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A Histologic Study of Two Technics of Autogenous Bone Grafts on Monkeys

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A HISTOLOGIC STUDY OF TWO TECHNICS OF AUTOGENOUS BONE GRAFTS ON MONKEYS

BY

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BIOGRAPHY

Aime F. Rivault was born the 27th of May, 1940 in Nantes (France).

He attended elementary school and high school in Angers (France) where he got his bachelor degree in experimental sciences in July, 1959.

He studied for one year at the Faculty of Science of Paris where he passed successfully the Certificate of Physics,Chemistry and Biology in October, 1960.

He entered the Dental School of Paris the same year and received his degree from the Faculty of Medicine in June, 1964.

He was drafted in the French Army for two years as a general practitioner. In 1966 he went back to Paris and taught part time in the Department of Prosthetics, Dental School of Paris as a clinical assistant.

In the fall of 1968 he left France and arrived in Chicago as a student in Oral Biology at the Graduate School of Loyola University.

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CHAPTER I

INTRODUCTION

After almost 50 years of experimentation, bone grafts in periodontics, are still a matter of controversy. To this day, no one has presented a technic or material which could bring unquestionable and predictable good results. Each technic appears limited not only in its indications, but also in its potentialities of success. However, it is clear, keeping into account the limits of our knowledge, that an increasing interest is noted in the use of autogenous bone grafts.[1] [2] [3] [4] [5]

Our research was undertaken to provide an initial histologic evaluation of two clinical technics recently developed. The question, if this bone source provides bone induction or mere matrix formation, must be observed at the histologic level in order to better evaluate the clinical procedures.

Nabers (1965-67)[6] [7] and Robinson (1969)[8] introduced the idea of cortical bone chips mixed with blood in the form of a "coagulum" to be introduced in bony defects of the periodontium. In 1968, Kramer[9] developed a technic using cancellous bone and its marrow content in these same areas or defects.

We thought it would be profitable to study histologically the merits of those two different types of grafts. We hope this research will provide some answers as far as their value is concerned, and will cast some insite on the cellular phenomenons involved in bone grafting. This should ultimately help the clinician in the choice of his osseous therapy as it applies to peridontal disease.

CHAPTER II

LITERATURE REVIEW

The first autogenous bone grafts reported in the field of periodontics were done by Hegedus[10] who published the results of six cases in 1923. He transplanted periosteo-cortical bands from the tibia to areas of "alveolar pyorrhea" in an attempt "to bring about a restitutio ad integrum". He advocated the temporary splinting of teeth before surgery. A flap was raised, the teeth were scaled and the granulation tissue was removed. The remaining bone substance was freshened to promote its adhesion to the transplant and the flap was sutured over the graft. The transplanted periosteum was believed to participate actively in the bone regeneration. Teeth were reported to return to firmness and, on radiographs, the remaining bone appeared post operatively healthier. However, it was difficult to evaluate a gain of supporting tissue from the graft itself.

Cross [11] [12] (1955 and 1957) presented a few cases of autografts together with homografts and later heterografts. Although his purpose was much less ambitious than the one of Hegedus, he presented interesting results in the treatment of wide open intrabony pockets, the only defect, he said,

which is amenable to grafting technics. He showed clinical and radiological evidences of success, almost five years post operatively for one case of autogenous graft. He was the first one to advocate the use of small pieces of cancellous bone and a systematic coverage with antibiotics at the time of implantation. In 1960[13] he published a new report based on the results of 68 cases treated with materials of autogenous, homogenous and heterogenous origins. The indication of his technic was extended to areas of bifurcation but only two cases of intrabony pockets were presented. Through the variety of his work it appeared difficult at that time to define bone grafts technics as a valuable and predictable procedure in periodontics. Such studies remain in fact on an experimental level. In 1934 Beule and Silver[14] published their first experiments of heterogenous grafts on animals then on humans[15]. Their study tended to show that graft particles accelerate osteogenesis and leads to a slightly greater amount of bone formation. Despite the fact that the graft sites could hardly be compared to those commonly encountered in periodontal diseases, their histologic findings on dogs allowed them to publish in 1949 a description of the basic phenomenons involved in bone grafting[16]. After the invasion of the grafted area by granulation tissue, a first

step of resorption of the bone particles was observed, followed by an osteoblastic phase organizing itself sometimes around the rements of bone fragments. An attempt to analyse the behavior and the part of the different group of cells was presented. A greater importance was given to the host cellular components than to the graft material which was supposed to provide only the prevailing mineral salts.

One year later, in 1950, Linghorne[17] published a series of studies on the repair of periodontal structures on dogs. In one of his experiments he surgically created intra osseous pockets and implanted small pieces of fresh autogenous cancellous bone. He described the same phenomenon of tissue organization, resorption and bone apposition as Beube.[16] In comparison with the control sites where no graft was performed, a definite increase in bone repair was noted. The origin of the different cellular elements, fibroglasts, cementoblasts, osteoblasts, is difficult to determine, but in his opinion, cellular differentiation depends mainly on the local environmental factors. He found reasons to ascertain that the grafted particles were used as a reservoir for mineral salts but also had a definite power of induction on the differentiation of the cells. In comparison with non grafted areas,

he said: "Without the appropriate environmental stimuli, cells with the potentiality to become osteoblasts did not differentiate into osteoblasts" and "it appears that the osteogenic effect of grafts is due less to their cellular content than to the calcified intercellular materials."

This statement could very well characterize the major idea which was behind the numerous attempts to graft all sorts of materials.[18][19][20][21] Schaffer[22][23] published a report utilizing cartilage implanted into the periodontium of rhesus monkeys, and in humans intra bony pockets. Then, noticing after Linghorne that particles of dentine or cementum could also induce bone formation around them, he experimented their osteogenic power on a dog and a rhesus monkey[24]. Like many of his predecessors, he pointed out that the material implanted was completely or partially resorbed, to be hypothetically reutilized by the invading cells to build new bone.

Meanwhile many scientists went on developing the fundamental research on bone biology[25] and on its interaction with the surrounding tissues[26]. But it is impossible in the context of this review to give a survey of those works. In vitro and in vivo tests were conceived to determine the biochemical and cellular mechanisms of bone tissue.[27] Every substance which seemed to participate in osteogenesis was

tested in an attempt to inhance our technics of grafting.[18] [29]

Because of the multiplicity and complexity of the mechanisms involved, it appeared that those studies which brought about an excellent scientific knowledge were also, most of the time, very deceiving on a clinical basis. And finally, with the tremendous research going on in biochemistry and physiology, the cells became a major subject of interest in the theories dealing with bone grafts. After Levender[30], who thought that an implant could liberate osteogenic substances to induce the mesenchymal cells to differentiate into osteoblasts, many workers hypothetised on the basis of their experiments[31]. Osteoblasts could come from three origins: from pre-existing osteoblasts or osteocytes[32], from the endosteum which lines the marrow cavities[33], and from undifferentiated mesenchymal cells which are found in close proximity of the capillaries[34]. Those three sources could also act together[35]. In the light of such a hypothesis, an autogenous graft of cancellous bone containing marrow appeared to bear the greatest chance of success. The reports of Shallhorn[36] on hip marrow biopsy implants are to this regard very encouraging.

Maximov[37] and other[31] showed that this material contains all of the cellular components which can be involved in osteogenesis. However, Burwell, after his experiments, hypothetized that to induce osteogenesis, a part of the marrow must undergo necrosis. This will release the proper inductors for differenciation of the surviving cells. Cushing[38] pointed out in the summary of its literature review on red marrow grafts, that such material do stimulate bone formation; but despite numerous studies, we do not know exactly why and how it works. According to a prevalent theory supported by Burwell[31]: "the cellular population of normal red marrow suggests strongly that the primitive cell in marrow which becomes a bone forming cell is derived from littoral cells lining its vascular sinusoids." But, if this is the case, such cells, belonging to the reticulo endothelial system, are found also along the blood vessels and capillaries in every tissue. In other words, the value of a graft may not lay in the nature or survival of its own cell population, but simply in its ability to release a certain substance which will induce any reticular cell coming from the neighboring tissues to become an osteoblast. Cushing[38] added that: "differentiation occurs as a result of an inductive signal seemingly initiated by products of necrosing marrow" but he also added

"similarly necrosing bone will perform the same function". Such double possibility is illustrated at the present time by the development of the two technics we have chosen to study.

In 1965, Nabers and O'Leary[6] introduced the use of autogenous bone chips. Nabers put the emphasis on the technical aspects of the problem, but despite the small cellular population of this cortical bone, he did remark that "in all probability the graft material still retains some of its vitality at the time of implantation". However Robinson[8] (1969) seems to think that the value of such a graft is elsewhere. He stated that "If it is necessary for the donor material to be completely resorbed before new formation can occur, the size of the graft material may be of major importance". From that point of view, the bone dust seems to be the best material when it is mixed with blood elements to facilitate the development of a granulation tissue. This tissue should bring with the numerous capillaries a sufficient amount of undifferentiated cells.

The other position is illustrated by the work of Kramer[9]. In 1966, he reported on the use of retrograde autogenous cancellous bone. The purpose of his technic is to push in the defect to be corrected trabeculae of the underlying bone. The idea is to bring in situ the marrow content

with the endosteum (possible source of osteoblasts) and some pre-existing blood vessels with their undifferentiated littoral cells. The procedure brings out a great deal of hemorrage which, according to Kramer, should maintain the viability of most of the transferred cells. In this case, the part played by the graft is not any more a passive one. It is expected to participate actively in the repair with its own cell population. The viability of those cells seems to be a primary concern for Kramer, and the bone lamellae are not merely a factor of induction but they constitute a support for the colonization and organization of the transplanted cells.

Our study should help to precise if these two theories have some real value, if they are opposed or complementary or if they are indifferently used by the organism to repair its osseous damages every time a bone transplant is performed.

CHAPTER III

MATERIALS AND METHODS

Four adult rhesus monkeys (Macaca mulatta mulatta), in good health, were utilized in this study. Through out the experiment, they maintained the physical parameters recorded at their arrival. They were three males and one female. They had complete dentitions with varying degrees of attrition and gingival inflammation. Periodontal disease was present in each one with calculus deposit and some times open contacts. The profile of bone resorption was radiographically regular and horizontal. On the molars the bifurcation was some times partially or completely involved. Each animal was operated on the four quadrants in succession. When control sites were prepared, this was done immediately after the completion of the regular experimental procedure on the opposite quadrant of the same side so that the monkey was not moved during surgery. Each animal was allowed a maximum time for recovery between two procedures. This had to be however compatible with the schedule previously established. The specimens were taken at 0 hour, 1 day, 3, 7, 11, 14, 17, 21, 30, 60, 90, 120, and 180 days postoperatively, and the controls at 14, 60, and 180 days.

The first monkey was an adult male weighing 7.2 kilograms; its gingiva was chronically inflammed along the margin and oedematous. There was some food debris and supra gingival calculus. The pockets had an average depth of two millimeters. Radiographs showed slight horizontal bone resorption. This animal provided for the 180 days experimental and control, 21 days and 7 days specimens.

The second monkey was an adult female of 5.5 kilograms. The occlusal abrasion was very severe and the first upper and lower molars were mobile. One had a decay on its mesial surface, favoring food impaction. There was a great amount of calculus and soft debris; the gingiva was oedematous, hyperplastic and receeded. Pockets were running from two to four millimeters. Resorption was important but the pattern of the crestal bone was regular and only crater like defects were found. This animal provided for the 120, 30, 17, and 3 days experimental specimens.

The third monkey was an adult male weighing 7 kilograms; the oral condition was relatively fair. There was a small amount of calculus and few signs of gingival inflammation; however, the average depth of the pockets was 3 millimeters interdentally. The bone architecture was close to normal despite a slight uniform resorption. This animal provided for the 90, 14, and 1 day experimental specimens

and the 60 day control.

Thirty minutes before surgery, the animal received an intramuscular injection of a tranquilizer (04 cc of Sernylan). Each surgical procedure was performed under strict conditions of asepsis.

The monkey was taken out of its cage and a retroalveolar radiograph and color transparancies were taken. The animal was placed on the surgical table and an intravenous injection of 90 milligrams of Diabutal was given for general anesthesia. The action was immediate. The body was placed on a cradle and the head positioned so that the respiration was not impaired and the accessibility was good.

After each member of the team had dressed with sterile gowns, masks and gloves, the monkey was draped in the usual manner and the surgical area was painted with a solution of Zepheran (1/1000 aqueous solution). The general aspect of the oral cavity was again recorded and all signs of disease were noted, particularly, color and consistency of the tissue, depth of the pockets and calculus formation. Immediately after, a scaling was performed; this was usually limited to the quadrant to be operated and there was no attempt to plane and polish the surfaces. This was mainly because of the difficulties encountered: rhesus monkey teeth are much

smaller than human ones and despite their thinness, the Gracey curettes were often almost too big for the interdental spaces. With a six millimeter diamond disc, teeth were noched on their buccal surface to record the level of the gingival margin.

The first incision was performed with a number 15 Bard-Parker blade. This incision was started at the disto-buccal angle of the second molar and carried to the mesial of the cuspid. The blade was maintained approximately in a parallel direction with the long axis of the teeth and followed the bottom of the gingival sulcus. The epithelial attachment and the connective tissue was severed so that the contact with the crestal bone was maintained. The papillae were sectioned in the same way. A vertical incision on the mesial aspect of the cuspid was carried from the free margin to the muco-gingival line. A full thickness flap was raised with a periosteal elevator and the root surfaces were again checked for possible pieces of calculus and tags of inflammatory tissue. When the field was clean, the graft sites were chosen. The spaces between the first and second premolar and between the second premolar and the first molar were usually selected. As a rule, the choice was made on the topography of the bone, the anatomy of the proximal teeth and the position of their roots. In the surgical preparation of the graft sites, the

idea was to create a defect comparable to the intrabony pockets usually amenable to graft technics; a standardized procedure was used. Fig. 1, 2, 3, 4, 5.

Using a round bur (number 4) at a low speed under continuous irrigation with sterile isotonic solution a vertical defect was created in the septal bone to a depth of 3 millimeters (measured with a periodontal probe). This defect had its opening on the buccal aspect of the col area or on the osseous floor of a bifurcation when this had been selected. Most of the time, one, sometimes two root surfaces were forming part of the walls of the defect. Two graft sites were created on each quadrant. Usually, the distal one was used for the "bone coagulum" and the other for the osseous "push up".

The following step was to obtain the osseous material for the graft. This was done by piercing with the same bur the cortical plate at about 2 millimeters apically to the bottom of the osseous defect selected for the "push up" technic. In this procedure, the bur was used at a very low speed without any irrigation. The bone chips were readily mixed with blood and placed in the "bone coagulum" graft site. A small spoon curette was used to carry the material which was sometimes pushed in the defect with an amalgam plugger.

However, the bone coagulum was never overcondensed. Great care was taken to fill the defect without compressing to avoid damage to the cells possibly present or overfill the defect. The graft material was usually part cortical and part cancelous bone.

The cortical bone in the area apical to the second graft site presented now a fenestration. Great care was always taken when preparing this cavity, to leave an average thickness of 1 millimeter of cancellous bone between it and the graft site. After the area was cleaned, an amalgam plugger was introduced through this apical hole and great pressure was exerted in a coronal direction just below the graft site. This brought a rupture of the bone trabeculae between the two cavities and the pieces were pushed gently to fill the coronal part of the osseous defect. The bleeding caused by the procedure was sufficient to fill the rest of the defect.

Then both grafts were checked; the surface area was cleaned with a gauze to make sure that nothing had moved, and pieces of bone around were removed. The flap was closed over in the following manner: no attempt was made to reapposition it apically at the level of the bone. Great care was given to cover the graft sites with soft tissue by suturing interdentally with 0000 interupted silk sutures. Each knot was

secured twice and the silk cut very short to avoid any pulling on the ply by the monkey. Two sutures were also closing the vertical incision.

Everything was then checked a last time and the oral cavity washed and cleaned. A post operative radiograph and color transparancies were taken. No periodontal dressing was used; it has been shown by many workers that any kind of device was promptly removed by the monkeys after their recovery from anesthesia. Our only concern was to suture tightly in such a way that the animal could not move it. Before complete recovery from anesthesia each monkey received an intramuscular injection of antibiotic (600 000 unities of Penicillin). When control sites were prepared on the opposite quadrant, the same procedure was followed. The difference was that both graft sites were washed out of any bone particle before the flap was closed. The whole procedure was performed in about 45 minutes. Each monkey was put on a liquid diet for 24 hours, followed by a soft diet for 3 days. After the fourth day, normal diet was given.

One week later, after an intramuscular injection of a tranquilizer, the sutures were removed. Clinical findings were recorded and a picture was taken. After this the animal was maintained until the next procedure or the time of sacrifice.

At sacrifice, each monkey was tranquilized, again radiographs and color pictures were taken, and was given a lethal intravenous injection (2 cc of solution).

The specimen was taken; on each quadrant the limits were traced by incising the soft tissues to the surface of the bone. Each specimen extended from the mesial of the cuspid to the distal of the first molar. Apically, the incision was running beyond the apices and the same was repeated on the palatal or lingual side. The bone was sectioned with an electric saw; each bloc was washed and tied up by a silk thread to a tag indicating the origin and other necessary information. Each was then fixed in 10% neutral Formalin and decalcified in Formic acid and Sodium citrate 50:50. Finally, every graft area was trimmed, embedded in paraffin, sectioned at 6 microns in a transversal plane (bucco-lingually) and stained with Hematoxilin-Eosin. From each experimental site all slides were studied then a representative one was selected for histologic analysis of each graft area.

CHAPTER IV

FINDINGS

A. CLINICAL OBSERVATIONS (Fig. 6, 7)

One week post operative, surface healing was almost completed. The lines of incisions were hardly visible; the gingival margin was still oedematous and epithelization was uncompleted in the interdental area. The rest of the tissue looked generally firm and pink. Most of the sutures were still in place, and removed at this time. The relationship of the gingival margin with the notches made on the buccal aspect of the teeth showed an apical displacement of the former one. This was, more so, due to a resolution of the oedema than to an apical migration of the epithelial attachment, for it was observed that the depth of the pockets preoperatively recorded had decreased. This resulted in a more appropriate relationship of the soft tissues with the teeth and the bone. However, with the time, the inflammation had a tendency to reoccur in the presence of food impaction and calculus formation. This was observed for all monkeys. Small variations in the degree of response were however noted, but this was related to the general condition of the oral cavity of each monkey and not to the procedure.

B. HISTOLOGIC OBSERVATIONS

1. EPITHELIUM

ZERO HOUR AND 24 HOURS POST-OPERATIVE

The epithelium was normal. Like in all subsequent specimens observed, there was some acanthosis and elongation of rete pegs. In one specimen (24 hours post-operative) some intercellular oedema could be observed in the same area.

3 DAYS POST-OPERATIVE (Fig. 8)

The tissue showed gradual signs of inflammatory reactions from the mucogingival junction to the marginal gingiva. There was intercellular oedema and increased mitotic activity. Many cells stained paler than normal and were atrophied in the area of the free margin.

7 DAYS POST-OPERATIVE

The epithelium showed a greater inflammatory reaction to the procedure than before. There was a decrease of the total thickness, partial loss of the keratin layer, intercellular oedema and, in the area of free margin, proliferation of some rete pegs in the depth of the connective tissue.

14 DAYS AND FOLLOWING

The specimens showed consistently a fairly normal configuration of the epithelium. However, on the 14 and 17

day specimens, the epithelial attachment was not yet properly repaired. The cells, in this area, were missing or degenerated and some ectopic epithelial proliferation could be seen at the level of the former incision. In one specimen (21 days) a few bones particles were included in the deepest epithelial projections. In all specimens, the lamina propria of the connective tissue was heavily infiltrated with lymphocytes. The epithelium bridged slowly on such a surface. Mitosis were generally seen in the germinal layer of the gingival crest and from this area new cells were migrating toward the teeth. Many dead epithelio cells were also observed. Even after a few layers of them had successfully bridged this critical area, intercellular oedema was often observed and in many cases some cells showed signs of necrosis. In one specimen (14 days) an increase in thickness of the epithelium could be observed in the area of the apical osseous fenestration of the "push up" technic. This observation was not repeated in the subsequent specimens.

2. CONNECTIVE TISSUE

In all specimens, oedema, increased vascularity, and infiltration by inflammatory cells were a constant finding in the marginal gingiva. In the rest of the tissue the reactions varied with time.

ZERO HOUR

Collagen fibers and blood vessels were normal in quantity and quality. Some inflammatory cells were found along capillaries and collagen bundles. At the line of incision, the tissue was disorganized.

24 HOURS (Fig. 9)

There was interstitial oedema, blood vessels were dilated and the number of inflammatory cells had increased. They were found around the capillaries and along the wound edges. In one area, the flap showed early signs of reattachment: some slender fibers staining like collagen were running from the connective tissue to the bone. Those fibers had supported a slight displacement of the flap during the processing.

3 DAYS (Fig. 8)

The signs of inflammation had not changed and the number of inflammatory cells stayed moderate. In the "push up" area, they concentrated mainly around the apical fenestration. At high magnification some cellular elements of the tissue overlying the graft areas showed necrosis. In the periodontal ligament, the blood vessels were dilated and a slight increase in the number of cells was observed. Between the flap and the bone a narrow band of blood clot was invaded by inflammatory cells.

7 DAYS (Fig. 10)

Some blood vessels were still dilated and in the "push up" area, inflammatory cells were concentrated at the apical fenestration. A fibrous scar had formed at the line of incision. In this area the new collagen fibers run parallel to the surface of the bone.

11 DAYS

In comparison with the preceding specimens there was a decreased density of the collagen fibers in the crestal area. The remaining fibers were staining paler than normal and they were forming an irregular pattern. However, the amount of inflammatory cells was moderate and the line of incision was hardly visible.

14 DAYS (Fig. 24)

The tissue had returned to normal except above the graft areas and at the apical fenestration of the "push up" specimen where there was still interstitial oedema, dilated blood vessels and inflammatory cells.

21 DAYS AND FOLLOWING

The tissue appeared normal, however, until the sixtieth day, an increased number of blood vessels and cells could be oberseved over the graft sites.

3. ALVEOLAR BONE AND MARROW TISSUE

In all monkeys the bone had comparable density of trabeculae and similar marrow contents. However, in the proximity of the graft sites each element showed specific changes which were the expression of an adaptation to the insult of the surgical procedure.

ZERO HOUR

The bone was normal: most of the lacunae surrounding the graft area contained osteocytes which appeared smaller than their osseous bed. The marrow spaces showed no change.

24 HOURS (Fig. 9)

The osteocytes in the bone surrounding the graft sites appeared enlarged. They were easily visible and filled most of their osseous bed. In the proximal marrow spaces the blood vessels were enlarged, and the cellular population had increased. Most of the cells around the capillaries were lymphocytes.

3 DAYS

Some lacunae close to the graft sites appeared empty. In the near marrow spaces, the number of cells had still increased. Besides inflammatory cells around dilated blood vessels, some others had a plae grey nucleus with a dark pin point of chromatin and were identified as undifferentiated mesenchymal cells. The osseous walls of these marrow spaces

were lined with a single row of mononuclear cells which were in greater numbers toward the graft site. In the crestal area of one graft, the inner surface of the cortical bone showed a few Howship lacunae but osteoclasts were not found.

7 DAYS (Fig. 12, 13)

Most of the lacunae in the bone around the graft sites were empty while the other ones showed enlarged osteocytes. In the marrow spaces the cellular population had increased. Along their walls osteoblasts and osteoclasts could be observed sometimes in adjacent sites. Osteoclasts were however in relatively small number, they concentrated mainly along the area of the walls facing the graft sites. Some other ones were found along the periodontal ligament and the crestal bone which both showed lacunae of resorption.

11 DAYS

Most of the lacunae along the graft sites were empty. In the proximal marrow tissue the number of cells along the walls had increased. In some areas, new bone had been formed. A few osteoclasts were still visible in the marrow spaces and along the osseous wall of the periodontal ligament. Many undifferentiated mesenchymal cells were gathered around the blood vessels.

14, 17, AND 21 DAYS (Fig. 11)

The bone lacunae around the graft sites appeared empty. In particular the cortical plate did not house any osteocytes. In the marrow spaces, various amounts of new bone had been deposited on the walls. There was a progressive decrease of cellular density in those spaces. The endosteal cells showed also a decrease in size. Osteoclasts were absent.

30 DAYS AND FOLLOWING

There was an increasing number of osteocytes in the lacunae around the graft sites. Marrow spaces showed a return to normal cellular density. On their walls, some times on the crestal area of the bone and along the periodontal ligament new bone was layed down. In some specimens where osteoclastic and osteoblastic activity had apparently ceased, an increase in vascularity could still be noted around the graft sites.

4. BONE COAGULUM GRAFT

ZERO HOUR (Fig. 14)

The intrabony defect presented clean edges sharply cut by the bur. It extended vertically between the buccal cortical plate of bone and the periodontal ligament. It was filled with numerous jagged bone chips, red blood cells and

a few white blood cells. At high magnification those three elements appeared to be fixed on a very thin mesh of fibrinoid like substance. In most of the bone chips the matrix had been damaged by the bur and most of the lacunae were empty.

1 DAY

The graft site was filled with the same kind of bone chips, red blood cells and a fibrinoid like clot. A great number of inflammatory cells had invaded the whole area; they seemed randomly distributed but were however more concentrated in the coronal area under the flap. At high magnification the architecture of the bone chips were difficult to identify as bone. Some areas showed cellular necrosis and serous exsudate.

3 DAYS (Fig. 15)

The inflammatory reaction was at its peak. At high magnification some of the inflammatory cells and most of the red blood cells appeared to be degenerating but, toward the bottom of the osseous defect, an increasing number of undifferentiated cells and young fibroblasts were scattered in the proximity of the proliferating capillaries coming from the marrow spaces and the periodontal ligament. Distinct fiber strands were present and gave the graft site some architecture. The bone chips were irregularly distributed and their matrix disorganized. Along the buccal aspect of the osseous wall, close to the coronal border, Howship lacunae

were observed. However osteoclasts were not found on any of the sections.

7 DAYS (Fig. 16, 17)

The osseous walls of the graft site were intact and the bone chips were embedded in the same net of slender fibers. The number of inflammatory cells and of red blood cells had greatly decreased. A few wide capillaries were seen throughout the area and the top of the graft was invaded by numerous undifferentiated cells forming a granulation tissue.

11 DAYS

The number of inflammatory cells had still decreased. They were replaced by macrophages and polymorphonuclear leucocytes. Almost all the red blood cells had disappeared and the space was filled with granulation tissue loosely organized. The bone chips were invested in it. Some of them stained differently from the rest of the bone giving a more bluish color.

14 DAYS (Fig. 18)

The granulation tissue organization seemed achieved. A young connective tissue had been formed with numerous fibroblasts which were bound to each other by their protoplasmic extensions. But the most striking feature was the new bone forming within the graft site. This osteogenic activity was

was more important along the walls. New bone was also forming and "growing out" around the graft particles which stained darker and showed at high magnification an increased deterioration of their matrix. At the junction between old and new bone no pattern of resorption could be seen. The amount of new bone decreased from the bottom to the coronal border of the graft. However, exception made for the most coronal ones, each graft particle was at this time surrounded by a continuous line of cells which had the characteristics of osteoblasts. The bottom of the graft site contained a few particles of cementum and dentin but the density of cells lined on their surface was lower than around the proximal bone chips and no bone formation could yet be observed.

17 DAYS

The amount of new bone had increased and the trabeculae lined up with numerous osteoblasts contained osteocytes. The rest of the graft area was filled with a young connective tissue, a few inflammatory cells and many capillaries. The graft material stained darker and appeared completely disorganized. It seemed to have lost its fibrilarity and cementing substance. The small particles appeared to "induce" more bone formation than the larger ones. A great number of osteoblasts were also concentrated in the crestal area where they had formed new bone. However, in this area no graft

particles could be seen.

21 DAYS (Fig. 19)

The different islands of new bone had fused together. The young connective tissue around them was highly vascularised. The bone graft particles stained darker and under high magnification they looked completely disorganized. In one area, close to the buccal wall, there was a few inflammatory cells with signs of necrosis and some osteoclasts.

30 DAYS

The graft area showed a decrease in cellular density. However, the repair was not achieved and in the coronal area the islands of bone had not yet fused together. In the new trabeculae some graft particles were visible but it was difficult to delineate most of them. They seemed to progressively disappear.

60 DAYS (Fig. 20)

The limits of the graft area were fading out. The staining property of the new bone was no longer different from the old one. The rements of the graft invested in the new bone had completely lost their fibrilarity and in the new marrow spaces the connective tissue had the characteristics of normal marrow tissue with loose and slender fibers housing large fat cells. Along their walls the endothelial

cells were sparse. Along the tooth, a new periodontal ligament had been formed and new cementum had been deposited on the dentin. The bone repair had stopped at the level of the inferior limit of the epithelium attachment. The connective tissue overlying the graft had the same characteristics of structure and density as a normal gingival tissue.

90 DAYS

The limits of the graft were almost impossible to trace. The bone stained like the rest of the alveolar process. The lines of growth in the new bone were visible. Some osteoblastic activity was still found along the walls of the marrow spaces close to the crest and in the periosteum of this same area. It was impossible to find any graft particle.

120 DAYS AND 180 DAYS

The bone repair was achieved; no osteoblastic activity could be found. The osseous area had healed without surface discrepancy. The new cortical bone appeared as dense and regular as the old one. No graft particles could be seen.

5. PUSHED UP BONE GRAFT

ZERO HOUR (Fig. 21)

The cavity carved during the procedure was slightly narrower between the coronal area and the apical fenestration. There, the walls showed rements of bone which had been

obviously fractured and torn off. The pushed pieces were accumulated in the crestal area. Collagen-like fibers were adhering to some of them, entrapping red blood cells and a few fibroblast-like cells. In the graft material, osteocytes could be seen in some of the lacunae. In the rest of the defect the blood clot had been washed out during the processing.

24 HOURS

The graft area was filled with a blood coagulum. It was invaded by a great number of inflammatory cells. Toward the coronal area where the graft material was concentrated, the inflammatory reaction was slightly higher. The bone particles were embedded in the fibrinous fibers and most of their lacunae were empty. Few fragments of cementum were found in the former area of the periodontal ligament which had been destroyed during the procedure.

3 DAYS (Fig. 22)

The disposition of the different elements was the same but the number of red blood cells had decreased. The inflammatory reaction had not changed. At high magnification, the lacunae in the particles of bone were empty.

7 DAYS

The graft site was heavily infiltrated by lymphocytes. At high magnification, all lacunae of the osseous graft were

empty.

11 DAYS

Through the coronal and apical openings, granulation tissue and numerous capillaries were invading the graft area. At the level of the bone graft, the blood coagulum was not changed and lymphocytes were still present with many polymorphonuclear leucocytes. At high magnification, all the lacunae in the grafted bone were empty. In the central area of the osseous cavity a beginning of osteogenic activity could be observed. The new bone was deposited on the walls and did not contain any graft particle. (Fig. 23)

14 DAYS (Fig. 24)

The graft area was completely invaded by young connective tissue and an important osteoblastic activity was developing in its apical part. In the coronal area where many graft particles were concentrated, the amount of new bone was much smaller. However, it was mainly found around the graft particles. The small fragments seemed to have more osteoblasts and more bone around them than the big ones.

17 DAYS

The central part of the graft area was undergoing necrosis. There was a heavy lymphocytic infiltration around some large pieces of bone with a few osteoclasts. But

elsewhere osteogenesis was going on. New trabeculae were deposited along the walls and around small bone particles. In the apical area the fenestration was partially closed by bone. In the new marrow spaces, the connective tissue was less dense than in other parts of the graft site.

21 DAYS

Most of the area was filled with new bone. However, on the coronal surface the osteogenic activity was minimal and few osteoclasts were seen around the most coronal particle. The central area contained 4 or 5 large fragments of cementum and dentin. There was very little bone formation on their surface and the connective tissue around was organized with heavier collagen fibers.

30 DAYS

The bone repair was far advanced and some new bone had been formed on the external surface of the crestal alveolar bone. The contour of the area was normal. In the proximity of the tooth, the graft particles were almost devoided of new bone except around the smallest ones. In the other parts, the bone grafts were embedded in the new trabeculae.

60 DAYS

The specimens showed a delay in repair. The center of the graft was well ossified but the apical opening was

not closed yet and the coronal area was not completed. Osteoblastic activity was still going on. Bone was deposited around many small graft particles. At high magnification the matrix of those particles showed a loss of fibrile and cementing material.

90 DAYS

The graft area was rebuilt. No osteoblastic activity could be seen on the coronal surface despite the presence of a craterlike defect. But in a few marrow spaces there was still some osteoblasts. In the other spaces the vascularisation was higher than normal but the connective tissue was fully differentiated in yellow marrow.

120 DAYS

The repair was achieved with a normal contour. In the marrow spaces there was still a higher vascularisation than in the rest of the alveolar bone and the endosteum was still visible. Some graft particles could be seen but they showed signs of disorganization of their matrix.

180 DAYS

The area of the graft was difficult to delineate. However the marrow spaces showed still a high blood supply. Some osteoblasts were seen in some of the spaces and on the coronal surface. It was very difficult to identify any graft particles.

6. CONTROL STUDY

"BONE COAGULUM" TYPE OF DEFECT

14 DAYS CONTROL (Fig. 26)

The osseous defect was invaded by a young connective tissue with fibroblasts, slender collagen fibers and blood vessels. A few minus bone particles were however present. In the apical area of the defect new bone had been laid down on the walls and tended to invade the connective tissue. Fig.27

60 DAYS CONTROL

The defect was filled with new bone. The trabeculae were still lined with osteoblasts. The connective tissue in the marrow spaces showed many blood vessels. On the surface there was a craterlike defect but osteogenesis was still present in this area.

180 DAYS CONTROL

The repair was achieved and no difference could be made between old and new bone.

"PUSH UP" TYPE OF DEFECT

14 DAYS CONTROL (Fig. 28)

The defect was invaded by a loose connective tissue but along the longitudinal axis of the cavity this tissue presented a denser arrangement of fibers running in parallel from one opening to the other. Along the walls new bone had

been deposited and the greatest formation was found in the apical area. A few small particles of bone were invested in the new trabeculae.

60 DAYS CONTROL (Fig. 30)

Osteogenesis was still going on in the coronal crater-like defect but the rest was repaired and the connective tissue of the marrow spaces was well differentiated.

180 DAYS CONTROL

The repair was completed.

CHAPTER V

DISCUSSION

The histologic pictures showed that, following surgery, the gingival tissue underwent minimal structural disorganization. (Fig. 8) The inflammatory reaction was mild enough to bring only changes which were essential to the repair process. In particular, despite the decrease in blood supply, the collagen fibers of the gingiva appeared to resist the injury. This was probably a consequence of the relatively small oedematous reaction which distented the tissue moderately. The permanence of these two components, epithelium and connective tissue, allowed the gingiva to sustain pressure and stresses during mastication and to carry out its role of protection.

Along the line of incision, an early reattachment of the flap was observed around the first three days. (Fig. 9) Classically, full flap procedures have been shown[39] to induce bone resorption. This removal of necrosed superficial lamellae is followed by repair with some apposition of bone which secures newly formed fibers on the cortical plate. (Fig. 31) Although we used the same type of flap, our specimens showed in some cases a different mechanism of repair.

It appeared that the raising of the flap with a blunt periosteal elevator allowed a part of the periosteum to remain on the bone. (Fig. 9) Consequently, when the flap was reapposed, some of the fibers were put in contact with remnants of periosteum. Under certain conditions a rebinding occurred rapidly between these two parts. Such a mechanism is possible as long as fibrils from both sides and fibroblasts are intact.

Healing by first intention occurred whenever the flap was ideally adapted to the underlying tissue as it was also shown by Caffesse[40]. The repair was faster and generated limited inflammation as it is well documented. Most important was the fact that very little bone resorption was observed in this type of healing. This also had the advantage of providing an immediate mature type of reattachment with groups of collagen fibers running perpendicular to the bone surface.

Healing by second intention was only found when the blood clot was in excess or in areas where the bone had been carefully scrapped off, mainly at the alveolar crestal border. In these areas, a granulation tissue developed first and fibroblasts differentiated to secrete new fibrils, collagen fibers were in majority deposited in a direction roughly parallel to the bone surface and some bone resorption occurred. (Fig. 10 and 32) The reorganization of the fibrous architect-

ure occurred over a period of 3 or 4 weeks. However, after complete repair, there was always a prevalence of the collagen bundles parallel to the vestibular surface of the alveolar bone. Such a finding agrees with the study of Melcher and Eastoe[41] who showed on normal human periodonteum that there is surprisingly few bundles which run perpendicularly to the bone surface.

The existence of the two mechanisms which provide for flap reattachment to the underlying structures consequently emphasizes the importance of controlling the bleeding when repositioning a flap in order to get a minimal clot formation which will not keep the fibers separated. Our findings re-enforce also the idea of using split thickness flaps in order to prevent bone resorption around a graft site.

In the osseous defects, a marked inflammatory reaction was observed only in the early period following injury. (Fig. 13) Each procedure probably damaged many of the cells pushed in the defects with the graft material. Because of the lack of an adequate blood supply, many cells of the coagulum underwent degenerative processes. Because of ischemia the ones which survived released catabolic substances which accumulated and were probably irritating to the surrounding tissues.

The importance of this phenomenon can be clearly understood when one realizes that the first cells which appeared were not macrophages but lymphocytes known for their property to neutralize toxins and enzymes. At the level of the bone the diffusion of those irritants and the lack of blood supply was evidenced by the enlargement of osteocytes near the graft areas. This reaction, observed in the first 24 hours, was followed by a shrinkage of the cells to a point that the bone lacunae looked empty. Under the higher magnification (x 1000) it was sometimes possible to find some trace of pale nuclei along the osseous walls but this picture became more rare after awhile. (Fig. 33) Such a disappearance of osteocytes has often been interpreted as a loss of vitality for the bone. However, the fact that they seemed to "reappear" around the 30th day challenges this hypothesis.

Osteocytes are in communication with each other by canaliculi which allow for fluid exchanges but not for cellular migration. When such a cell, known to maintain the normal chemistry of bone, vanishes and then "reappears", this has to be translated in terms of metabolic disturbances. It is possible that immediately after injury, the osteocytes had enough energy (during 24 hours) to increase their metabolic exchange with the environment in an attempt to preserve the integrity of the osseous tissue. Shortly after, nutrient

supply probably became scarce and more toxic substances started to diffuse through the canaliculi. Consequently they could not compensate any longer for the injury and gradually reduced their exchanges, losing materials and shrinking. Later, when a normal environment was re-established, they appeared to return gradually to function and a normal size. Such an explanation may be controversial for it is based on observations of different specimens and at different times. However, the fact that the bone did not show any significant resorption and maintained its staining property when osteocytes were invisible is a first evidence of its survival.

Despite the amount of injury, this resistance of the osteocytes had apparently a beneficial effect on the repair process. In particular, the balance of factors favorable to bone induction could not be upset in the osseous defects where no osteoclastic activity was observed at any time. Gradually, in a lapse of time of about one week, all connective tissue elements were mobilized and adapted to the new local conditions created by the surgical procedure. This adaptation involved mainly the vascular elements[42] and the undifferentiated mesenchymal cells. Both proliferated toward the graft areas, closely following the recession of the inflammatory stage. Although the first signs of endothelial

proliferation were observed three days postoperative (Fig. 15) at the level of the periodontal ligament, we found in the subsequent specimens that capillaries branched out from the marrow spaces as well as from the connective tissue of the gingiva. (Fig. 17 and 34)

The origin of the undifferentiated mesenchymal cells is still a matter of controversy; Maximov and others believe that they form a special group of cells which arise in the vicinity of the capillaries. Our observations agree with this theory. In the first days they were very close to the vessel walls and it was difficult to differentiate them from the endothelial cells. (Fig. 15) However, on the 11 and 14 days specimens when the capillaries had already invaded the defects, they were in so great number in the coronal areas that they appeared to proliferate independently from the capillaries. (Fig. 17)

Morphologically it was clear that all cells which, at this time, entered the graft areas were undifferentiated mesenchymal cells. They were first observed in the central part of the defects coming from the marrow spaces and the periodontal ligament, but the greatest number came from the overlaying gingival connective tissue. Apparently this was a consequence of the topography of the graft sites. The

contact zones with the periodontal ligament or the marrow spaces were very limited, but with the gingival tissue the contact was immediately on the surface openings of the defects. Consequently, once the flap was reattached, it appeared to supply more undifferentiated cells than other structures. This contrasts with the findings of Retief and Dreyer[43] who found that bone defects in the mandible of rats were filled mainly with endosteal cells.

However, Urist and McLean[44] have shown that two types of cells are generally involved in bone formation. The first type is found in the cambium layer of the periosteum. By proliferation it is supposed to give an osteogenic type of cell. However, we did not observe any particular activity in the periosteum. It is likely that this tissue provided for capillaries and cells but it probably played a minor part in our experiments for it was not covering the defects and was only found on their edges.

The cells which invaded the graft from those areas did not present any particular features and when osteoblasts-like cells were found this was always along the bone surface or in specific areas where they had been preceded by undifferentiated cells which were showing gradual signs of differentiation. (Fig. 23) When the granulation tissue initiated

its differentiation many cells also showed intermediate figures between undifferentiated mesenchymal cells and fibroblasts. Those observations tend to demonstrate again that the mesenchymal cells are the source of many specialized cellular elements. On the other hand, the fact that they invaded the graft mainly through the large openings of the osseous defects emphasizes the need for keeping a mature and well vascularized connective tissue over those areas. In such cases flaps should always be sutured tight to heal by first intention in order to protect the area during the first phase of inflammation. This would allow them to reattach more rapidly and to participate actively in the repair process of the osseous grafts.

The primary role given to the undifferentiated mesenchymal cells sets the problem of their induction. It is known (Melcher[45]) that those cells will produce generally soft connective tissue, however if they meet the proper type of induction they will differentiate in osteoblasts. The nature of the stimuli involved in this process is not known but its action is called regional induction. We always observed their differentiation along the walls of the defects, (Fig. 23) or around the graft particles. (Fig. 35) This

suggests that in the graft sites an osteogenic stimulus was present along the osseous walls and on the surface of the graft material. In the 11 days "push up" specimen, for instance, some of the mesenchymal cells had differentiated into osteoblasts in two areas, namely along the walls where a thin layer of osteoid tissue was present, and in the granulation tissue at some distance from the walls. In the latter formation no graft material was observed and the granulation tissue surrounding them did not appear different from the rest. (Fig 23) The same phenomenon was also observed in the 14 days control specimens. We can hypothetize that an "osteogenic factor" originated in the alveolar bone lining the defects and was able to diffuse a certain distance in the granulation tissue.

The existence of such a mechanism has been demonstrated by Goldhaber [46] with living osseous tissue. But the induction observed with the graft particles appeared to be different. This material stimulated only the cells in contact with it. There was no proof of the diffusion of an osteogenic substance. It is possible that this was related to the loss of vitality of the graft material.

Two types of stimuli may also be present. The living osseous tissue having continuous exchanges with its surroundings; its metabolites diffuse and one of them probably acts

as an osteogenic factor. In the case of the graft material it is more likely that according to Linghorne[17] its mineralized constituants have the same action. The latter appeared weaker than the former, however the graft particles proved to be beneficial to the repair. In particular both 14 days control specimens showed a marked delay in the amount of bone formation when compared with the grafted areas (Fig. 18 and 24) and, in the superficial areas of other specimens where osteogenesis was delayed, osteoblasts were more often found around the graft particles than along the walls of the defects.

Another interesting phenomenon was the degree of induction manifested by the various types of graft particles. The fragments of cementum and dentine which were accidentally introduced with the graft did not induce bone formation. (Fig. 36) They were finally invested in the bone but as a consequence to the osteogenic activity which independently developed in the surrounding area, finally they showed only minor signs of disorganization and appeared to be indifferently accepted by the surrounding bone.

On the other hand, the bone particles showed varying fates according to their physical characteristics. All of them exhibited signs of gradual disorganization while osteoblasts appeared on their surface and new bone was formed.

(Fig. 37) This arrangement realized a particular type of picture called involucrum. Such a phenomenon has been described in bone repair following osteomyelitis. It is a circumferential formation of bone which develops around a necrosing center. (Fig. 38) The simultaneous presence of bone resorption in the center and of bone apposition in the periphery suggests that the materials from the graft particles were reutilized by the surrounding osteoblasts. Those two mechanisms being biologically opposed, it is not possible to attribute their development to one type of cell which would have to function both ways. As pointed out by Young[47], osteoblasts are only capable to synthetize bone and cannot consequently be engaged at the same time in its removal despite the fact that osteoclasts are admittedly issued from the fusion of osteoblasts.[48] However, as some osteoblasts became rapidly invested in the newly formed bone, it is possible that those cells which have ceased to synthetize bone got involved in the removal of the graft material. Some authors like Belanger[48] have indeed shown that osteocytes produce proteolytic enzymes and are able, under certain conditions, to remove bone.

To re-enforce the theory of immediate reutilization of degraded bone materials by the surrounding osteoblasts the amount of bone which was formed on a given particle appeared

to be proportional to the degree of resorption of this particle. The smaller and thinner ones resulted in being the best type of graft material. (Fig. 37) Their rapid resorption produced probably a local increase of calcium salts which have been shown by Weinmann and Sicher to favor the formation of bone in atypical areas. From that point of view, the bone coagulum technic provided for the best material.

It appears that the action of the steel bur created a homogenous cluster of bone particles which averaged a length of 100 microns as measured with an optical micrometer. Most important was their thinness of about 10 microns. For a small volume these particles offered a large surface. This had the advantage to make them readily susceptible to hydrolysing enzymes which dissolved their cementing substances and liberated their minerals. After the initial phase of involucrum formation, we observed their rapid removal and replacement by new bone. Most of all this permitted a great number of mesenchymal cells to be in contact with the graft material and to gain benefit of their osteogenic stimulus. The only disadvantage to their thinness was that most of them appeared to have been somewhat disorganized and torn off by the bur at the time of their formation. Consequently the cells they could contain had been also damaged or destroyed.

This excluded the possible involvement of those cells in the osteogenic process or in their utilization as stimuli to induce other cells (Goldhaber)[46].

In comparison, the "push up" technic resulted in the formation of larger fragments which often averaged 1 millimeter in length. The way they were formed and their thickness offered a better guarantee for the survival of the cells they contained. They showed the same inductive property as bone chips and undifferentiated mesenchymal cells became osteoblasts at their contact. However they were disorganized very slowly and some were still visible in the late specimens (4 months postoperative). When the areas were almost completely repaired their remnants appeared as large involucri which could be a factor of fragility for the bone. But most of all, the "push up" technic brought a small quantity of graft material. (Fig. 21) Moreover the relative small surface of each particle with regard to their volume offered to a limited number of cells the possibility to differentiate into osteoblasts. We believe that such a factor would be more detrimental in one or two wall osseous defects or three wall ones with a large opening. In our experiments this problem did not result in failure because the type of defect selected could heal without the help of the grafts (control specimens).

However it appeared significant that in two instances (7 and 21 days push up specimens) local signs of necrosis developed around some of the larger fragments. Also in most of the specimens some delay in bone formation was observed around large particles in comparison to the smaller ones. Obviously those larger fragments (more than 1 millimeter in length) were acting as irritants and the organism was rejecting them. We could not clarify the reasons for such a reaction but the presence of lymphocytes suggested that toxic substances were released. It is possible that they originated at the level of the osteocytes which were enclosed in the graft particles. Because of the transplant procedure those cells were submitted to a greater amount of injury than the ones observed in the alveolar walls and they probably died because of a total lack of nutrients. Consequently, they may have released enzymes or other substances which behaved as irritants diffusing slowly through the canaliculi. It is also possible that those particles constituted, because of their size, an obstacle to the normal development of the capillary system which invaded the area before the granulation tissue.

After Cross[13] and Robinson[8] the size of the bone particles appears to be of primary importance. As with the grafts used, induction occurred only by contact. It follows

that the best technic should provide for the greatest number of thinner particles. It appears also that their condensation in the defects may be necessary for a better result but great care should be taken not to stick too many particles together for some space seems to be necessary for the granulation tissue to develop rapidly.

Every act should be executed with the idea that the osteogenic stimuli initiated by the alveolar bone or the graft particles can be easily suppressed if detrimental factors are allowed to develop in a graft area.

Among them one of the most important appears to be linked to the shape of the defect. Even in our experiment where we created small cavities ideally designed for repair to occur, we observed that, everything else being excluded, osteogenesis was delayed and progressed slower in the superficial areas of the graft sites. In particular the apical fenestration and the coronal opening of the push up specimens were always the last to be repaired. The 14 days push up control specimen permitted to demonstrate that the topography of the defects could be by itself detrimental to the repair process. In this case, the transformation of the granulation tissue in a fibrous type was interpreted as a phenomenon of maturation which has been shown by Melcher and Irving (50) to

inhibit osteogenesis. It is possible that the use of an apical fenestration increasing the area of contact between the graft and the surrounding gingival connective tissue modified the equilibrium established between the osteogenic factors in the graft site and the inhibiting ones in the gingiva. As shown by Mulholland and Prichard[51] it appears that the presence of fibrous connective tissue tends to inhibit osteogenesis in areas of intramembraneous bone repair.

The delay in repair was more important in the coronal areas where precisely chronic inflammation was always present because of the proximity of the tooth surface and its bacterial irritants. It is quite possible that a relation exists between these two facts. Clinicians should always manage to eliminate or control to the limit of their possibilities all the factors which, directly or indirectly, maintain some chronic inflammation in a flap covering a bone graft. As inflammation cannot be avoided in the first days following surgery, the administration of antibiotics during the first week postoperative may be advisable.

CHAPTER VI

CONCLUSIONS

Bone grafts should be completely covered by soft tissue flaps, besides the periodontal ligament and the marrow spaces the connective tissue of the gingiva provides for an important number of capillaries and undifferentiated mesnchymal cells which invade the defects.

The osteogenic stimulus, which induce the undifferentiated mesenchymal cells to become osteoblasts, originates in the osseous walls of the defects as well as on the graft material. In this regard, the use of an apical fenestration in the push up technic may be detrimental to the osteogenic induction.

The graft material undergoes necrosis and its components appear to be used for the build up of the new bone. In all probabilities the original small number of osteogenic cells, brought with the bone graft, does not survive the transplantation.

The size of the graft particles appears to be of major importance: thin bone chips of 100 microns, as found in the bone coagulum technic, provided for a sooner and greater osteogenic activity than thick particles ten times bigger which may initiate localized inflammatory reactions.

As osteogenic induction appeared to occur by contact with the graft material, the greatest number of bone particles should be provided. But "overpacking" may result in delay or failure by holding up the formation of the necessary amount of granulation tissue.

All factors of irritation to the area should be kept under control, for the delay observed in the healing of the coronal parts appears to be linked with the persistence of chronic inflammation near the epithelial attachment.

CHAPTER VII

SUMMARY

Two technics of autogenous bone grafts were used in surgically prepared intraosseous defects. Four adults Rhesus monkeys provided for 32 specimens from zero hour to 6 months postoperative. There was 13 specimens for each type of graft and 6 others were used as controls.

A description of the reactions observed at the level of the different components of the periodontium was given. Some of the factors involved in the repair process were discussed. An attempt was made to bring out the significance of the most prominent histologic features characterizing the two types of graft. The merits and disadvantages of each technic were analyzed and an insite to their clinical aspects was provided.

The "bone coagulum" technic appeared to better support and stimulate osteogenesis than the "push up" technic.

CHAPTER VIII

FIGURES

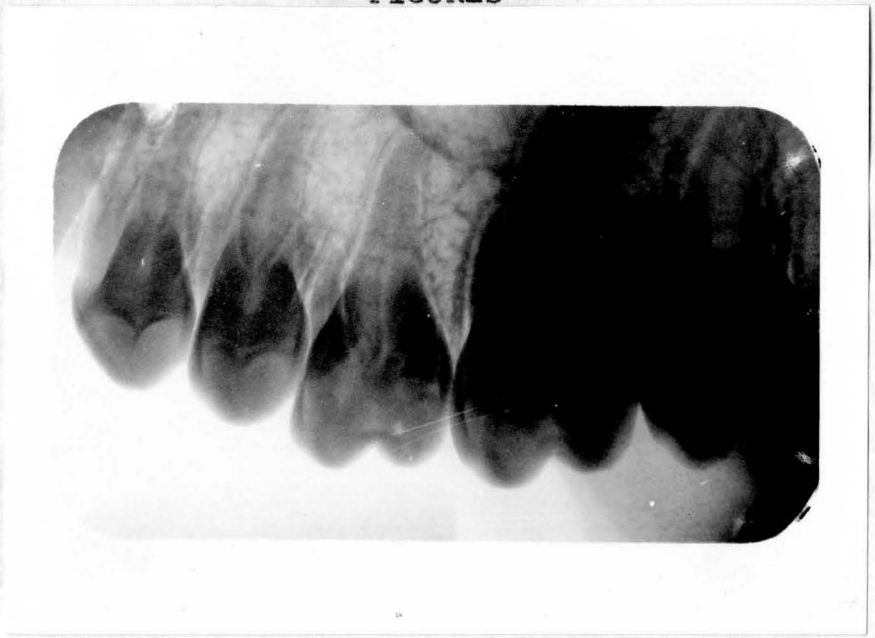

Fig. 1 Preoperative radiograph showing moderate amount of bone resorbtion.

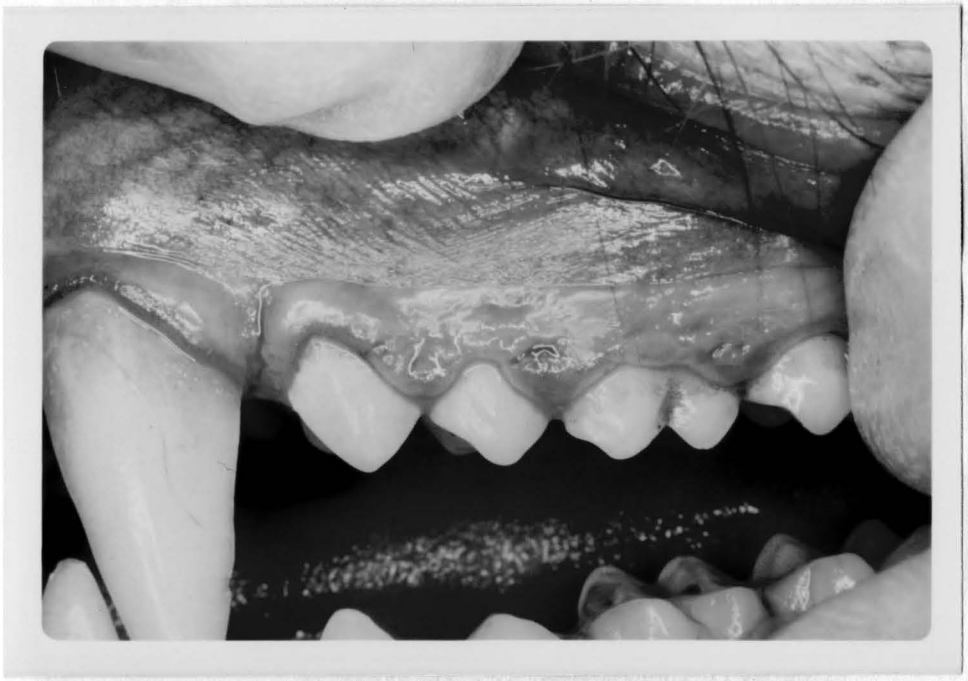

Fig. 2 Preoperative view. The amount of calculus is moderate but gingival inflammation is present. Pocket depth in this case was averaging 3 millimeters. (original magnification x 1)

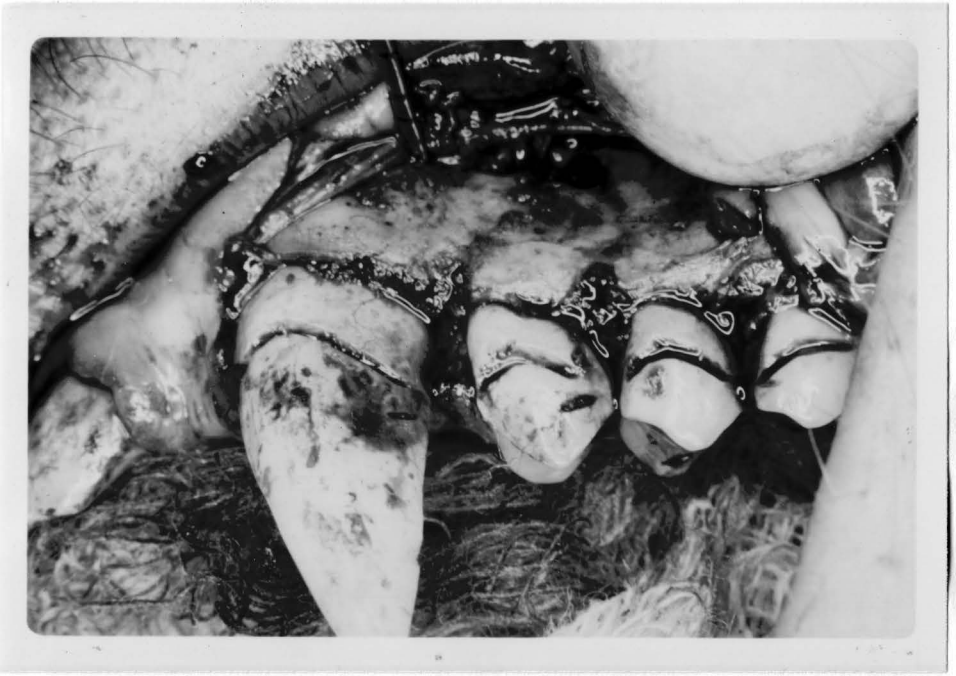

Fig. 3 The 2 grafting technics have been performed. The push up technic is located between first and second premolar; the bone coagulum technic between second premolar and first molar. (original magnification x 1)

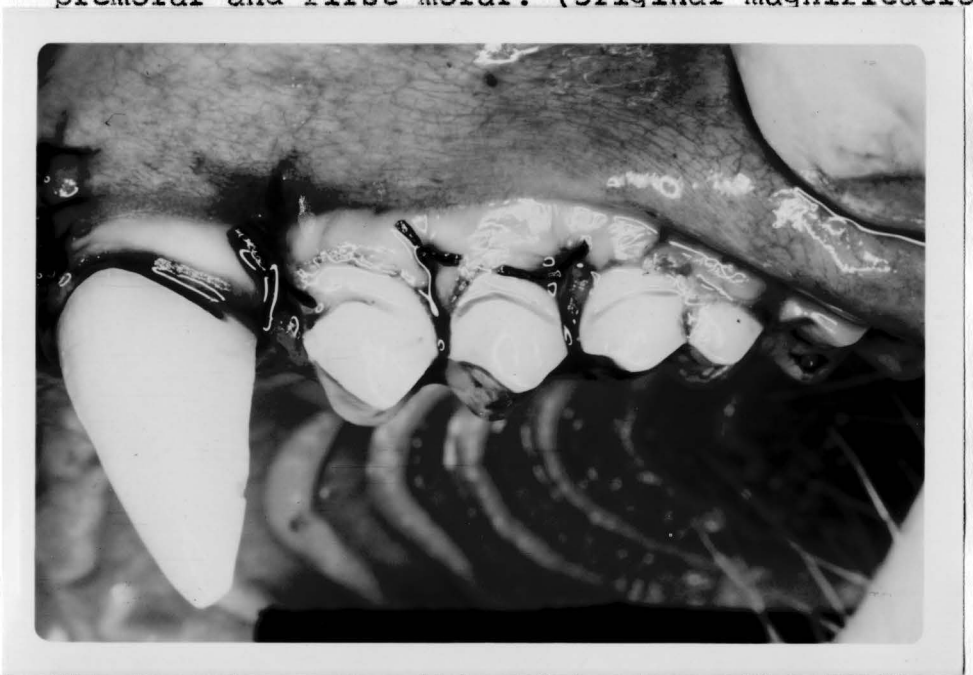

Fig. 4 The flap is reapposition to cover the grafts as completely as possible (original magnification x 1)

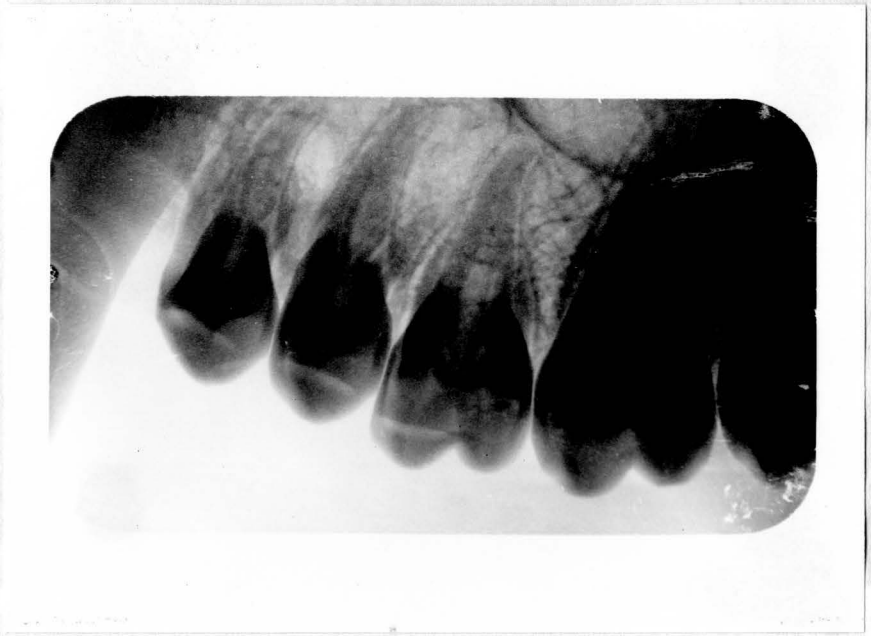

Fig. 5 Postoperative radiograph of the preceding specimen. The site for the push up technic is visible between the premolars. (original magnification x 1)

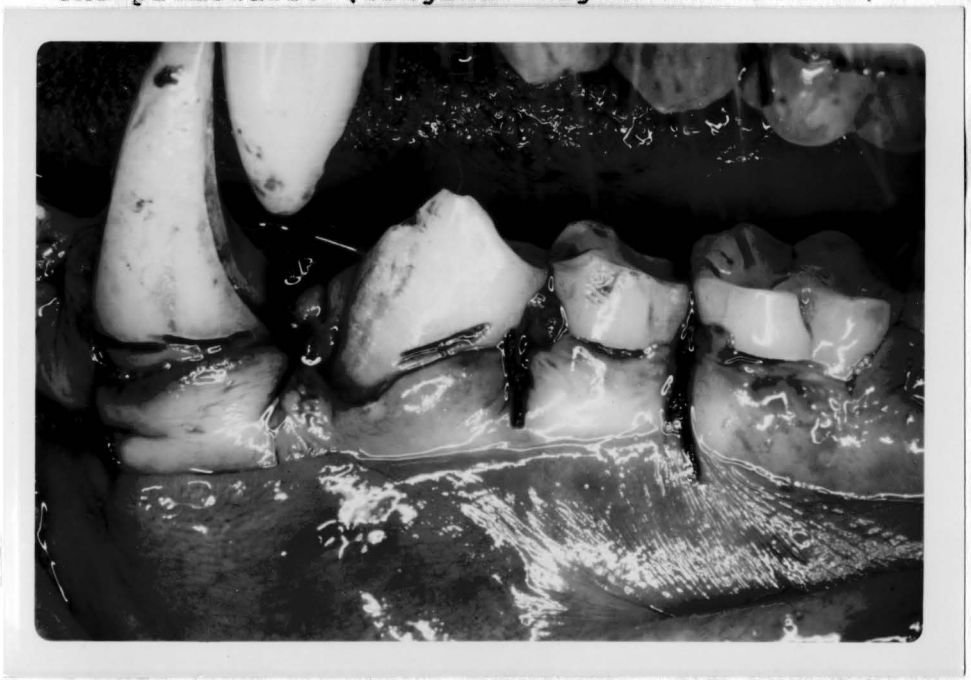

Fig. 6 0 hour postoperative. Note the relationship of the free margin with the notches on the teeth. (original magnification x 1)

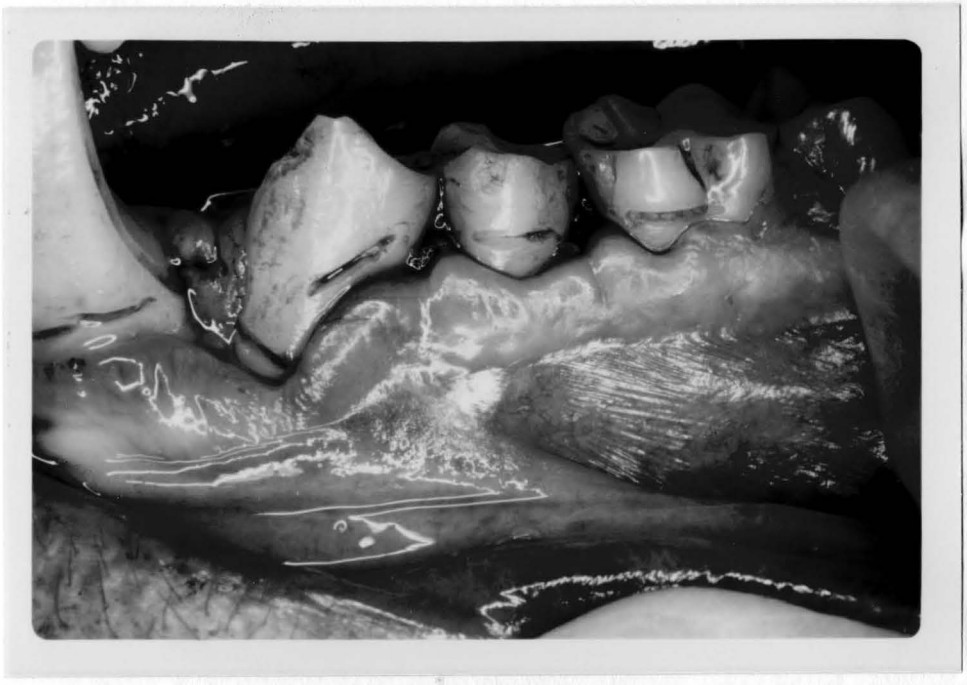

Fig. 7 One week postoperative view of the previous specimen . Dispite the oedema, the free gingiva has receded assuming a better relationship with the underlying bone. (original magnification x 1)

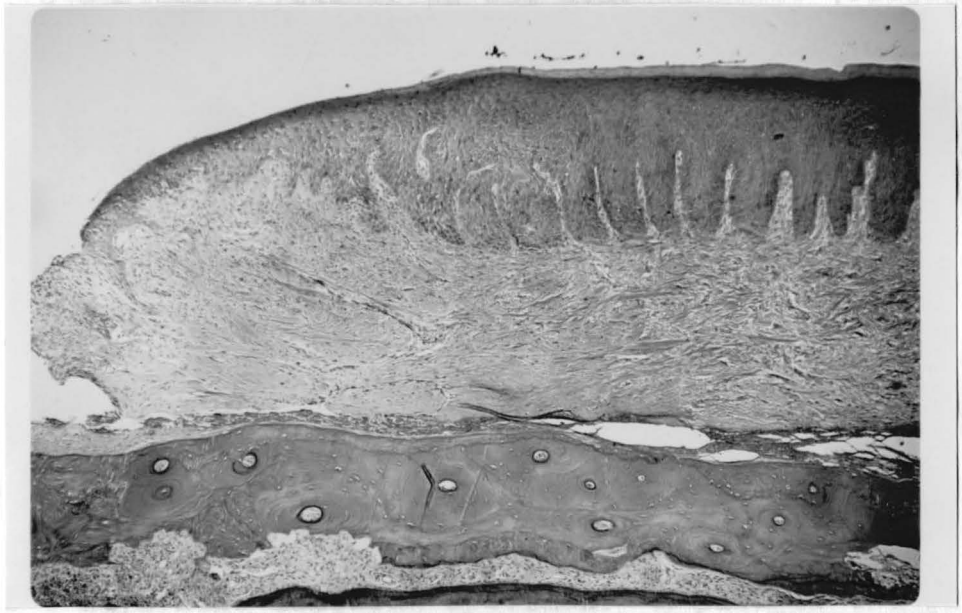

Fig. 8 3 days bone coagulum specimen. The acanthotic epithelium shows signs of atrophy in the area of the free margin - the connective tissue is moderately inflammed - the blood clot is invaded by inflammatory cells along the line of incision. (original mag. x 40)

Fig. 9 1 day - bone coagulum specimen. An early reattachment of collagen fibers can be observed at the former level of separation between the flap and the alveolar bone. Fibroblasts can be seen between the fibers - osteocytes are clearly visible in the bone lamellae (original magnification x 100)

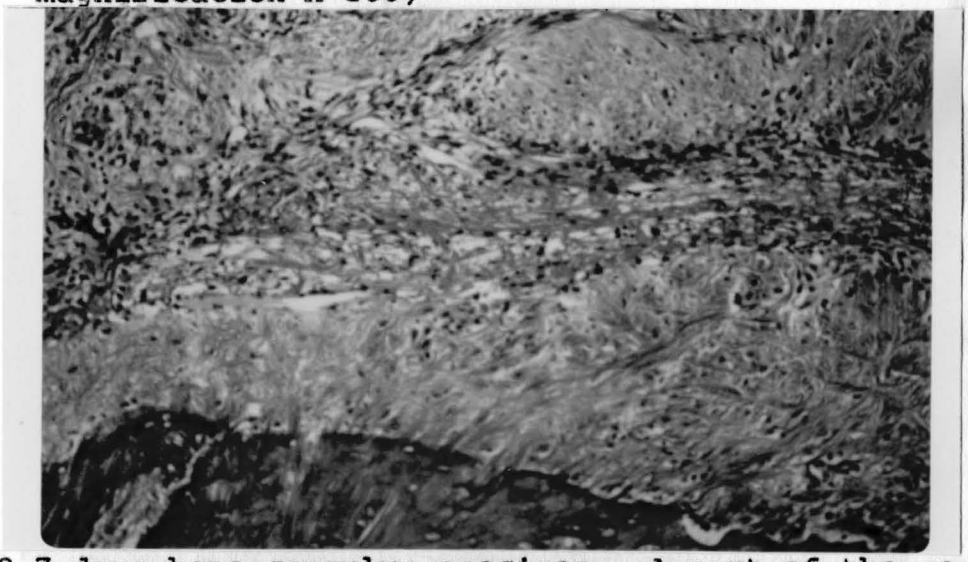

Fig. 10 7 days bone coagulum specimen. A part of the periosteum has been left intact on the bone - the fibrous scar clearly visible with collagen fibers running parallel to the bone (original magnification x 100)

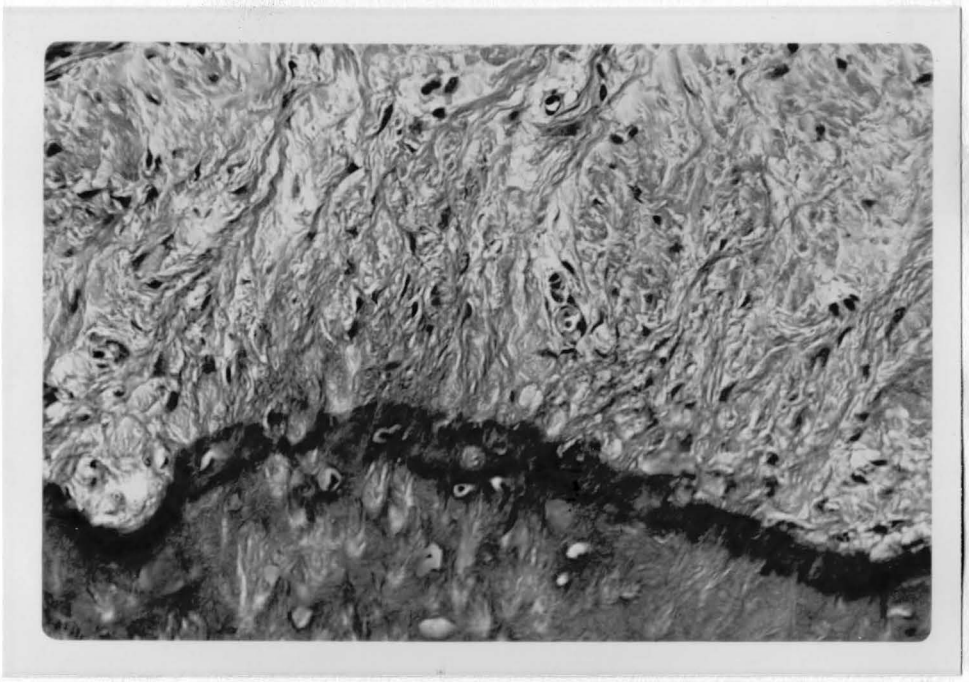

Fig. 11 17 days - bone coagulum specimen. At the level of the flap reattachment the collagen fibers have been re-orientated and are mostly perpendicular to the bone surface - a new layer of bone has been deposited. Deeper in the bone the lacunae are empty (original magnification x 100)

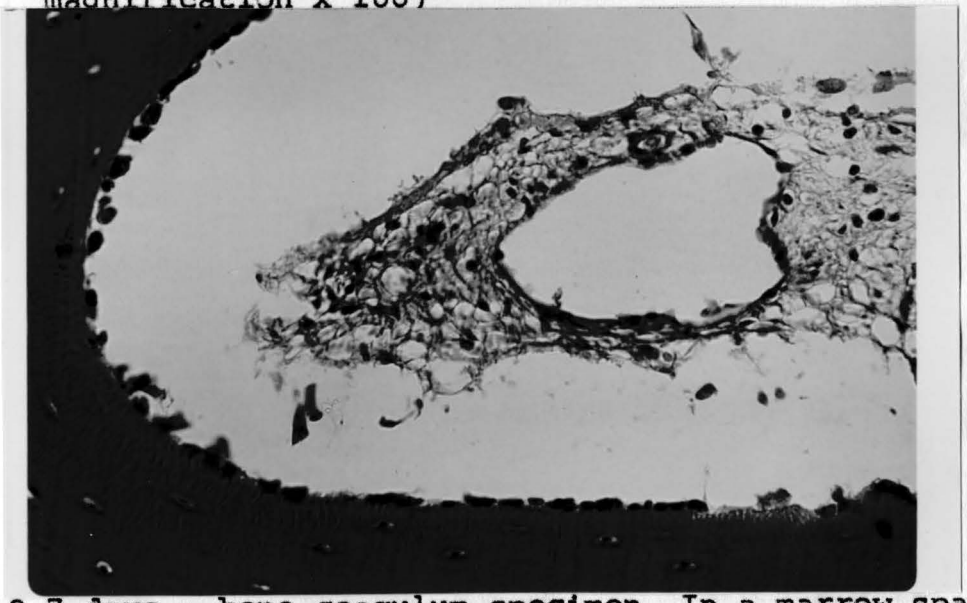

Fig. 12 7 days - bone coagulum specimen. In a marrow space close to the graft the endsteal cells start to differentiate in osteoblasts (original magnification x 250)

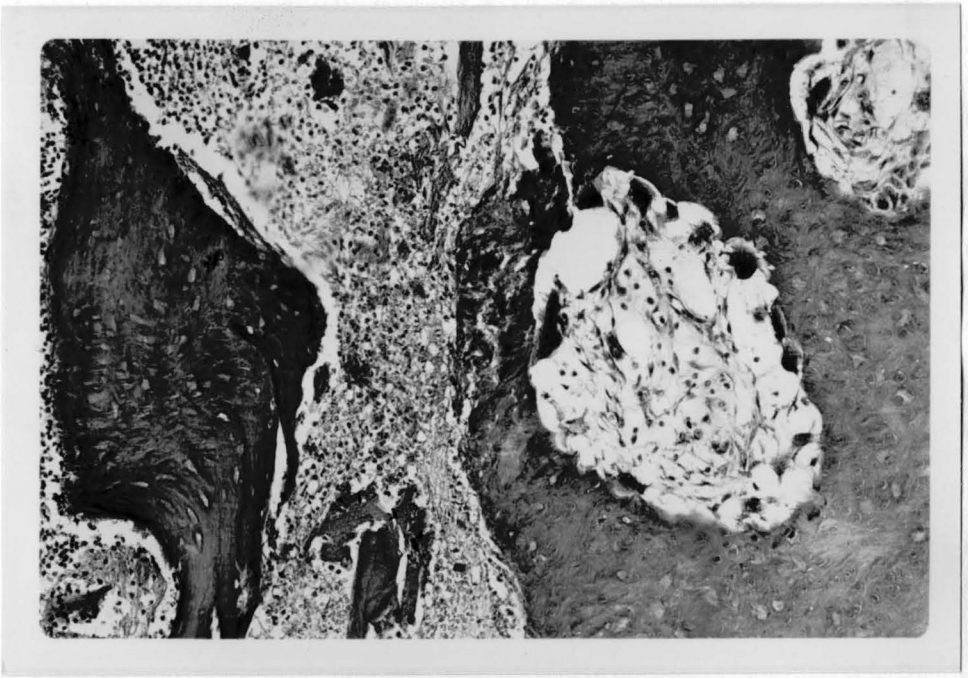

Fig. 13 7 days push up specimen - on the left the graft area is heavily infiltrated with inflammatory cells - a graft particle shows empty lacunae - on the right osteoclasts are resorbing the osseous walls of a marrow space (original magnification x 250)

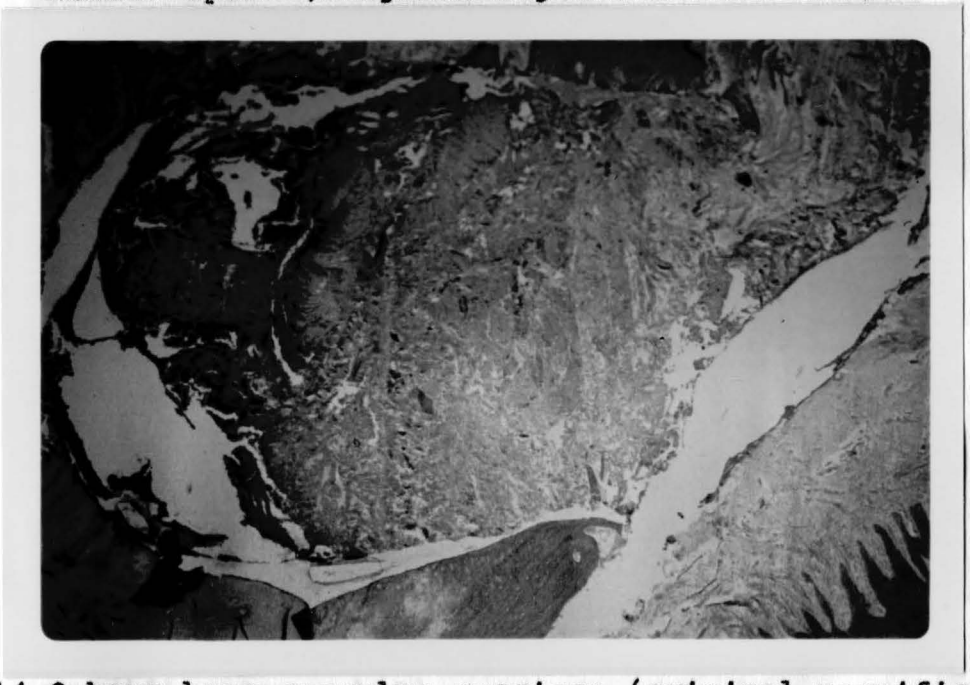

Fig. 14 0 hour bone coagulum specimen (original magnificatio x 10)

Fig. 15 3 days bone coagulum specimen. Capillaries are invading the bottom of the graft site from the periodontal ligament. A few undifferentiated mesenchymal cells are present (original magnification x 40)

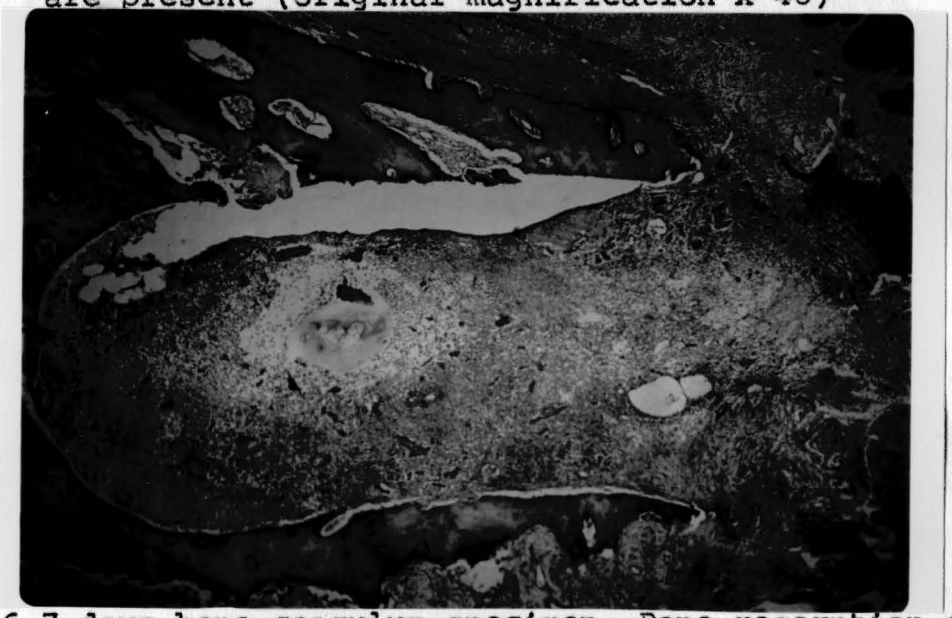

Fig. 16 7 days bone coagulum specimen. Bone resorption occurs along the osseous wall of the peridontal ligament. The buccal plate of bone appears to be intact (original magnification x 100)

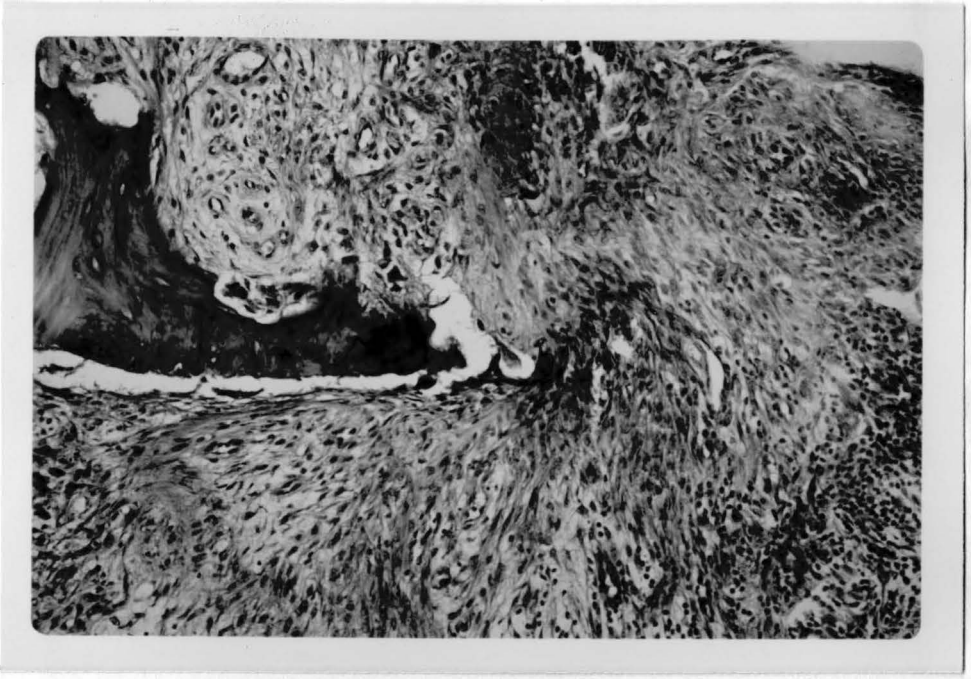

Fig. 17 7 days bone coagulum specimen. Undifferentiated mesenchymal cells are invading the coronal part of the graft site (magnification x 100)

Fig. 18 14 days bone coagulum specimen. The granulation tissue is organized, new bone is forming along or near the osseous wall of the graft site - the bone graft stains blue. (original magnification x 10)

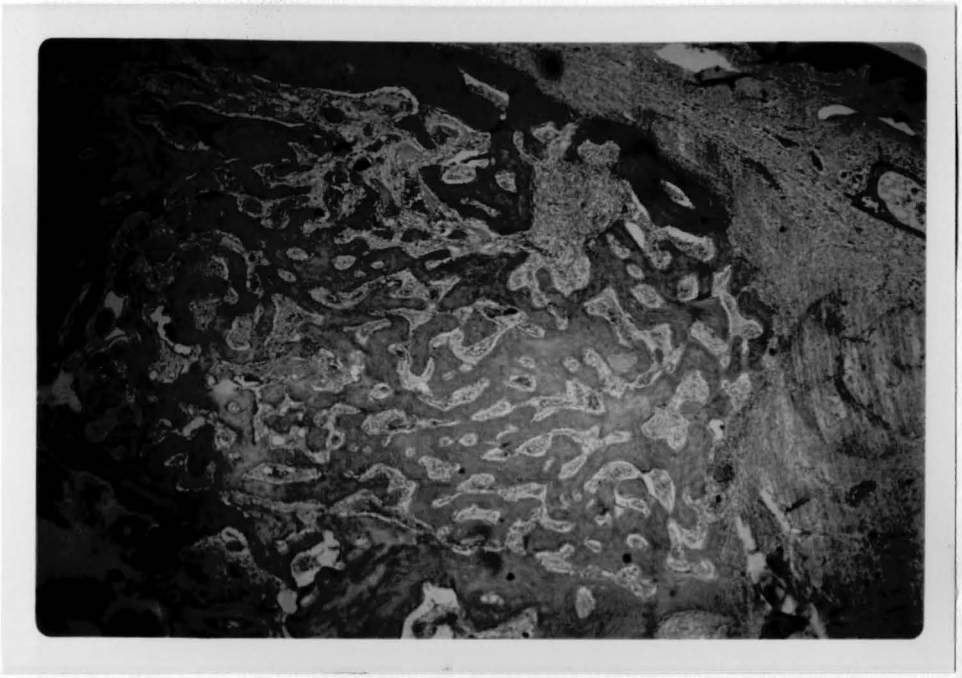

Fig. 19 21 days bone coagulum specimen. The islands of new bone have fused together (original magnification x 10)

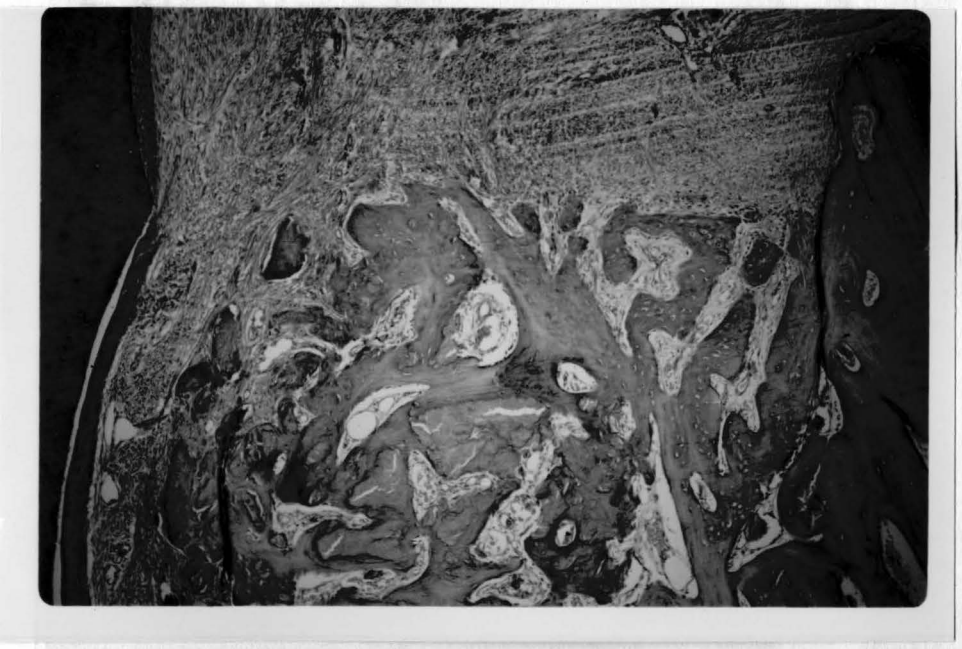

Fig. 20 60 days bone coagulum specimen. The graft material which has lost its fibrilarity is imbedded in the new bone - there is a residual intrabony defect probably due to the apical migration of the epithelium attachment along the root surface (original mag. x 40)

Fig. 21 0 hour push up specimen. The graft particles are located in the coronal area of the defect (original magnification x 10)

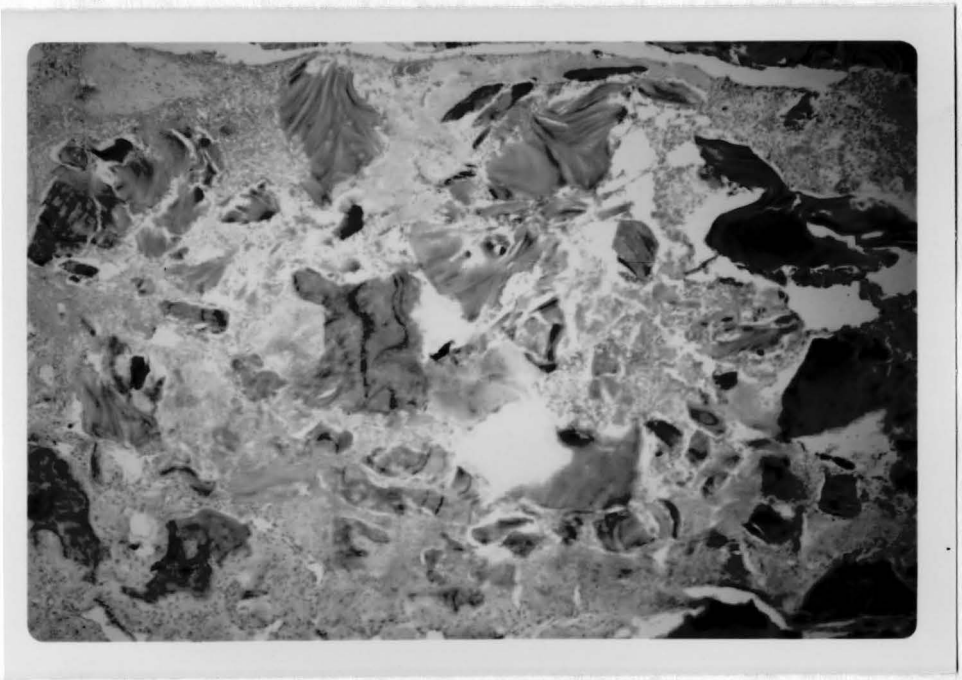

Fig. 22 3 days push up specimen. The graft particles are embedded in the fibrinous coagulum; on the right a part of the alveolar bone can be seen (original mag. x 40)

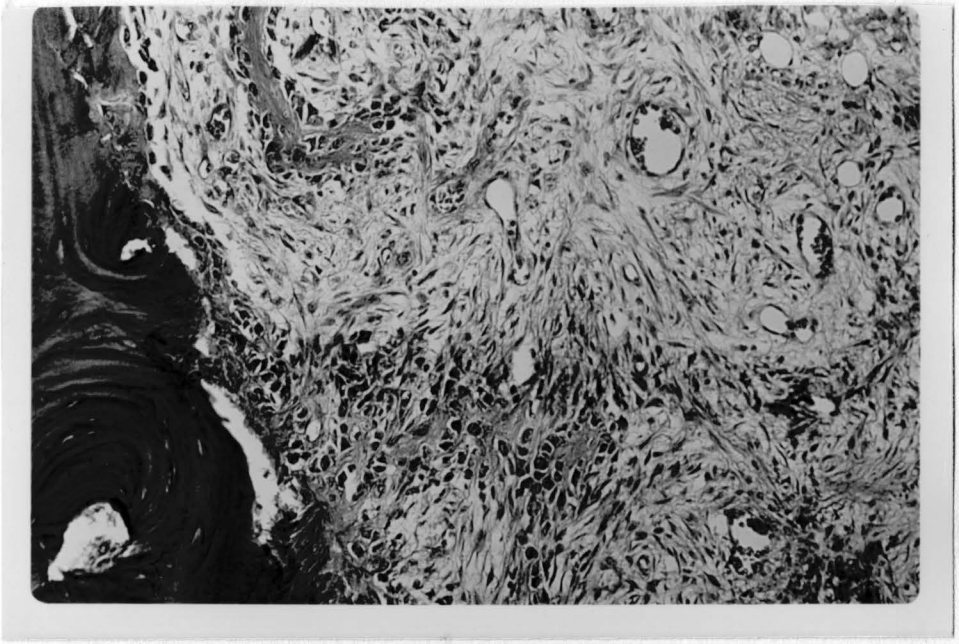

Fig. 23 11 days push up specimen. Osteophytic formation develops near the osseous walls in the central area of the defect. There is no graft particle. Osteoblasts are only seen where bone is being formed. (Original mag. x 250)

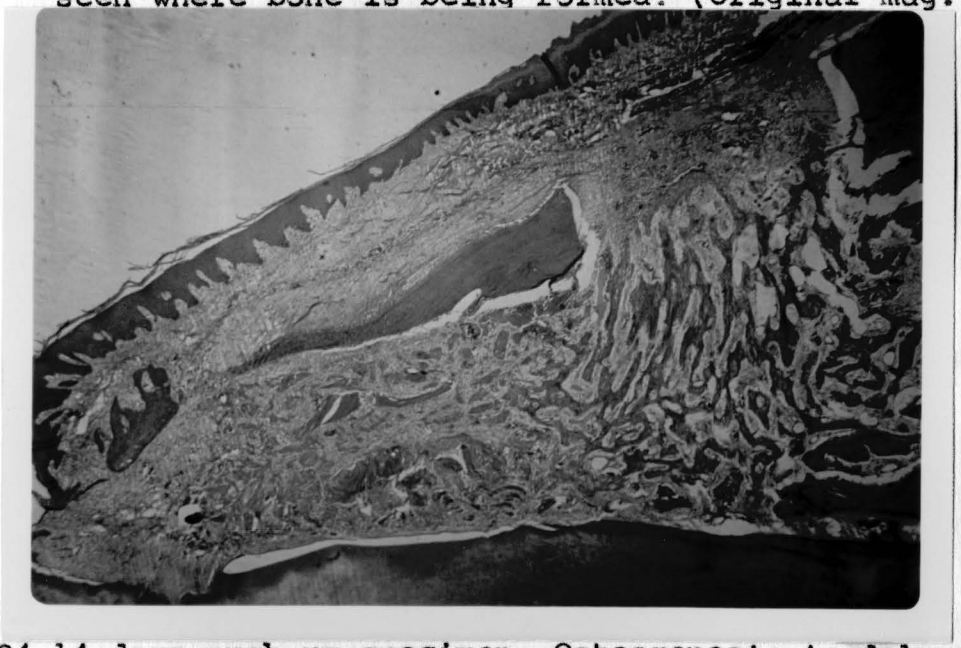

Fig. 24 14 days push up specimen. Osteogenesis is delayed in both apical and coronal areas above them the gingival connective tissue appears inflammed and highly vascularized. (original magnification x 10)

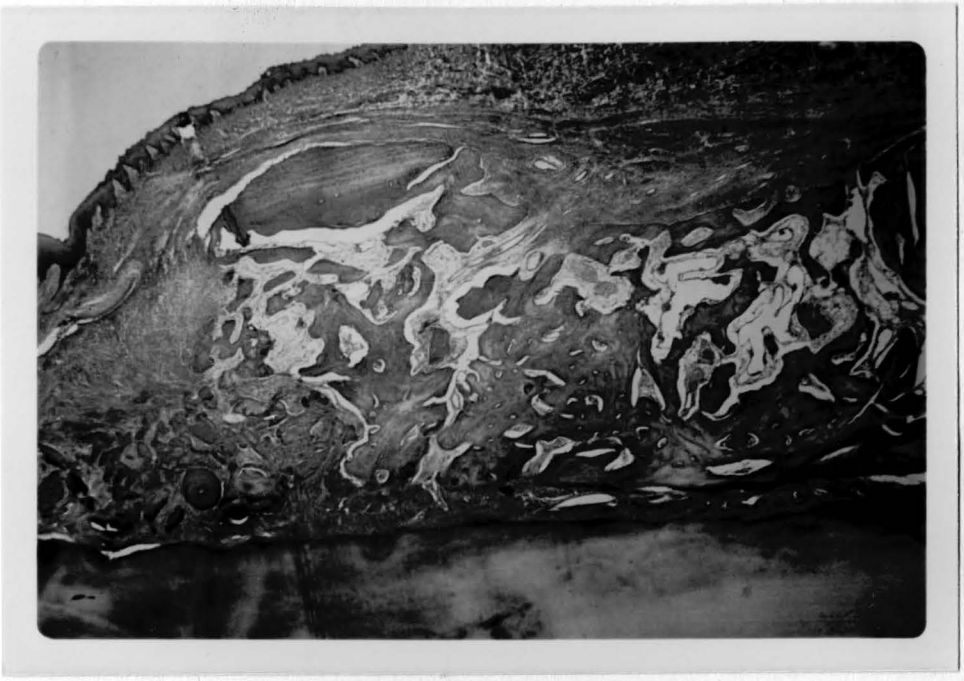

Fig. 25 60 days push up specimen. Repair is not yet completed in the coronal and apical areas of the graft site (original magnification x 10)

Fig. 26 14 days bone coagulum control specimen (original magnification x 10)

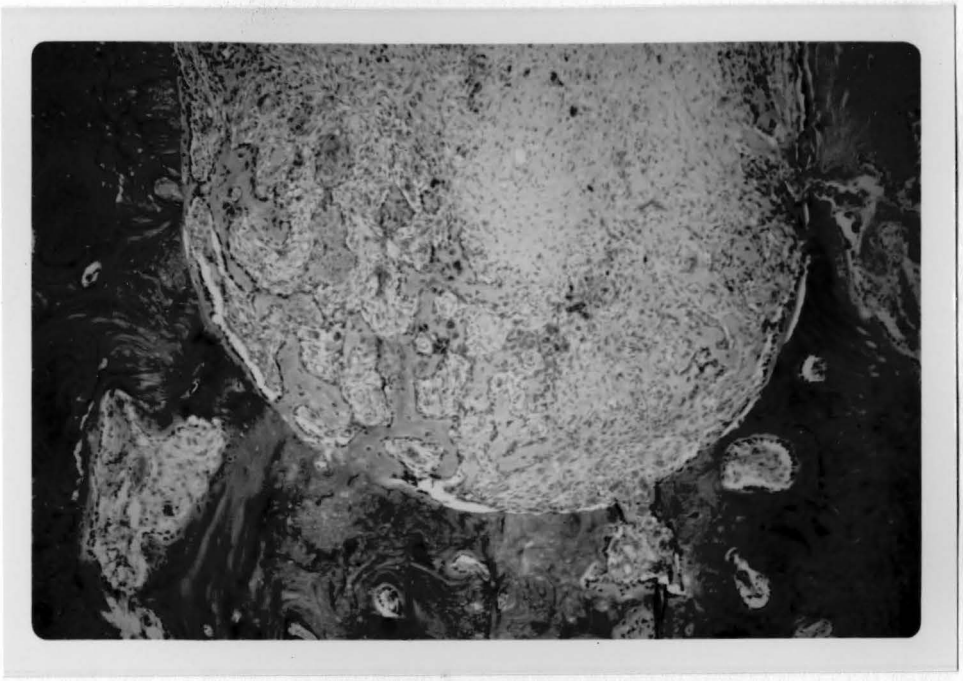

Fig. 27 14 days bone coagulum control specimen. Osteophytic formation is developing from the walls of the defect. (original magnification x 10)

Fig. 28 14 days push up control specimen. The granulation tissue shows a particular fibrous arrangement (original mag. x 10)

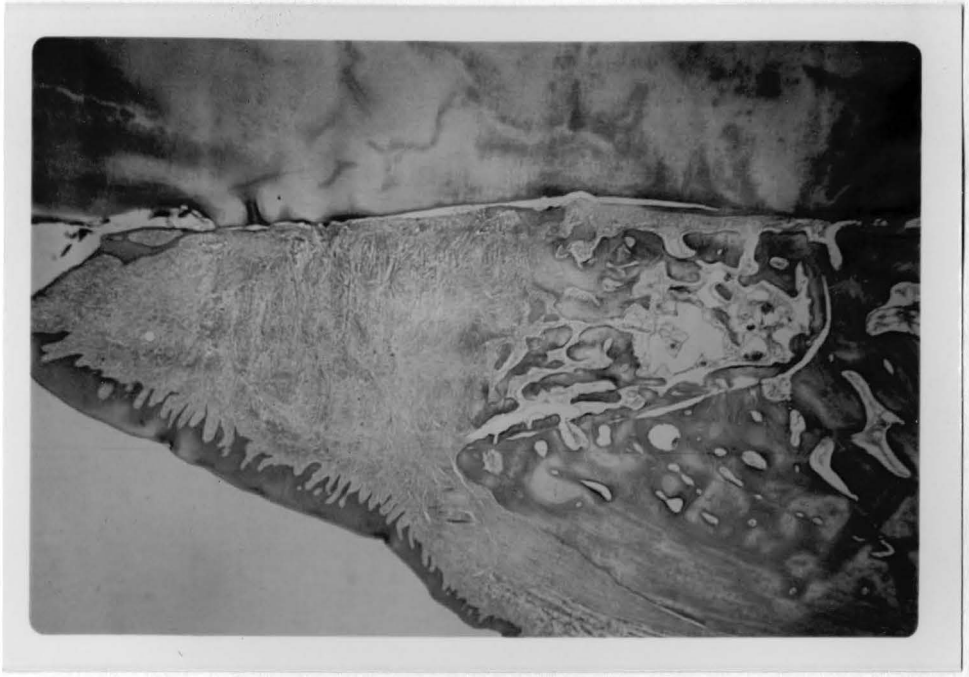

Fig. 29 60 days bone coagulum control specimen (original magnification x 10)

Fig. 30 60 days push up control specimen (original mag. x 10)

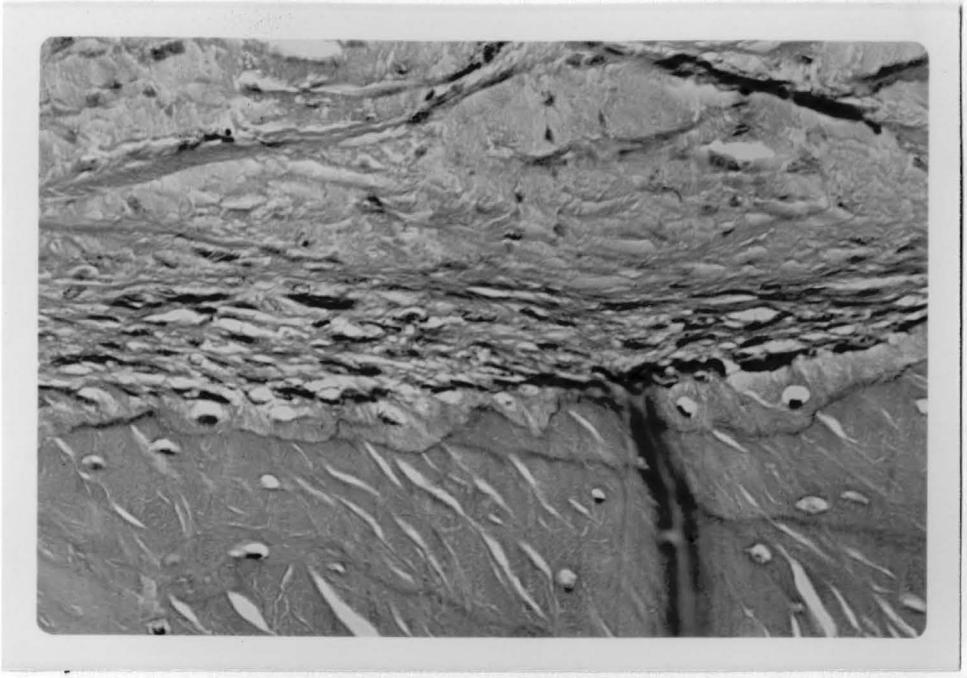

Fig. 31 7 days bone coagulum specimen. New bone has been laid down along the cortical plate of bone to secure newly formed fibers (original magnification x 100)

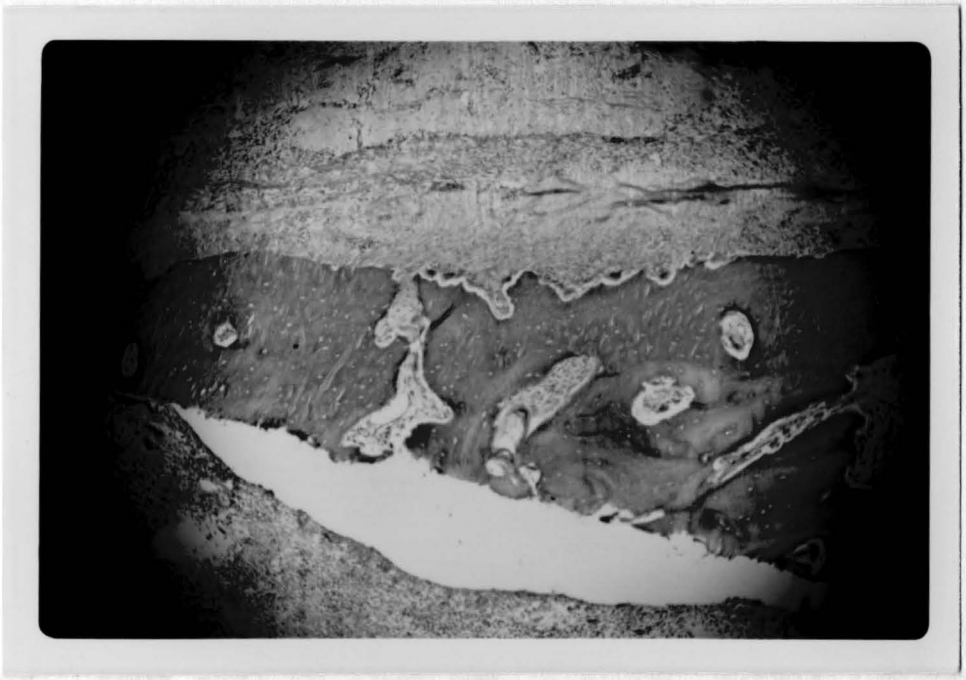

Fig. 32 7 days bone coagulum specimen - Bone resorption is still going on under some parts of the flap. (original magnification x 100)

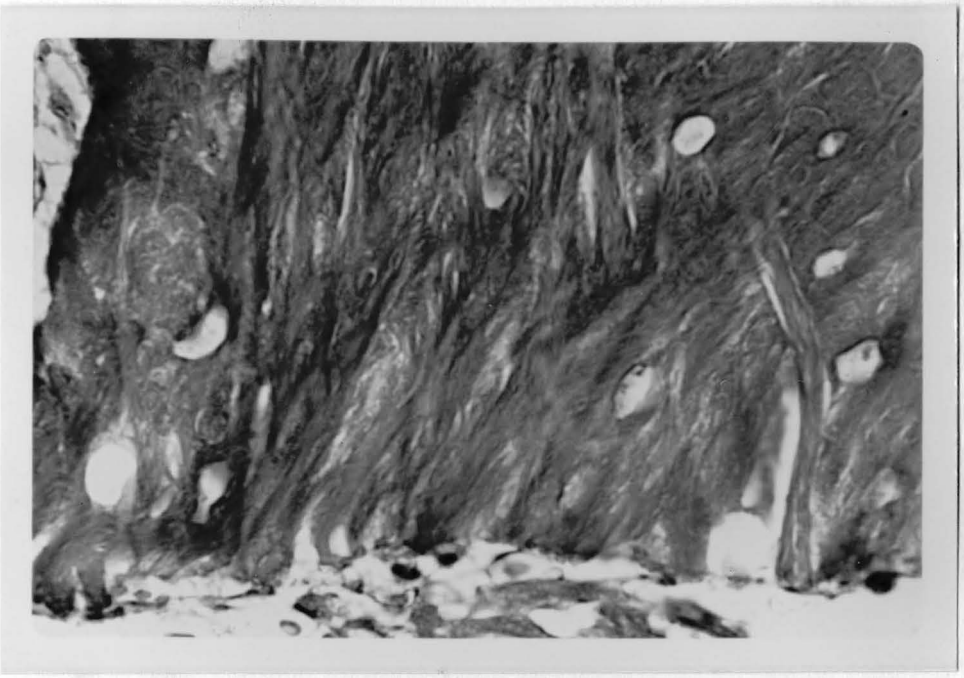

Fig. 33 7 days bone coagulum specimen. This picture represents a part of the alveolar bone which lines the graft site. Most of the lacunae are empty (original mag. x 400)

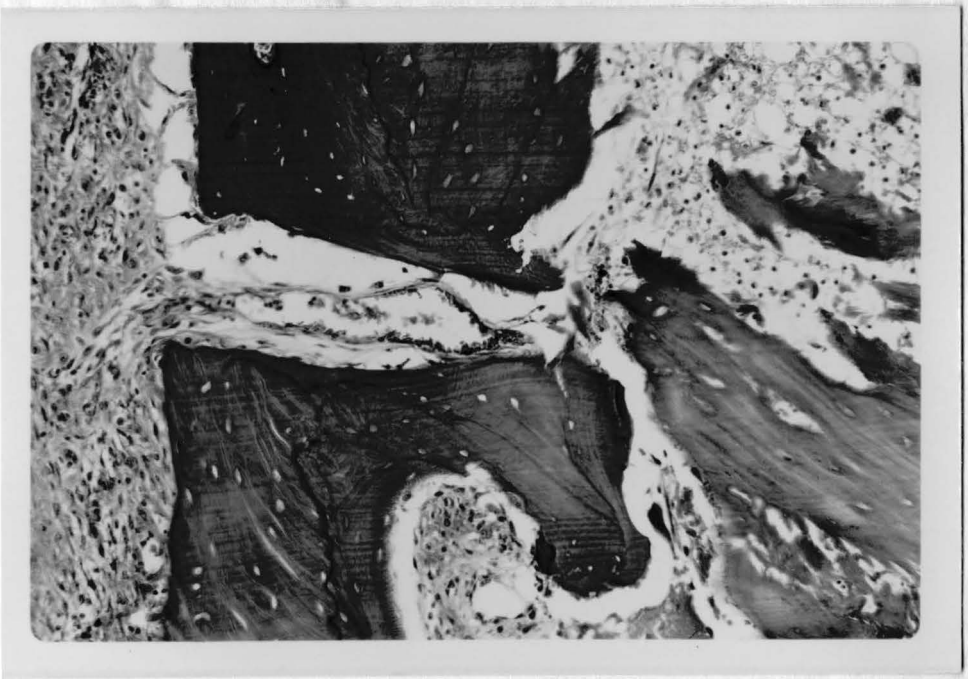

Fig. 34 7 days push up specimen. A blood vessel from the gingival tissue is reaching the graft area on the right through the cortical plate of bone (Original mag. x 100)

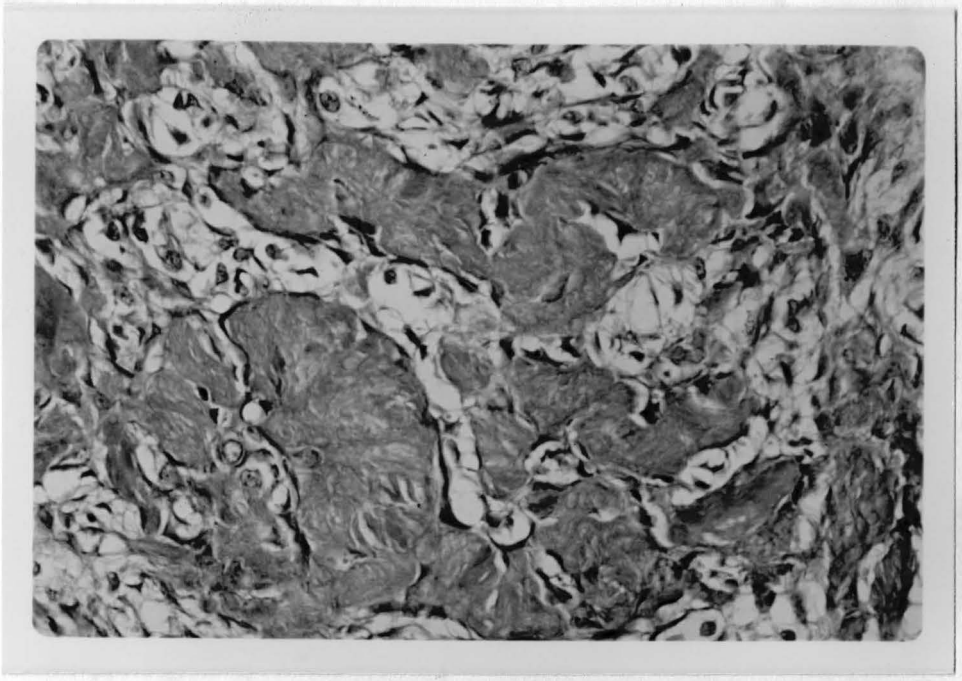

Fig. 35 14 days bone coagulum specimen. The graft particles are being gradually lined with osteoblasts (original magnification x 250)

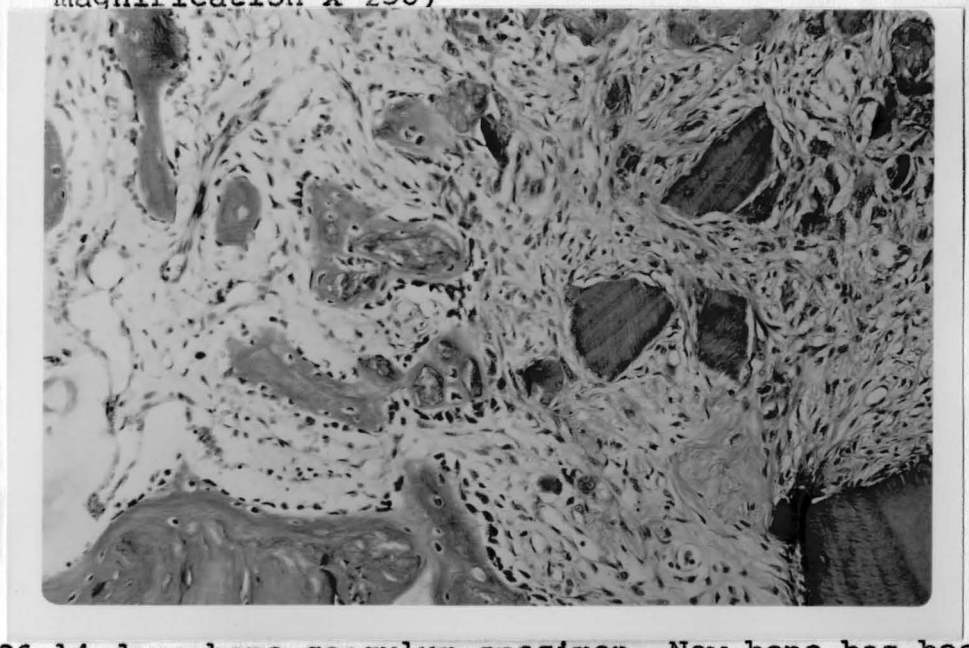

Fig. 36 14 days bone coagulum specimen. New bone has been formed on the osseous walls and the graft particles at the bottom of the defect. 3 particles of cementum can be seen. They are not lined neither by osteoblasts nor by bone (original magnification x 100)

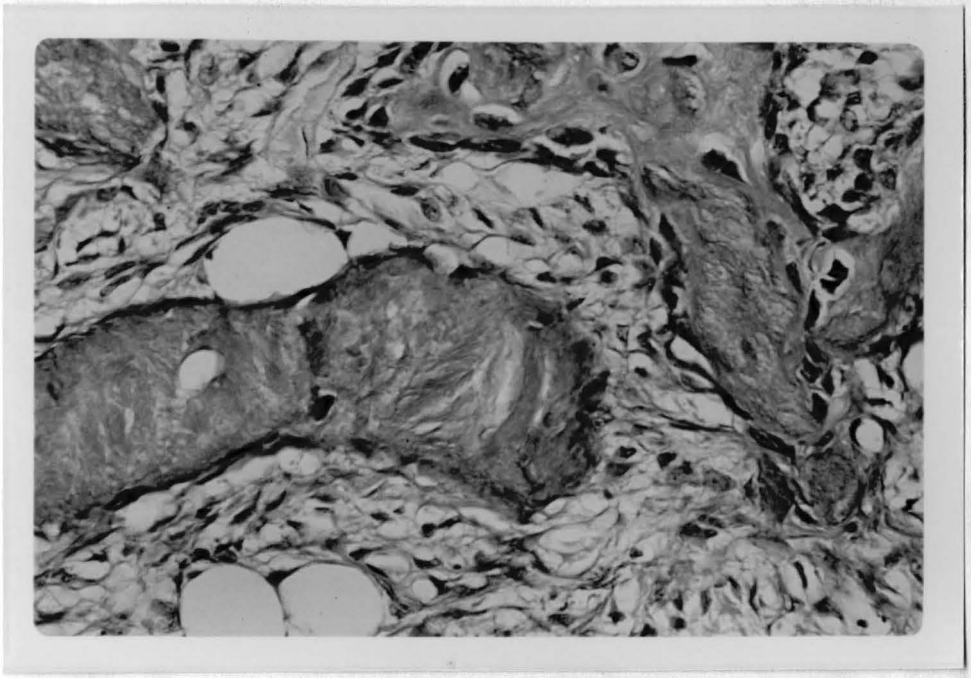

Fig. 37 14 days bone coagulum specimen. The bone particles show signs of advanced disorganization. New bone is being laid down on the smallest ones (original magnification x 250)

Fig. 38 14 days bone coagulum specimen. Typical picture of involucrum. Bone is being laid down around a necrosing center (original magnification x 400)

Fig. 39 21 days push up specimen. Inflammatory cells are present around 2 larger bone fragments. They appear to inhibit osteogenesis around them. A few osteoclasts can be seen on the surface of the graft (original magnification x 100)

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APPROVAL SHEET

This thesis, submitted by Aime F. Rivault, has been read and approved by 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

Signature of Advisor