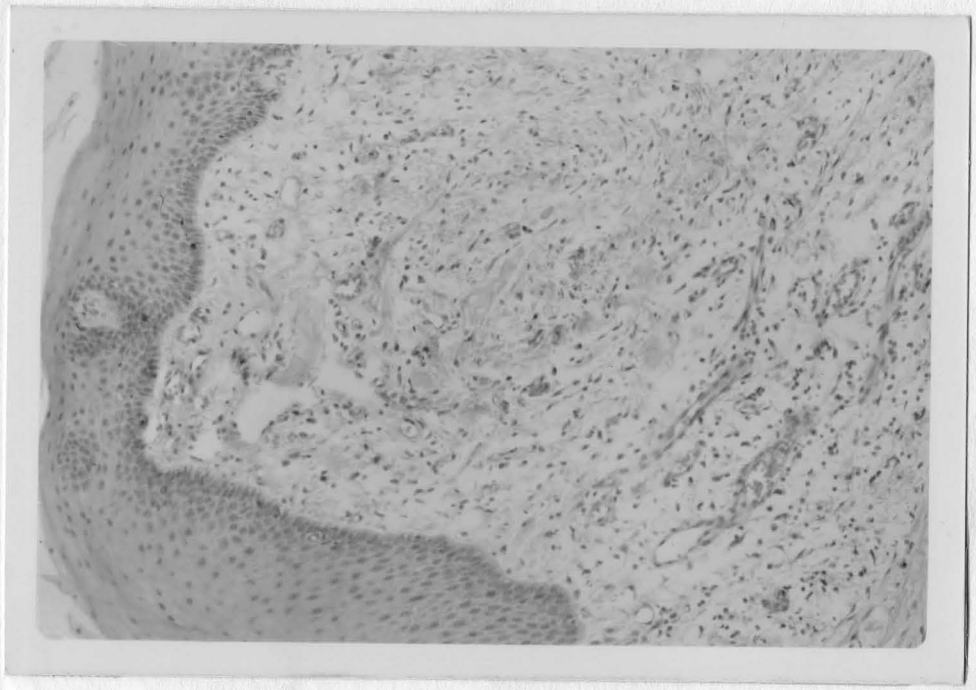
Note the lighter staining area below the osteoclastic activity, perhaps as early as eight days we are beginning to see osteoblastic activity on the buccal surface.

FIGURE NO. 15

100X: Seventeen Day Specimen - Interphase Area

Interphase area showing rapidly maturing well organized connective tissue. Sinusoids are disappearing but an abundance of capillaries are still present. Note the alighment of the connective tissue fibers parallel to the surface of the alveolar bone now extending into the graft itself.

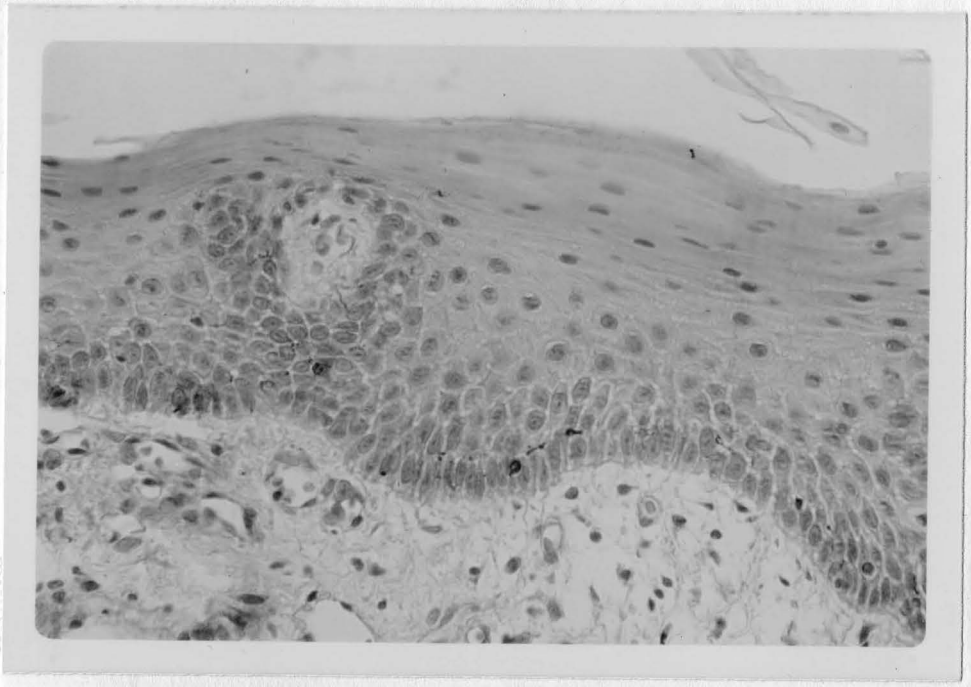
FIGURE NO. 16



100X: Seventeen Day - Epithelium

Note healthier appearing, thicker epithelium still lacking rete pegs.

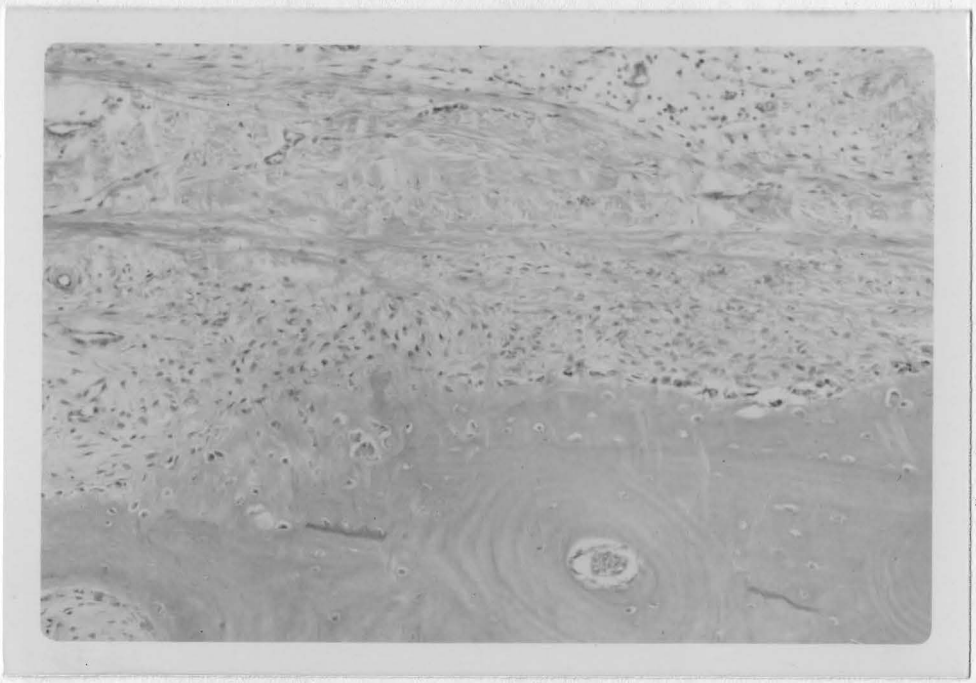
FIGURE NO. 17



250C: Seventeen Day Specimen - Epithelium

Epithelium showing return of mitosis in basal cell layer.

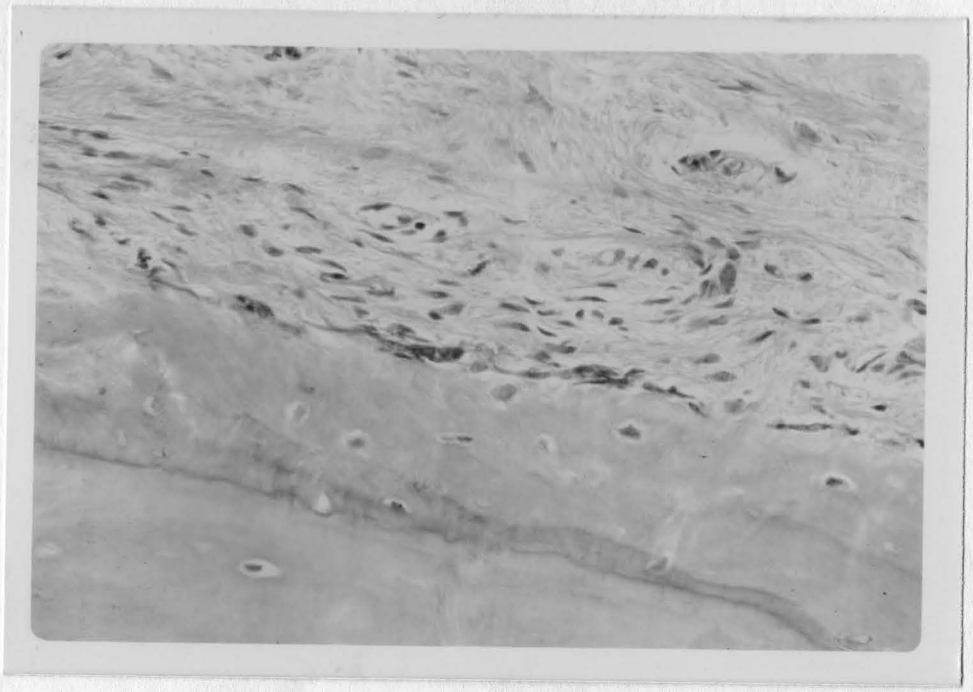
FIGURE NO. 18



100X: Seventeen Day Specimen

Surface of alveolar bone underlying graft showing an abundance of new bone along the entire surface of the specimen.

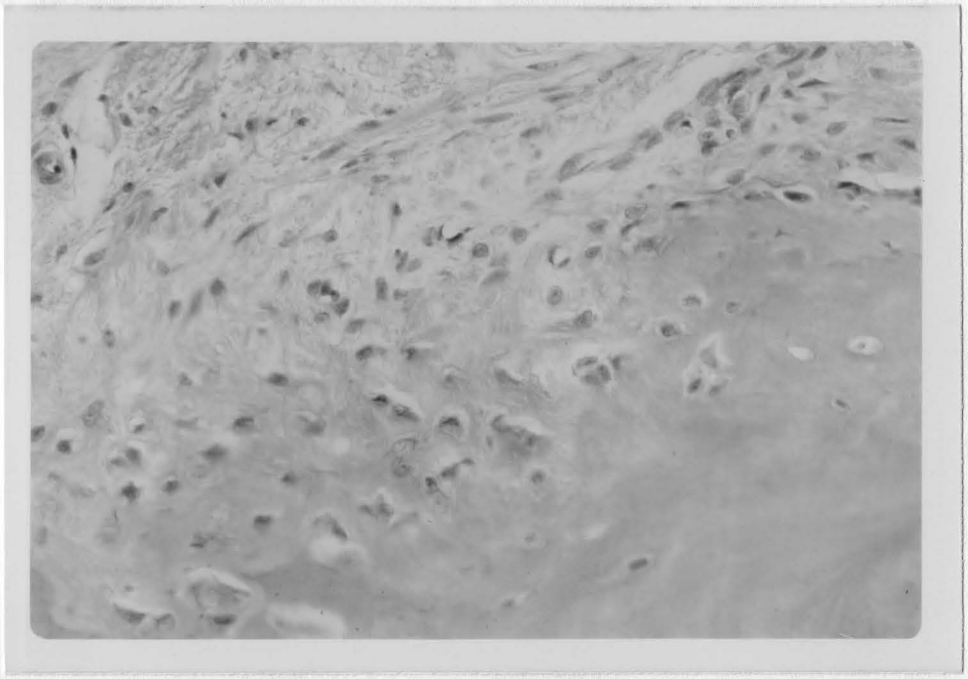
FIGURE NO. 19



250X: Seventeen Day Specimen

Seventeen day sample of labial plate underlying graft. Note the uniformity of thickness of new bone.

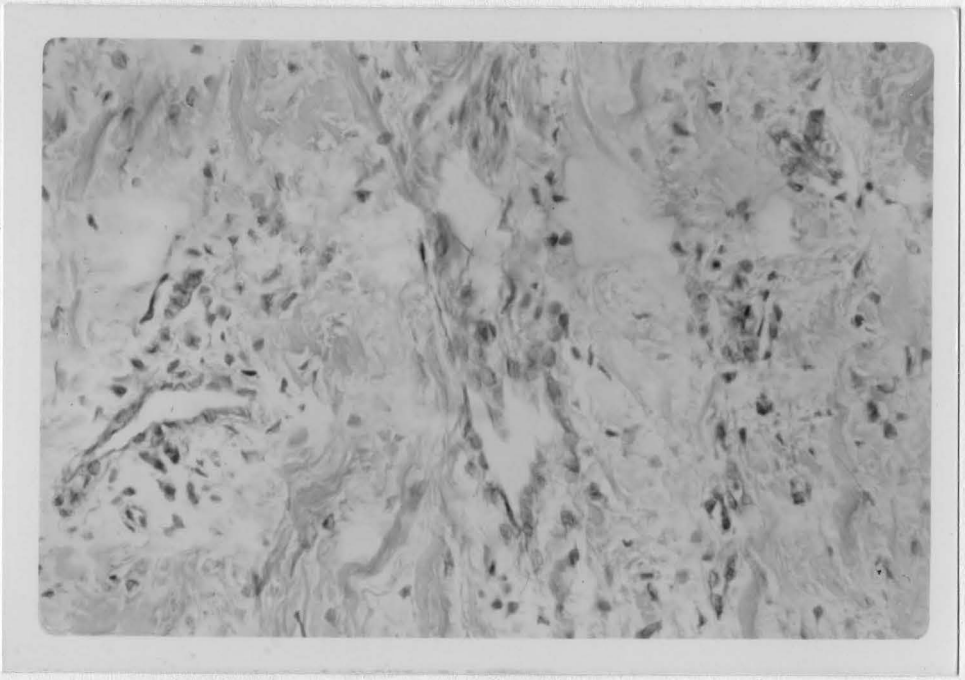
FIGURE NO. 20



400X: Seventeen Day Specimen

Seventeen day specimen showing osteoid tissue. Rapidity of osteogenisis depicted by abundance of osteoid tissue.

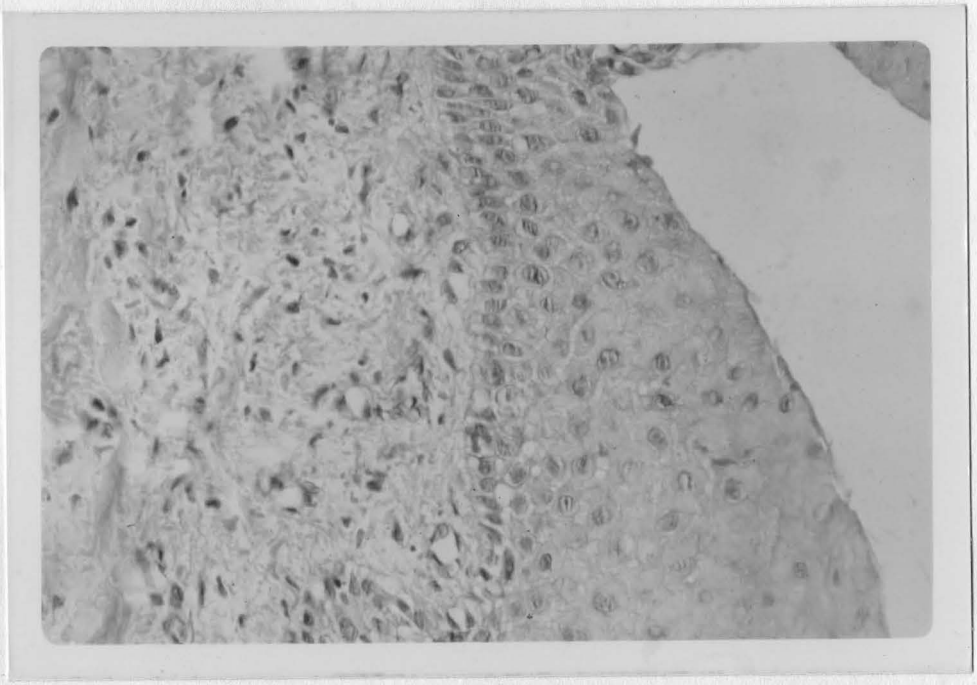
FIGURE NO. 21



205X: Twenty-three Day Specimen - Interphase

Interphase showing maturing connective tissue with fibers oriented parallel with the surface of the alveolar bone. Degenerating sinusoids are in evidence.

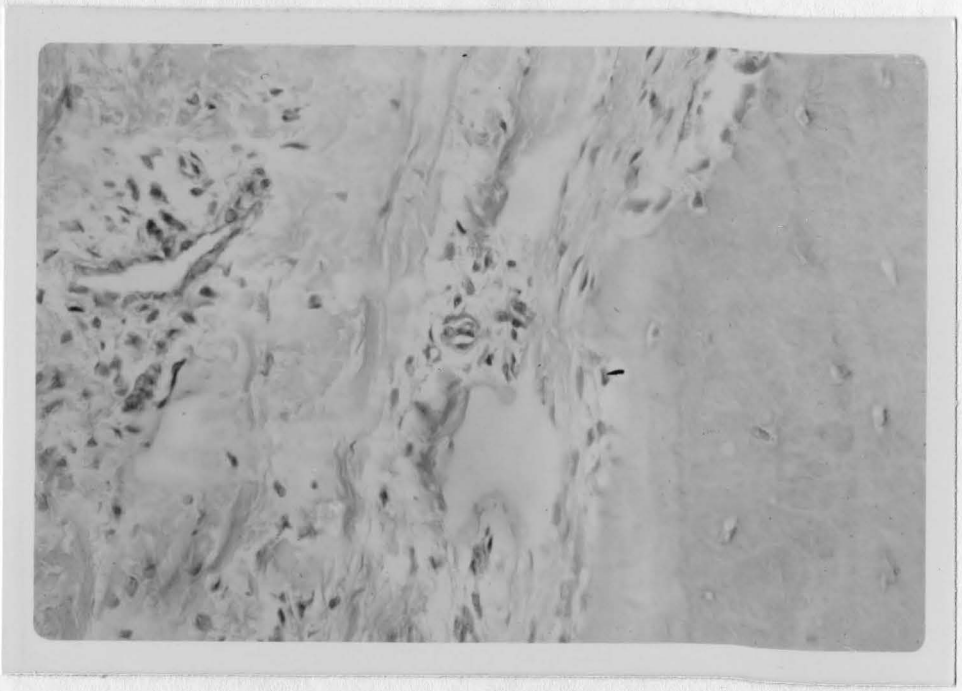
FIGURE NO. 22



250X: Twenty-three Day Specimen - Epithelium

Note thickening epithelium showing regeneration of rete pegs and polarization of basal cells.

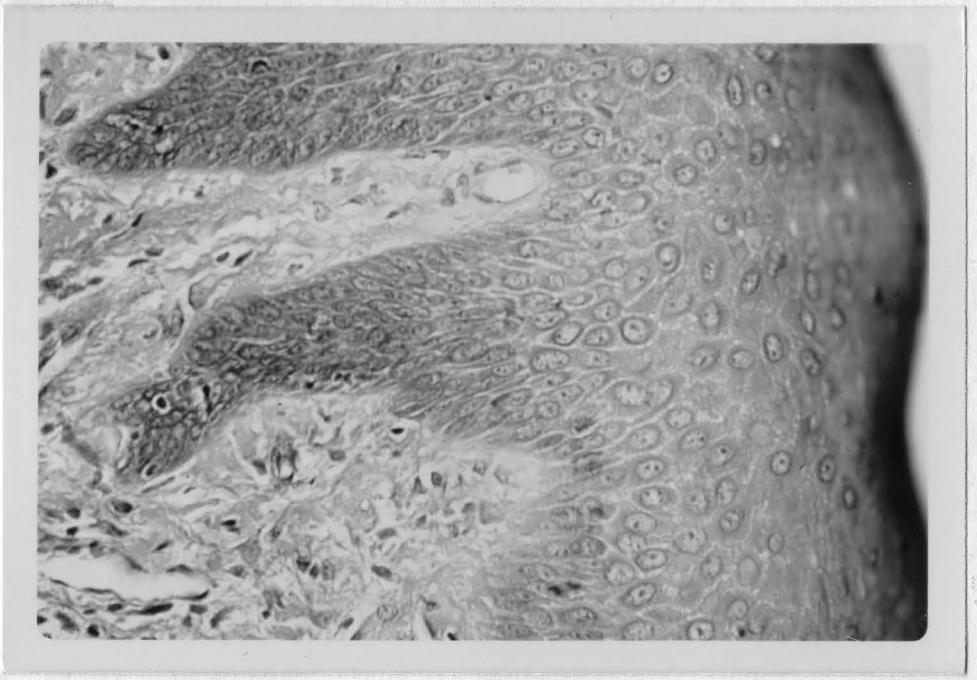
FIGURE NO. 23



250X: Twenty-three Day Specimen

Note the surface buildup of alveolar bone labial surface.

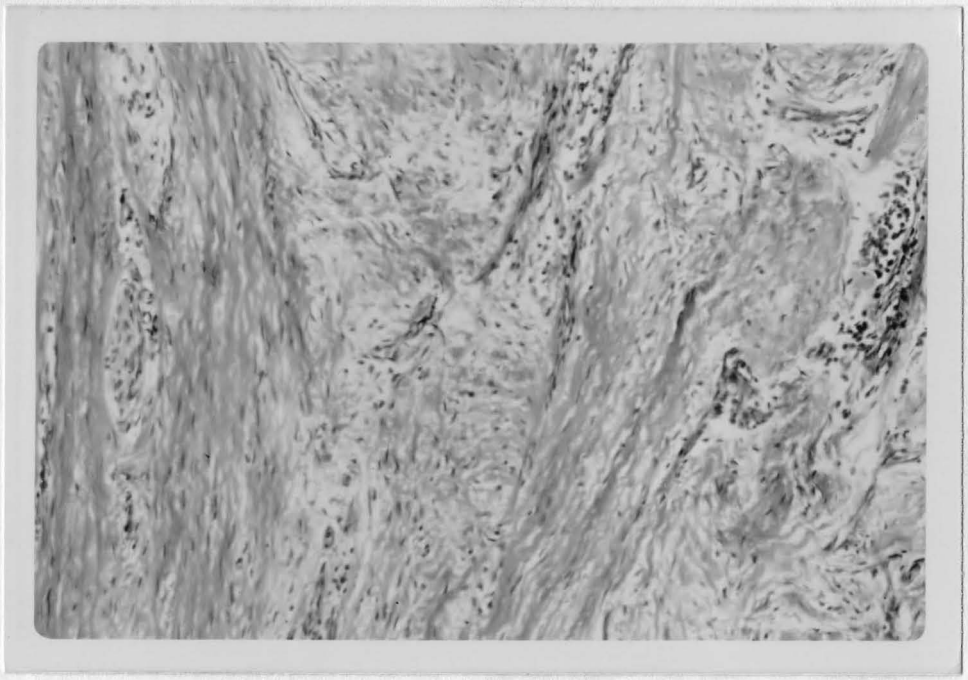
FIGURE NO. 24



250X: Forty-nine Day Specimen

Normal appearing stratified squamous epithelium.

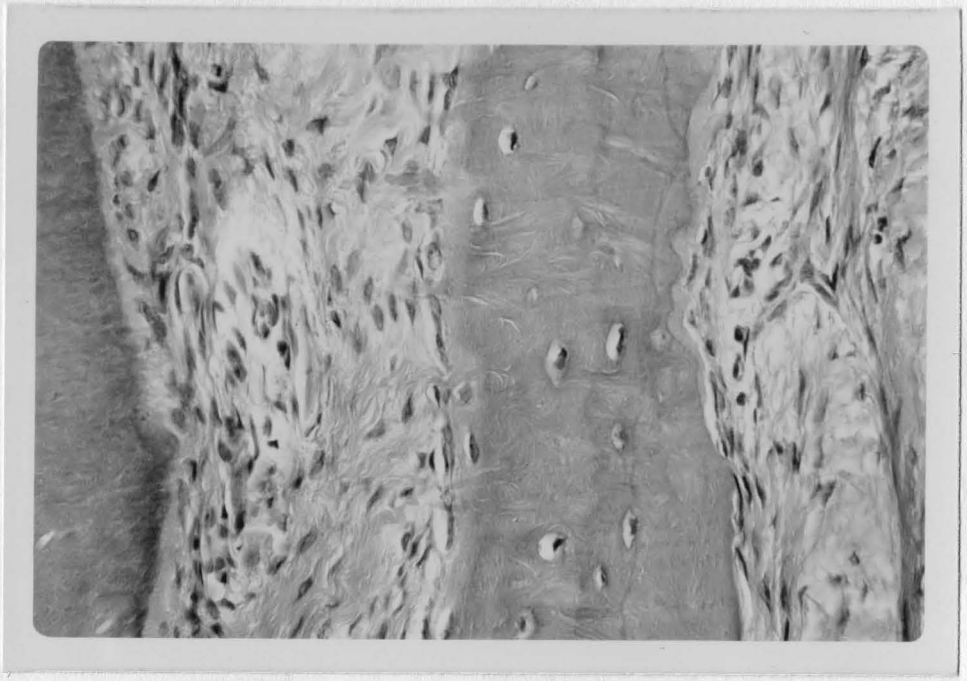
FIGURE NO. 25



100X: Forty-nine Day Specimen - Interphase

Interphase area showing fully matured connective tissue and bundles of collagen fibers.

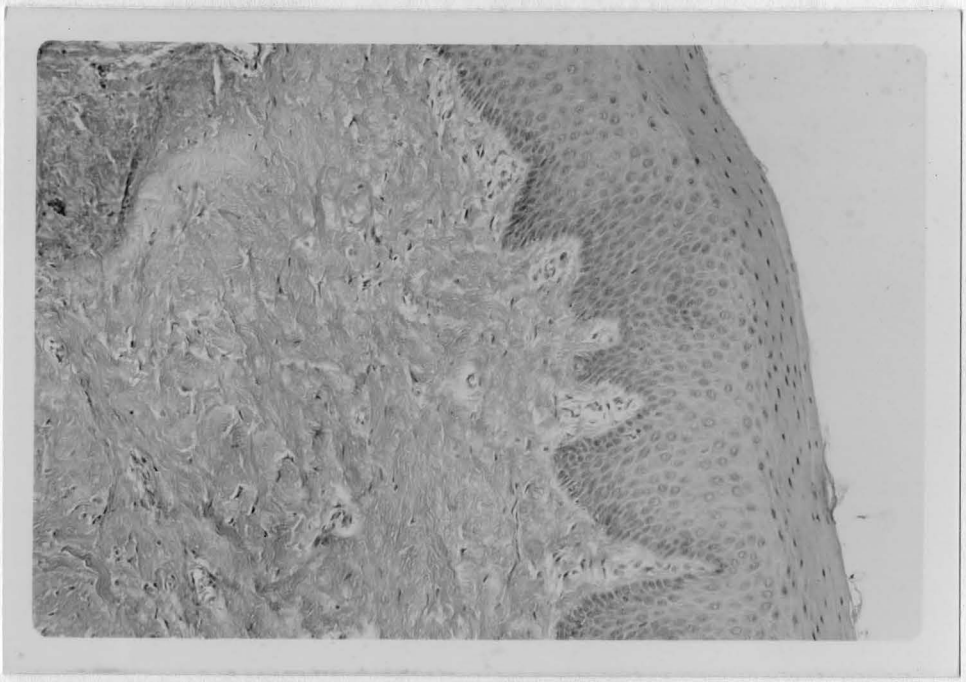
FIGURE NO. 26



250X: Forty-nine Day Specimen - Periodontal Ligament

Periodontal ligament side showing no osteoclastic activity. Mature bone is evident on both the periodontal ligament and labial sides.

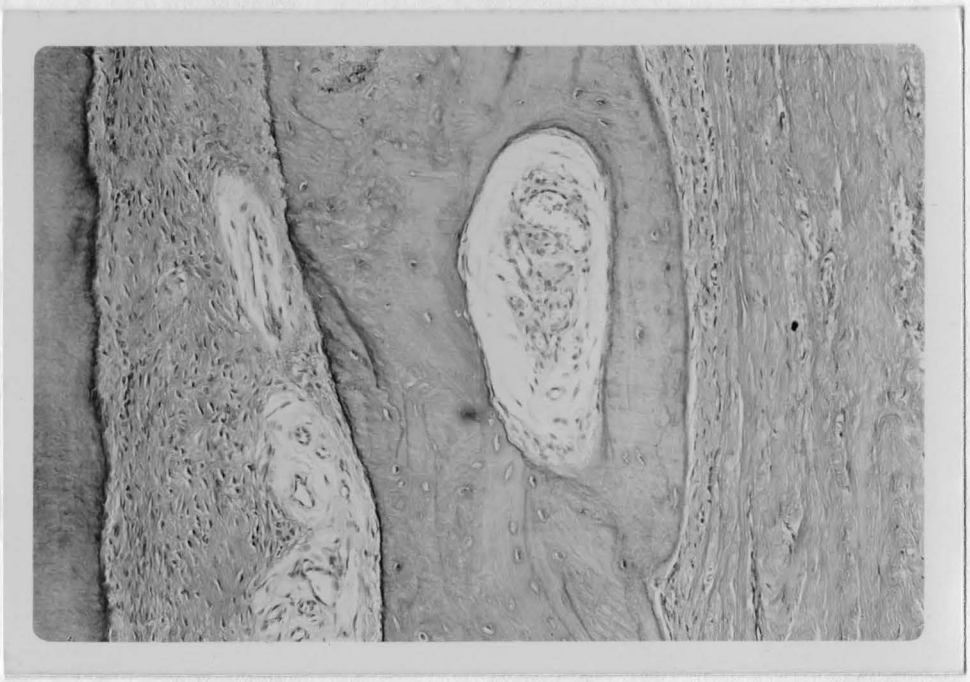
FIGURE NO. 27



100X: Six Month Specimen

Six month specimen displaying gingiva and connective tissue.

FIGURE NO. 28



100X: Six Month Specimen

Periodontal ligament space : Note the persistence of the connective tissue fibers maintained their parallelism to the labial plate.

Summary of Histological Evidence by Gargiulo and Arrocha

4th Day	Capillary formation at the graft-bed interphase and beginning formation of a new connective tissue attachment was noted.
7th Day	Less delineation between graft and bed with marked invasion of capillaries into the lower areas of the graft was noted. Some of these capillaries were said to appear "sinusoid".
10th Day	A greater vascular invasion of graft and early connective tissue organization of the interphase area was noted. Mitotic activity was seen in the epithelial portion of the graft.
14th Day	Specimen showed very little demarcation of interphase zone and connective tissue was beginning to mature. The surface of the graft showed keratinization. Capillaries were still more numerous than would be considered normal.
21st Day	Interphase had lost delineation completely and connective tissue organization was complete.

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APPROVAL SHEET

The thesis submitted by Dr. R.C. Brackett has been read and approved by four members 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.

5-21-70
Date

Arthur W. Hangin
Signature of Advisor