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Fresh and Frozen Iliac Crest Transplants in Primates: A Sequential Histologic Study

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FRESH AND FROZEN ILIAC CREST TRANSPLANTS IN PRIMATES:

A SEQUENTIAL HISTOLOGIC STUDY

by

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DEDICATION

To my father, the late Andrew Joseph Morris, who did not live to see the completion of my formal education, but who stimulated its happening.

To my wife, Sherrian, for her patience, love, and understanding.

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CHAPTER I

INTRODUCTION

Osseous defects are frequently found in patients requiring periodontal therapy. Attempts to correct these lesions have been numerous and varied. These include reshaping the alveolar process via osteoplasty and osteoectomy, new gingival attachment procedures through curettage, and bone grafting techniques.

In cases of advanced periodontal disease the morphology of the osseous defects usually does not lend itself to corrective treatment by new attachment procedures. When osseous resection techniques are employed to recontour the inconsistent bony margins and crater defects a physiologic osseous architecture is obtained, although at a more apical level in relation to the dentition. The objectives of periodontal

therapy are attained, however this occurs at the expense of mature connective tissues so vitally needed for support of the functioning dentition.

One of the most significant trends in periodontal therapy has been the development of procedures designed to replace lost or diseased periodontal structures, and the use of osseous tissue autografts in bone defects resulting from periodontal disease represents a major effort in this direction. Elimination or reduction of osseous defects by the deposition of new alveolar bone, periodontal ligament, and cementum utilizing an implant technique would produce a more desirable result, and therefore represent the ideal therapeutic approach to these lesions.

The feasibility of utilizing cancellous bone and marrow from the ilium in the correction of periodontal osseous defects has been recently demonstrated[1][2][3][4]. Although both fresh and frozen autogenous iliac transplants have been employed in periodontal therapy, their histologic evaluation is limited, and to date, no study has been undertaken to compare their healing phenomena. The purpose of this investigation is to study, on a

histologic level, the sequential healing phenomena
of fresh and frozen autogenous iliac transplants placed
into chronic periodontal osseous defects. and to compare
these events to see if any significant difference may
be attributed to the preservation of the autogenous
bone material.

CHAPTER II

REVIEW OF THE LITERATURE

A. AUTOGENOUS BONE GRAFTS

The first autogenous bone grafts employed in the
treatment of periodontal disease were reported by
Hegedus[5] in 1923. He published the results of six
cases in which he transplanted periosteo-cortical bands
from the tibia to areas of "alveolar pyorrhea" in an
attempt to bring about a _restitutio ad integrum_.
Following the removal of a section of the tibia (with
intact periosteum) buccal and lingual mucoperiosteal
flaps overlying the involved periodontium were reflected.
The teeth were scaled, the granulation tissue was
removed, and the remaining bone substance was "freshened"
to promote its adhesion to the transplant. The donor
bone was then placed on the recipient bed, and the

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mucoperiosteal flaps were sutured over the graft. Hegedus attributed his clinical success, based on decreased tooth mobility, eradication of pockets, and radiographic evidence, to the bone-building and regenerating property of the periosteum.

Beube and Silvers[6], in 1934, demonstrated that heterogeneous devitalized bone-powder, placed into surgical cavities in the alveolar bone of dogs, produced new bone formation. Following surgical preparation of the bone defects, sheep bone-powder was placed in the experimental sites while the control sites were left untreated. Histologic sections demonstrated complete bone formation in the experimental sites, while no bone formation occurred in the control sites. Although the graft sites were not periodontal defects and the graft material was not autogenous, their study demonstrated that graft particles do indeed accelerate osteogenesis, and they concluded that the role of the grafted bone was to supply calcium for the calcification of the granulation tissue constituting the healing process.

One year later, Beube and Silvers were also

successful in treating human periodontal defects by implanting boiled bovine bone-powder into the osseous lesions[7]. They attributed this success to the maintenance of the blood clot and reasoned that the fibrin of the clot has a great affinity for calcium and, therefore, acts as a scaffold and binder for the bone-powder, which can then be reutilized by the cells involved in the repair of the osseous defect.

The idea that the calcium content of the bone-powder was a significant ingredient required by the repairing tissues to form bone and cementum was also supported by Murray[8]. His experiments, reported in 1938, supported the idea of direct utilization of calcium and he again cited the potential affinity of fibrin in the clot for calcium as a mean of retention.

Beube[9] continued his experiments with heterogenous bone grafts of boiled bovine bone-powder. In 1949, he studied the healing phenomena in surgically created osseous defects in dogs and stated that the bone-powder showed consistently a hastening of the repair process. This study also provided a histologic discription of the healing sequence. Following an initial acute

inflammatory response, a subacute inflammatory response occurs characterized by the proliferation of young fibroblasts and the gradual appearance of new capillaries. Then, after organization of the granulation tissue, osteoclastic resorption of the bone begins before osteoblastic activity occurs. He noted that the bone-powder did not alter the repair sequence, but definitely accelerated the rate of healing resulting in more rapid deposition of bone and cementum as compared to that of the controls. Beube also asserted that the cementoblasts were one and the same cell derived from fibroblasts.

Cross[10][11][12][13][14] published several reports on the use of bone implants in periodontal disease. He utilized autogenous, homogenous, and heterogenous bone and presented striking roentgenographic evidence to support his claim of bone regeneration. He advocated preliminary curettage of the root surface and soft tissue wall, as well as immobilization of the involved teeth. He was the first to advocate grafting small fragments of autogenous and homogenous bone to minimize the possibility of sequestration and also routine antibiotic coverage to avoid postsurgical infection.

Linghorne and O'Connell[15], in 1951, published the results of their studies on the repair of periodontal tissues in dogs. Utilizing surgically created periodontal osseous defects, they employed autogenous cortical and cancellous bone grafts along with fragments of dentin and cementum. By histologic comparison of their findings with that of control defects in which no grafts were placed, they found a significant increase in bone and cementum repair in the grafted areas. Again, it was concluded that the presence of calcified tissues appeared to be a factor for the differentiation of osteoblasts and cementoblasts. They demonstrated that this differentiation occurred only in the regions where calcified tissues were being resorbed and, therefore, felt that it may well be the presence of resorbing calcified tissue that provides the stimulus for the differentiation of these cells. Also, because no essential difference was observed between the grafts of bone and those of tooth structures they suggested that the osteogenic effect of grafts is due less to their cellular content than to the calcified intercellular material.

Linghorn described the same sequence of repair as that reported by Beube[9]. Following the organization of granulation tissue, resorption of the grafted particles began before new trabeculae were formed. They demonstrated that grafts seemed to encourage osteogenesis by stimulating the growth of new trabeculae and by acting as new islands of ossification. They also concluded from their study that the origin of the osteoblasts and cementoblasts was probably the undifferentiated mesenchymal cells emanating from the bony margins of the wound, rather than from the connective tissue of the gingiva.

The evidence of bone regeneration utilizing grafted calcified tissue has motivated researchers in the field of periodontics to graft various other mineralized substances. Attempts to graft "anorganic bone" (ethylenediamine extracted bone)[16], plaster of Paris[17], os purum[18], and cartilage[19], have demonstrated that dissimilar materials apparently have the capacity to stimulate some osteogenic response in alveolar bone. None of these materials, however, can be used consistently in periodontics with acceptable results.

Levander[20], in 1940, had grafted bone marrow only into the soft tissue of rabbits. New bone was formed in the soft tissue, although morphological analysis of the tissue demonstrated that the grafted marrow cells had died. He maintained it was highly unlikely that bone fragments played an important role in the formation of new bone or that any of the hematopoietic cells contributed directly to bone formation. Instead Levander felt that the implanted necrosing marrow liberated an inductive substance which stimulated the undifferentiated mesenchymal cells of the recipient site to differentiate into osteoblasts.

Further research by Goldhaber[21] on the problem of bone induction implies the presence of an osteogenic inducing substance. Allogeneic neonatal skull bone was implanted in the subcutaneous tissue of immunized mice, both, within a millipore diffusion chamber and free. New vital bone free of any homograft reaction was found on the host side of the filter, while the control bone implanted freely was found to be necrosed, surrounded by inflammatory cells, with no evidence of new bone formation. The formation of new bone found

on the host side of the diffusion chambers certainly
lends credence to the existence of a cell-free inducing
system.

It has been shown by many researchers, therefore,
that bone grafts will form bone in a variety of
heterotopic sites in mammals. Subsequently, the induction
powers of these grafts became a major area of research,
with the origin of the osteoblasts the main subject
of interest. The osteoblasts within the graft have
three possible origins; they may arise from pre-existing
osteoblasts[22], from the endosteum lining the marrow
cavities[23], and from perivascular undifferentiated
mesenchymal cells[24]. These three sources, of course,
may also act concomitantly[24].

Burwell[25] presented evidence, in 1964, that a
specific stimulus to osteoblastic differentiation is
present within bone marrow and that the necrosis of
marrow will liberate this stimulus. He felt, then,
that marrow transplanted to a heterotopic site produces
bone because osteogenic substances liberated from its
necrotic part induces osteoblastic differentiation
in primitive cells derived from its surviving portions.

He stated further, that if osteogenic inductors are present in marrow, the generally accepted place for their residence would be the cells lining the medullary sinusoids or the reticular cells.

Cushing[26], in his excellent review on the potential for induction of osteogenesis of autogenous red marrow grafts noted that, although such material does stimulate bone formation, we do not yet understand the mechanisms involved despite numerous studies. He concluded, however, that the great potential of this tissue to form bone appears to be due to the availability of source cells lining the vascular sinusoids which have the propensity to differentiate into osteoblasts. He added that this differentiation occurs as a result of an inductive signal seemingly initiated by products of necrosing marrow, and that similarly, necrosing bone will perform the same function.

McLean and Urist[27] stated that recent observations demonstrate, more conclusively than ever before, that the proteins of the matrix of bone and dentin contain the precursor of the inducing substance. They demonstrated that when bone or dentin is decalcified,

lyophilized, and implanted in the belly of a muscle, a bone-induction system is set up over a period of three weeks. All the available interstitial spaces, old vascular channels, or dentinal tubules are repopulated with lymphocytes, plasma cells, wandering histiocytes, and mesenchymal cells derived from migratory cells of the circulating blood. Later, the surfaces are covered with ingrowing capillary sprouts, fibrous connective tissue, and giant cells. The interaction of mesodermal cells and mesodermal derivatives in excavation chambers in old matrix during the process of resorption induces differentiation of preosteoblasts, osteoblasts, and new bone. The sinusoidal source cells that Cushing referred to, therefore, are the same undifferentiated mesenchymal cells found along blood vessels and capillaries in all tissues. The real value of a graft, then may not necessarily be the survival of its own cell population but instead, its ability to release a certain inducing substance capable of causing the differentiation of a primitive reticular cell derived from adjacent host tissue into an active osteoblast.

Nabers and O'Leary[28][29], in a clinical study

in 1965, introduced the concept of utilizing autogenous bone chips removed during routine osteoplasty and osteoectomy procedures as a graft material for periodontal osseous defects. Their study was not designed as a controlled research endeavor, however, based on clinical and radiographic evidence they demonstrated osseous regeneration in all eight cases attempted. The authors concluded that not only is the graft material readily available and compatible with the receiving tissues, but in all probability still retains some of its vitality at the time of implantation.

Stimulated by the work of Nabers and O'Leary, Robinson[30], in 1969, first described his "osseous coagulum" technique in which autogenous cortical bone chips mixed with blood were used as an implant material. He based his apparent clinical success on two assumptions: 1) the smaller the particle size of the donor bone, the more certain are its resorption and replacement; and 2) mineralized fragments can induce osteogenesis as other investigators have reported.

Rivault[31], in 1969, and Coverly[32], in 1972, presented sequential histologic studies of the healing

phenomena in periodontal osseous defects corrected by
the osseous coagulum precedure. They both demonstrated
conclusively that a more rapid osseous repair occurred
in the defect sites receiving the graft than in control
defects in which no coagulum had been placed. Both
studies noted that the graft material undergoes necrosis
before osteogenesis begins, but that at ten to fourteen
days osteogenesis appears on the grafted particles as
well as the osseous walls of the defect. These studies
also demonstrated, both clinically and histologically,
that chronic periodontitis can be successfully
simulated in rhesus monkeys by the method they employed.
After surgically preparing an osseous defect, they
placed a wooden irritant into the defect for a period
of seven days. This served as a chronic irritant
while also allowing the ingress of bacteria and bacterial
toxins. In this way osseous defects can be created
with a marked degree of similarity to one another,
subsequently rendered into chronic lesions for healing
studies, and serve as excellent experimental models in
the study of surgical osseous therapy.

Rosenberg[33] has recently demonstrated successful

results using free osseous tissue autografts of cancellous bone and marrow obtained from intraoral sources, and implanted in human periodontal osseous defects. The donor sites most commonly employed were the mandibular retromolar region, the maxillary tuberosity area, edentulous ridges, and buccal ledges and plates. The author presented no histologic evidence, but based his observations and clinical impressions on photographs, radiographs, and probing of over four-hundred periodontal osseous lesions managed with free osseous tissue autografts. Although the results offered are certainly promising, the author found one of the limitations of this implant procedure to be the inability to obtain adequate amounts of implant material to fill multiple and deeper defects.

Schallhorn[1][2], however, reported that the iliac crest served as a source of autogenous cancellous bone and marrow that is not only more accessible but is capable of supplying an unlimited amount of implant material. Also, while favorable results have been produced with various types of implants, there is growing evidence that autogenous hematopoietic marrow

in cancellous bone is presently the most optimal material available for bone grafting procedures[25][34][35][36][37][38][39][40][41][26][42]. Schallhorn therefore utilized fresh autogenous iliac crest material for implantation into interproximal periodontal osseous defects, and his five month re-entry procedure demonstrated that the bony craters were completely filled with new osseous tissue. This study was limited, however, in that it was only a case report, and also because it lacked histologic evaluation.

Investigations utilizing autogenous bone implants preserved by freezing techniques have also been reported. The preservation of the graft material is discussed in the following section of this review. DeBruzer and Kabisch[43] had reported in 1955 that fresh autogenous transplants of periosteum, cortical bone, and marrow were a better source of osteogenic material than either frozen autografts, fresh homografts, or frozen homografts. Boyne and Yeager[39], however, reported conflicting findings in 1969. Utilizing programmed freezing techniques to preserve autogenous bone marrow, they found that following implantation the frozen marrow both stimulated and supported osseous proliferation. Furthermore,

their study showed no difference between the osteogenic
potential of fresh or frozen bone marrow. Although
these studies did not involve autogenous bone grafts
placed into periodontal osseous defects, the latter
emphasized the feasibility of using properly preserved
tissue for such defects in clinical patients.

Subsequently, in 1970, Schallhorn, Hiatt, and Boyce[4]
reported their findings after utilizing autogenous
marrow and cancellous bone from the ilium, to correct
a total of 182 periodontal osseous defects in 52 patients.
Although some fresh iliac transplants were used, the
majority of the transplants were preserved by freezing
and stored until ready for implantation for the sake of
convenience. Following thorough root planing and complete
debridement of the osseous defects, which were exposed
by full mucoperiosteal flaps, the graft material was
placed snugly into the defects using an overfill
approach; the flaps were subsequently replaced for
complete coverage of the implant. Prophylactic antibiotics
were used in all cases. This well controlled study,
which utilized osseous defects normally not amenable
to "reattachment procedures", found that all of the

two-wall osseous defects exhibited complete fill to the coronal margin of the existing bony walls, and many cases demonstrated bone regeneration coronal to the existing bony walls. The study also found that, to a lesser degree, furcation and one-wall osseous defects were completely or partially corrected. These findings, which were based on clinical probings, radiographs, and re-entry surgical procedures, constantly observed early closing of the pre-existing periodontal pockets, but more importantly they demonstrated the potential of iliac transplants to gain crestal apposition of bone beyond the preoperative coronal bony margins. The authors attributed this apparent inductive effect of the implant at a site removed from the immediate area of implantation (i.e. 1-3mm. more coronally) to the ensuing cellular breakdown and release of some inducing substance which may cause osteoblastic differentiation from surrounding cellular elements. Again, this hypothesis is in accord with the one promulgated by Burwell[25]. They concluded that the utilization of iliac transplants in a typical dental office environment is certainly feasible; however, until further histologic

material becomes available we must remain guarded as to the nature of repair of implant cases.

The mechanism of repair following implantation therapy is little understood, although several theories have been suggested, and so remains a fertile area for further research. By investigating the repair phenomena at the histologic level of both fresh and frozen autogenous iliac grafts utilized in chronic periodontal osseous defects, our study should provide additional information in regard to their healing and clinical application.

B. TISSUE PRESERVATION

Smith, Polge, and Parkes[44][45] classically described a freezing technique that has since been utilized, with slight modification, to preserve a wide variety of mammalian tissues. Using a slow freezing technique with glycerol as a cryoprotective agent they were successful in preserving bull spermatozoa at -79^0C. After thawing at $+40^0$C. the spermatozoa were not only motile but possessed full functional capacity and unaltered genetic qualities. Smith[46] asserted that, with the aid of glycerol, a wide variety of living cells and tissues

could be stored for long periods at low temperatures for future use in experimental, diagnostic, or therapeutic medicine.

Pegg[47], in 1964, elaborated on a controlled freezing technique which he had previous success with[48][49]. Glycerol (15%) was again utilized as the cryoprotective agent in a slow freeze, rapid thaw technique, and his _in vitro_ and _in vivo_ findings provided conclusive proof that this method of human marrow preservation was an effective one. Pegg noted however, because he had found cellular deterioration under light microscopy during storage (-79^0C.), that long storage periods may require lower storage temperatures and possibly the use of dimethyl sulfoxide (DMSO) for cryopreservation.

Subsequently, Malinin _et al_[50] demonstrated that glycerolized human bone marrow cells stored in dry ice (-79^0C.) were almost entirely destroyed after 3 years. Virtually all cells showed nuclear vacuolation, pyknosis, and karyolysis. By comparison, bone marrow cells with DMSO in liquid nitrogen (-196^0C.) were largely intact after 3 year storage.

Recent work by Rubinstein and Trobaugh[51] describes

a technique for the preservation of hematopoetic tissue,
with an ultrastructural description of the frozen and
thawed tissues. Their study demonstrated the presence
of "presumptive stem cells" in a suspension of bone
marrow cells, frozen to -196^0C., that exhibited a repopu-
lation potential similar to that of fresh marrow.

Boyne and Yeager[39], in 1969, reported on the osteo-
genic potential of frozen autogenous marrow compared to
similarly procured fresh autogenous marrow. Their pro-
grammed freezing technique employed liquid nitrogen stor-
age (-196^0C.) with DMSO as the cryoprotective agent.
Following mandibular implantation it was found that the
frozen marrow possessed the ability to stimulate and sup-
port osseous proliferation on the host bone surface.
Furthermore, no clinical difference was found between the
osteogenic potential of frozen marrow and that of freshly
obtained marrow tissue.

The ultrastructure of bone marrow prior to and after
the programmed freezing technique described by Boyne and
Yeager[39] was studied by Barkin and Newman[52]. Their
findings with electron microscopy revealed various
degrees of cellular destruction, however reticular and

blast cells were observed to be viable (on the basis of morphologic criteria) and therefore support this technique of programmed freezing.

Kromer[53], in 1956, reported the first utilization of frozen bone in periodontal therapy. He had treated 13 cases of intrabony pockets with deep frozen bone, kept in 1:1000 merthiolate solution, and was successful in 8 cases. Although the author did not describe the freezing and storage technique in detail, his work remains classic in the field of periodontal bone grafts.

Schallhorn, Hiatt, and Boyce[1][4] have demonstrated the feasibility of using frozen autogenous hip marrow in cancellous bone for the correction of periodontal osseous defects. The technique employed was similar to that described by Pegg[47], utilizing 25% glycerol in minimum essential media and a slow freeze technique. This was a crude modification of the basic cooling curve described by Smith and Polge[45] and consisted of allowing the iliac crest biopsy core to reach equilibrium in the glycerol solution at room temperature, placing the vial in a refrigerator for several hours, and finally storing it in a low temperature freezer (-79^0C.) The results of the study

confirmed that frozen iliac cancellous bone and marrow implants have future potential. The authors concluded that the apparent success of the graft lies in the proper preservation of its cellular structure until it is implanted.

C. CRYOINJURY

Tissue preservation at low temperatures has been the object of considerable research during the past two decades. Since the introduction of glycerol[44] and dimethyl sulfoxide (DMSO)[54] as cryoprotective agents, the feasibility of prolonged preservation of viable cell suspensions by freezing has been repeatedly demonstrated. Progress in this area however, has been hindered by limited knowledge both of the mechanism of damage during freeze-preservation and of the mechanism of the cryoprotection[55].

Retention of tissue structure and function has been shown to be dependent on the rate and manner of freezing and thawing, as well as on the concentration and nature of the protective agent[56]. Factors implicated in cell damage include: intracellular ice crystal formation[57], toxic effects from pretreatment with cryoprotective

agents[58][59], increases of intracellular electrolyte
concentration[60][55], the effect of osmotic stress on
membranes[61], different vapor pressures resulting in
water desorption and redistribution[62], and direct
cryoinjury by the catabolic action of lysosomal enzymes[63].

Steere[64] used freeze-etching to study, by direct
visualization, the presence of ice crystals in bacteria,
intestinal epithelium of monkeys, red blood cells, and
heart tissue of dogs and mice. He felt this was the best
technique for studing the fine structure of freezing
damage to cells, and used photomicrographs to illustrate
membrane and organelle distruction by ice crystal formation.
Steere noted that increasing concentrations of glycerol
(up to 40%) resulted in decreasing amounts of ice crystal
formation. Mazur[65] has derived a quantitative expression
that has been used to predict the probability of the
formation of intracellular ice in several cell systems.

Sherman[58] utilized mouse skin, protected with 5%
and 15% concentrations of both DMSO and glycerol, to
conduct autograft experiments comparing the survival of
frozen-thawed transplants to unfrozen control transplants.
The findings demonstrated that the cryoprotective agents

could, themselves, cause cellular damage which contributed to the loss of several mouse skin transplants. In addition, the 5% solutions were found to be less toxic than the 15% solutions. Sherman asserted that the loss of transplants which occurred was due to exposure to glycerol and DMSO during pretreatment, and not to deleterious alterations induced by freezing and thawing.

More recently, Meryman[59] reported that glycerol produced an abrupt fall in the membrane resting potential of frog muscles without recovery following return to isotonic medium. He noted that penetrating cryoprotective agents require high concentrations to achieve protection, but at these concentrations they exhibit toxicity.

Barchi, et al.[55], discussed the theory concerning the role of cell electrolyte concentration in mediating cellular damage during freezing. Exposure of cells to slow freezing procedures is known to induce cellular dehydration secondary to the formation of ice crystals in the extracellular areas. The immediate result of this process is an increase in intracellular electrolyte concentration and possible pH shifts due to crystallization of slightly soluble buffer salts. The change in pH and

electrolyte concentrations can induce the denaturation of lipid protein complexes[66], and is thought to damage the cell by interacting with intracellular lipoprotein membranes[57].

Farant, et al.[60], demonstrated that the use of glycerol as a cryoprotective agent decreased the amount of intracellular ice formation, resulting in a decreased build up in the concentration of electrolytes during freezing. They felt that the direct action of high concentrations of electrolytes and other solutes, and osmotic swelling during thawing were two possible factors that would damage cells during slow freezing.

Meryman[56] had proposed that it was not the absolute concentration of electrolytes which was responsible for cellular damage, but rather the osmotic pressure produced by increasing the concentration of nonpenetrating extracellular solutes. He later studied freezing injury to fresh human erythrocytes[61] and concluded that this increasing stress is related to the osmotic loss of cell liquid phase and an associated cell volume reduction. At some limiting volume, the stress becomes sufficient to cause a sudden loss of membrane integrity and an influx

of extracellular solution. The limiting stress appears to be unrelated to the absolute concentration or identity of the extracellular solute (provided it is nontoxic), and unrelated to the character of the intracellular solvent or the concentration of intracellular solutes. It is related to the proportion of cell liquid phase removed. There is evidence for a progressive development of stress up to the critical level. There is also evidence that the loss of permeability characteristics, when it occurs, is basically reversible but that immediately subsequent events can render it irreversible.

Meryman[61] stated that hypertonic stress is undoubtedly the principle cause of freezing injury in living cells when ice formation is extracellular. He favors the hypothesis that the stress of hypertonic suspension results from a resistance of the cell to unrestrained volume reduction, that the failure of the cell to shrink freely results in an osmotic gradient across the cell membrane, with an inward osmotic gradient balanced by the mechanical resistance of the cell. In other words, he proposed a "minimum cell volume" hypothesis in which membrane damage results from osmotic stress

imposed on the cell membrane when the cell volume is decreased beyond a minimum tolerable size[67].

Litvan[62] has proposed a new hypothesis to explain the mechanism of cryoinjury that does not deny the importance of other proposed mechanisms, but rather suggests the primary causes leading to the creation of conditions under which other mechanisms may become injurious. His hypothesis is based on the assumption that intracellular water remains liquid below 0^{0}C. and therefore its vapor pressure (vp) is greater than that of ice. On cooling, extracellular ice forms and a vp difference is created which increases with decreasing temperatures. A spontaneous process of water desorption (gradual release of bound water) and subsequent redistribution is prompted by the nonequilibrium state. Injury is the result of dehydration at slow cooling rates and membrane rupture at rapid rates. Penetrating cryoprotective agents, therefore, improve survival rate by diminishing the migration rate and vp difference. Litvan noted that this hypothesis is compatible with the most important modes of freezing injury already proposed, but suggested it was the basic phenomenon from which the others resulted.

According to Litvan[62], the same considerations also
apply to the process of thawing, which is perhaps even
more injurious than freezing. On warming (2-3 min.,
$37-40^0C$.), there may be an incomplete disappearance of ice
crystals, and during this period the unfrozen cells can
warm to well above 0^0C. Under these conditions large
deleterious vp differences may exist, aggravated by the
relatively high temperature at which water diffusion is
rapid.

Persidsky[63] studied the effects of cryopreservation
on bone marrow cells from rats to determine if lysosomal
activation could be involved as a possible mechanism in
cryoinjury. By comparing the degree of cell viability
between frozen bone marrow cells treated with trypan blue
(lysosome enzyme inhibitor) and frozen untreated bone
marrow cells (control) it was found that the treated bone
marrow cells exhibited a much greater recovery after
cryopreservation. Persidsky felt that this improvement
in cell preservation resulted directly from the inhibition
of lysosomal enzymes by trypan blue, and prevention
thereby of their catabolic activity against cells. He
concluded that activation of lysosomal enzymes by freezing

and thawing could be a primary cause of cell damage.
Consequently, cryoinjury of cells, particularly of those
with high lysosomal content, may be caused directly
by the catabolic action of lysosomal hydrolases.

CHAPTER III

MATERIALS AND METHODS

A. EXPERIMENTAL DESIGN

Three adult female rhesus monkeys (Macaca mulatta)
were utilized as experimental models in this study. Through-
out the experiment they maintained the physical parameters
recorded at their arrival, and appeared to remain in good
health. Each animal demonstrated a full complement of
teeth with similar periodontal findings. Calculus deposits
as well as plaque and food debris were found, and the
teeth showed varying degrees of attrition. All present-
ed with a slight marginal chronic gingivitis. The papil-
lae were slightly erythematous, while the gingiva was firm
in consistency and pink in color. The sulcus depth on the
buccal and interproximal surfaces varied between one and
three millimeters (Figure 1).

The two maxillary quadrants in each of two of the animals were utilized as experimental sites, while the two maxillary and two mandibular quadrants of the third animal were used as experimental sites. Thus, in total six maxillary quadrants and two mandibular quadrants were included in the study. Each one of these quadrants served as a site for two two-walled surgically produced osseous defects. One was corrected by a fresh and one was corrected by a frozen autogenous iliac crest transplant. A third two-walled defect was created in three of these quadrants to serve as a control, and its preparation or correction (i.e. curettage only, no transplant) was accomplished immediately following the preparation or correction of the analogous graft defects within the same quadrant.

A predetermined schedule was followed so that at sacrifice both a fresh transplant and a frozen transplant was obtained 0, 3, 7, 14, 21, 28, 42, and 56 days postoperatively. Each time interval, therefore, provided an experimental defect that had been corrected by a fresh graft and a frozen graft. Control defects were obtained at 7, 21, and 42 days postoperatively.

One monkey, which weighed 4.4 kilograms, provided intraoral graft specimens of 0 and 14 days postoperatively. Another, weighing 4.2 kilograms, provided intraoral graft specimens of 3 and 28 days postoperatively. The third monkey, which weighed 5.2 kilograms, provided intraoral graft specimens of 7, 21, 42, and 56 days postoperatively. The third animal also provided the control specimens.

Ten minutes prior to the time of surgery the monkey received an intramuscular injection of 10mg. Sernylan[*] for sedation. Occasionally during the procedures an additional 5mg. Sernylan was given intramuscularly for more complete sedation. When the animal appeared sedated in the cage, it was removed and placed on the surgical table, and the head was positioned in such a manner that respiration would not be obstructed but adequate accessibility was afforded.

Throughout the course of each procedure strict conditions of asepsis were maintained, pertinent clinical observations were recorded, and Kodachrome slides were obtained.

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*Parke, Davis, and Co., Detroit, Michigan

B. GENERAL PREPARATION

The preparation of the osseous defects and later their correction required that full mucoperiosteal flaps be reflected to expose the underlying alveolar bone. An intrasulcular incision to the alveolar crest, from the mesial aspect of the second molar to the mesial aspect of the cuspid, was carefully performed with a #15 Bard-Parker blade. The incision was scalloped such that all interdental papillae were preserved in order to provide primary closure of the surgical wound. A small periosteal elevator was then used to reflect the buccal full mucoperiosteal flap, carefully avoiding any inadvertant laceration of this tissue. Small curved tissue scissors were then utilized for removing tissue tags from the inner surface of the flap. Gracey curettes were then employed to remove all interproximal granulation tissue and to thoroughly plane the exposed root surfaces.

C. PREPARATION OF THE DEFECTS

The sites chosen for the osseous defects to be prepared were the interproximal osseous septae mesial and distal to the second bicuspid. These areas were easily observed,

presented little or no access problem, provided a sufficient amount of interproximal bone in which the osseous defects could be prepared, and were protected postoperatively to some degree by interproximal contacts between the adjacent teeth. On two occasions (3 and 42 day specimens), because of the close approximation of the roots of the first and second bicuspids, the interproximal osseous septae mesial and distal to the first molar were chosen as the defect sites. On one occasion (56 day specimen), again because of an inadequate amount of interproximal bone present, the defect sites selected were the mesial of the first bicuspid and the distal of the first molar. The sites chosen for the control defects were the interproximal osseous structures distal to the first molar (7 and 21 day specimens) and distal to the second molar (42 day specimen).

Two-walled osseous defects were then created in the selected sites. A #2 round bur in a slow speed dental handpiece was used to penetrate the interproximal alveolar crest apically to a depth of 3mm. This depth was carefully obtained by repeated measurements with a calibrated periodontometer. The apical penetration of the bur was performed immediately adjacent to the root surface of one of the

adjacent teeth resulting in either a mesial wall of cementum and a distal wall of bone, or vice versa. The buccal cortical plate was then removed to the same depth with the rotary cutting instrument. The two-walled osseous defect was therefore characterized by either a mesial wall of cementum with distal and lingual walls of bone, or a distal wall of cementum with mesial and lingual walls of bone (Figure 2). All osseous removal was performed with isotonic saline irrigation in order to minimize the damage incurred, by the surrounding osseous tissue, from the increased temperature produced by the rotating bur.

D. INTRODUCTION OF CHRONIC IRRITANT

A wooden toothpick approximately 6mm. in length was introduced into each defect to serve as a chronic irritant. The irritant extended from the apical end of the defect to a level slightly below the occlusal table of the adjacent teeth. It therefore served as a source of chronic irritation to the osseous defect while also allowing the ingress of bacteria and bacterial toxins. In this manner, osseous lesions such as those seen in human periodontitis would be simulated.

The buccal mucoperiosteal flap was then reapproximated to its original position and interrupted interproximal sutures (4-0 silk) were used to secure the tissue at this level. By utilizing this type of suture the wooden irritants were stabilized in their position, and the interdental papillae were reapposed directly over the interproximal osseous defects. The loose ends of the suture material were cut very short (Figure 3).

E. POSTOPERATIVE CARE

Following each surgical procedure the monkey received antibiotics before being returned to the cage. An intramuscular injection of 200,000 units Distrycillin A.S.[*] was given followed by an intramuscular injection of 400,000 units Bicillin[**]. It was specified that the animal receive no food for the first 24 hours postsurgically, a soft diet for the following 3 days, and a normal diet thereafter.

F. REMOVAL OF IRRITANT

Seven days following the creation of the osseous defects, the animal was sedated with an intramuscular

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*E. R. Squibb and Sons
**Wyeth

injection of 10mg. Sernylan and the sutures and wooden irritants were carefully removed. All clinical observations were recorded (Figure 4).

G. PROCUREMENT OF GRAFT TISSUE

The technique used to obtain iliac crest graft material is the method described by Dragoo and Irwin in 1972[68]. Seven days prior to the scheduled time for the correction of the defects, iliac crest material was obtained for preservation by controlled freezing. Following sedation the operative site overlying the anterior superior iliac spine and crest was shaved, scrubbed with surgical soap, and Betadine antiseptic germicide* was applied to the skin. The area was then isolated with sterile towels.

The desired landmarks were located by finger palpation, and an incision to bone was made with a #15 Bard-Parker blade, beginning at the anterior superior iliac spine and continued dorsally over the iliac crest for approximately 5mm. Small curved tissue scissors were used to dissect the surrounding tissue, leaving the cortical bone of the iliac crest exposed (Figure 6).

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*Purdue Frederick Co.

A Turkel Trephine needle* was then utilized to obtain multiple cores of cancellous bone from the iliac crest. The outer needle was carefully aligned to follow the medial-posterior inclination of the ilium, and was then rotated slightly to penetrate the periosteum and cortical bone. The inner trephine needle was then inserted into the outer needle, and with slight pressure and rotation it penetrated the remaining cortical bone and entered cancellous bone. The inner needle, which then contained a core of bone, was slowly withdrawn while the outer needle remained embedded in the cortical bone of the anterior crest. An inner needle stylet was inserted into the inner needle trephine to push out the core of cancellous bone into a small freezing vial containing Hank's balanced solution with 12% glycerol (Figures 7 and 8). The technique was repeated until the desired number of cores had been obtained, usually 2 or 3, and the outer needle was then withdrawn. Silk suture material (4-0) was used to close the wound, and Betadine solution was again applied to the surface. The same postoperative antibiotics were administered as described previously, and the animal was returned to the cage. In

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*Turkel Instrument Co., Detroit, Michigan

all cases the hip sutures were removed seven days following
their placement.

The vials containing the cores of osseous tissue in
Hank's solution and glycerol were immediately placed in an
insulated container, packed with ice (0^0 C.), and transport-
ed to Rush-Presbyterian St. Luke's Medical Center, Chicago,
for controlled freezing. These specimens would later serve
as the frozen autogenous bone graft material. At the time
of defect correction, the fresh autogenous bone graft
material was obtained from the contralateral iliac crest
utilizing the same procedure.

H. FREEZING AND THAWING THE TISSUE

Controlled freezing of the specimens was accomplished
by placing the freezing vials into a Linde BF-4-1 freezing
chamber* and flooding the cooling chamber with vaporized
nitrogen. The tissue was frozen to -12^0 C. at a rate of
3^0 C. per minute. Immediately following the release of
the latent heat of fusion, the tissue was frozen to -100^0 C.
at a rate of 2^0 C. per minute. The freezing vials were then
rapidly transferred to a liquid nitrogen storage tank

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*Honeywell Lab. Products

(-196^0 C.) where they were maintained for 7 days.

One hour prior to the surgical correction of the osseous defects, the frozen vials were placed in an insulated container, packed with frozen CO_2 (-79^0 C.), and transported to Loyola University. Shortly thereafter, when the surgical procedure had progressed to the point where the bone graft was indicated, the vials were immersed in a 40^0 C. water bath and thawed for 2 minutes.

I. DEGLYCEROLIZATION

After thawing, the vials were opened and the contents poured into separate sterile beakers. Immediately thereafter, at 2 minute intervals, the following amounts of sterile 35% glucose and 6% Dextran solution were added to each beaker; 0.5cc. glucose; 0.75cc. Dextran; 2.50cc. Dextran; 3.00cc. Dextran; and 2.25cc. Dextran.

The cancellous bone cores, following deglycerolization, were immediately utilized in the bone graft procedure.

J. CORRECTION OF THE DEFECTS

Exactly 30 days following the preparation of the two-walled osseous defects they were corrected with a fresh or

a frozen autogenous bone graft, or by curettage (Figure 5).

Fresh cancellous iliac bone had to be obtained just prior to the intraoral surgery. Procuring the fresh bone cores was achieved in an identical manner as that described in section G. of this chapter. The contralateral hip served as the donor site however, and the fresh bone cores obtained were placed in a beaker containing sterile isotonic saline solution as a holding media.

The same surgical procedures and considerations outlined previously were used to expose the osseous defects for surgical correction. A buccal mucoperiosteal flap was reflected, the root surfaces of the teeth thoroughly planed, and the granulation tissue in the defects and surrounding areas meticulously removed by curettage (Figure 9).

The frozen bone cores were then thawed and deglycerolized according to the protocol described. Both fresh and frozen cancellous bone cores were therefore ready for intraoral grafting. A #15 Bard-Parker blade was used to slice the cancellous bone cores into small sections (approximately 0.5mm x 0.5mm.), for this would facilitate placement of the graft material and assure a more complete filling of the defects.

After the surgically prepared osseous defects were again inspected to assure complete removal of granulation tissue, the iliac cancellous bone was placed into the defects. A standardized procedure followed for all grafts was the placement of fresh bone material into the mesial defect, and frozen bone material into the distal defect. The cancellous bone was firmly packed into the defects until they were filled, but no attempt was made to overfill them (Figure 10). The periodic use of gauze squares removed the excess hemorrhage and assured a more complete fill.

The control defects underwent the same procedure as the graft recipient sites, however after they were curetted of all granulation tissue they received no graft.

Following the surgical correction of the defects the mucoperiosteal flap was reapposed, and firmly fixed over the osseous lesions with interproximal interrupted sutures of 4-0 silk (Figure 11). The same postoperative antibiotics and diet instructions as previously described were given.

Seven days after the correction of the defects the animal was sedated and the sutures removed (Figure 12). The monkey was then maintained until the next procedure, or until the scheduled sacrifice (Figure 13).

K. COLLECTION OF SPECIMENS

Following sedation the animal was given an intraperito-
neal injection of a lethal dose of Totaltox[*]. Block sec-
tions that included the experimental sites were then
obtained. After cutting each section free from the sur-
rounding structures with an electric Stryker saw, it was
washed with water, tagged for identification, and placed
into a jar containing 10% formalin for fixation.

L. PREPARATION FOR HISTOLOGIC EXAMINATION

Following adequate fixation (2 weeks) each specimen
was decalcified in formic acid and sodium citrate (4 weeks),
trimmed, embedded in paraffin, sectioned at 7 microns in
a transverse buccolingual plane, and stained with hema-
toxylin and eosin (H and E). The slides from each exper-
imental site were studied, and a representative histologic
section was selected for detailed histologic analysis.

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*Chicago Veterinary Supply, Chicago

CHAPTER IV

FINDINGS

A. CLINICAL OBSERVATIONS

The gingival tissues of the experimental animals were
characterized by a slight marginal chronic gingivitis
at the initiation of the study (Figure 1). The papillae
were slightly erythematous and the sulcus depth varied
between one and three millimeters. Seven days following
the creation of the osseous defects and placement of the
irritants the gingiva demonstrated marked inflammation.
The marginal and papillary gingiva was edematous,
erythematous, and displayed easy bleeding upon manipulation
(Figure 4). Thirty days postoperatively, at the time
of defect correction a decreased amount of inflammation,
compared to 7 days postoperatively, was noted. The
marginal and papillary gingiva, however, were still

characterized by edema, erythema, boggy consistency, and easy bleeding upon probing. The gingival sulci at the defect sites measured five millimeters in depth (Figure 5).

The osseous topography also demonstrated notable changes at the time of defect correction. Additional bone resorption was consistently observed, when compared to the original osseous defect, and the characteristic two-walled defects (Figure 2) demonstrated loss of adjacent buccal cortical plate mesially and distally, resulting in a defect with a V-shaped appearance (Figure 9).

Seven days following the correction of the defects the marginal gingiva was rolled in appearance and the papillae were edematous and slightly boggy in consistency. Adjacent areas were more firm and pink however, indicative of the normal healing process (Figure 12). From this time interval to the completion of the experiment the health of the gingival tissues continued to improve in appearance as the healing process continued (Figure 13).

B. HISTOLOGIC OBSERVATIONS

1. INTRODUCTION

Epithelial changes throughout the experiment were

generally inflammatory in nature, reflecting the response of this tissue to the surgical insult and to the chronic gingivitis that was present.

Connective tissue changes were also chiefly inflammatory in nature, characterized by edema, vascular dilation, inflammatory cell infiltration, with progressive maturation of the granulation tissue and organization of the gingival fibers.

Histologic changes of the alveolar bone were most obvious adjacent to the defect sites and the surface of the adjacent cortical plate.

The defect sites with autogenous bone grafts demonstrated remarkable histologic changes as the repair process occurred.

2. FRESH TWO-WALLED GRAFT

    a. EPITHELIUM

        0 Day

            Keratinized stratified squamous epithelium demonstrates slight intercellular edema and acanthosis.

        3 Day

            The block section representing the 3 day fresh autogenous bone graft was unfortunately lost.

7 Day (Figure 18)

Keratinized stratified squamous epithelium demonstrates slight intercellular edema, acanthosis, dyskeratosis, and hyperplasia. There is an apical migration of epithelium along the inner aspect of the flap, and in places the proliferating strand of epithelial cells is in contact with fragments of the graft. A polymorphonuclear leukocytic infiltration is seen near the incision site.

14 Day (Figure 21)

Keratinized stratified squamous epithelium is present with slight intercellular edema and a polymorphonuclear leukocytic infiltration near the site of incision. Further apical migration of epithelium is present which is in contact with the young granulation tissue. The proliferating epithelium surrounds some of the exfoliating graft fragments.

21 Days

Keratinized stratified squamous epithelium with slight intercellular edema and a decreased

polymorphonuclear leukocytic infiltration near
the incision site is present. Epithelial
proliferation surrounds chronic inflammatory tissue
within the free gingiva, and local areas of
increased intercellular edema are present within
this proliferated epithelium.

28, 42, and 56 Days (Figures 34 and 36)

Keratinized stratified squamous epithelium
with only slight intercellular edema is present.
The epithelium is closely adapted to the tooth
surface and presents a normal epithelial attach-
ment.

b. CONNECTIVE TISSUE

0 Day (Figures 14 and 15)

The full mucoperiosteal flap is completely
detached. The connective tissue is unremarkable
in appearance with slight vascular dilatation
present.

7 Day (Figure 18)

The connective tissue presents marked vascular
dilatation, interstitial edema, and a subacute
inflammatory infiltrate. Areas of collagen loss

are apparent where the ground substance appears amorphous. Fibroblasts are proliferating and show large nuclei and nucleoli. Young capillaries and fibroblasts are seen emanating from the connective tissue border to the adjacent graft area. The connective tissue is attached to the underlying cortical plate by a well organized clot.

14 Day (Figure 21)

The connective tissue presents interstitial edema, vascular dilatation, and loss of collagen fibers. The inflammatory infiltrate consists primarily of lymphocytes and plasma cells. Macrophages and proliferating fibroblasts are present, however the intensity of the inflammatory response at this time began to decrease slightly.

21 Day

The connective tissue presents only slight interstitial edema. Proliferating fibroblasts from the connective tissue into the healing graft site give an appearance of continuity between the connective tissue and the underlying osseous tissue. Collagen fibers demonstrate definite

organization. Vascular dilatation is present
and a chronic inflammatory response is seen
only in the most coronal part of the connective
tissue where the overlying epithelium is prolif-
erating. An inflammatory infiltrate of lymphocytes
and plasma cells is present in this coronal
portion.

28 Day (Figure 30)

Slight interstitial edema and vascular dila-
tation is present, and is similar to the 21 day
specimen, however the collagen fibers show very
good orientation. The inflammatory response
present is one typical of chronic gingivitis
coupled with the exfoliation of graft fragments.
The lamina propria in the sulcular region because
of these exfoliating fragments, demonstrates
increased interstitial edema, vascular dilatation,
loss of collagen, and a subacute inflammatory cell
infiltrate.

42 and 56 Day (Figures 34 and 36)

The flap wound is repaired and the histologic
observations are the typical inflammatory response

found in chronic gingivitis. Well organized gin-
gival fibers, particularly the circular group and
the alveolar-gingival group of the gingival
fiber apparatus, are well demonstrated. The lamina
propria of the sulcular and col area presents
slight interstitial edema, vascular dilatation,
loss of collagen, and a chronic inflammatory
cellular infiltrate.

c. ALVEOLAR BONE

0 Day

The alveolar bone adjacent to the defect shows
repair from the initial trauma of defect prepara-
tion. Basophilic reversal lines are seen indi-
cating recent osteogenic activity, osteocytes
are present in lacunae in the newly formed bone,
and osteoid is present. Marrow spaces adjacent
to the defect present increased vascularity and
fibrous tissue proliferation when compared to
distant marrow spaces, and demonstrate osteoblastic
and osteoclastic remodeling simultaneously.

7 Day (Figure 18)

The marrow spaces present increased fibroblas-
tic activity. Both osteoclastic and osteoblastic

activity is observed as bone remodeling proceeds.

14 Day

The alveolar wall of the defect presents increased fibrous tissue proliferation with new bone formation both toward the marrow spaces and on the surface. New trabeculae of bone are formed into the defect area which extend to the graft fragments. Osteoclastic activity is present along the defect wall.

21 Day

The alveolar bone shows apposition on the surface of the cortical plate and the crestal area. The marrow spaces are reduced in size because of new bone formation, and fewer osteoclasts are seen. Cementoid is seen on the dentinal surface of the tooth, however odontoclasts are also observed on the dentin coronal to the cementoid apposition.

28 Day

The histologic appearance of the aveolar bone is similar to the previous section but shows further reduction in the diameter of the marrow spaces.

The defect is characterized by osteoblastic activity with new trabeculae projecting into the graft area. Cementoid apposition is demonstrated on the injured dentinal surface of the tooth.

42 and 56 Days

Further osteoblastic activity is observed along the defect wall, and the alveolar crest and buccal surface show additional bone apposition and remodeling. Trabeculae of bone projecting from the walls of the defect are confluent with the remodeling graft fragments. Cellular cementum and cementoid apposition exist on the dentinal surfaces of the teeth, however the 42 day specimen also exhibits odontoclasts on the dentin surface.

d. FRESH CANCELLOUS BONE AND MARROW GRAFT

0 Day (Figures 14 and 15)

The graft material is seen in contact with the surrounding alveolar bone, periodontal ligament, tooth, and overlying flap. The grafted cancellous bone shows viable osteocytes within lacunae, with a highly cellular fibrous connective tissue surrounding the trabeculae. Some extravasated red blood cells are present.

7 Day (Figures 18 and 19)

The defect site receiving the graft presents a fibrinopurulent exudate at the coronal aspect containing numerous polymorphonuclear leukocytes and lymphocytes. A few graft fragments are being exfoliated. The graft fragments present empty lacunae and some fragments are surrounded by necrotic debris, primarily fragmented polymorphonuclear leukocytes and lymphocytes. The defect site is characterized by a proliferation of young fibrous connective tissue and new capillaries. Areas of fatty marrow are also seen. Some bone fragments are enveloped in proliferating fibroblasts and young capillaries. Osteoclastic activity can be seen on these fragments.

14 Day (Figures 21 and 22)

A well organized cellular fibrous connective tissue surrounds all graft fragments. Numerous young fibroblasts and proliferating capillaries are present. At the coronal aspect of the defect some exfoliating bone fragments are surrounded by proliferating epithelium and a chronic

inflammatory infiltrate. Osteoclasts are abundant in the graft area while at the same time osteo- blasts and osteoid tissue are seen, with new bone trabeculae formation. Devitalized graft fragments are surrounded by both bone resorption and apposi- tion on their periphery.

21 Day (Figures 26 and 27)

The repairing defect site demonstrates bone graft fragments scattered throughout the young fibrous connective tissue which still shows abundant capillary proliferation. Devitalized bone fragments are surrounded by osteoblasts and osteoid tissue, and a dense cellular infiltrate on the cortical bone surface resembles young periosteum. Although osteoclasts are present they are fewer in number. Cementoid is deposited on the dentin surface that faced the graft, how- ever odontoclasts were also present and active dentin resorption is demonstrated.

28 Day (Figure 30)

The graft site is filled with young trabeculae of bone among which more basophilic graft particles

are scattered. Active bone remodeling is occurring
but the number of osteoclasts has decreased. The
marrow spaces are filled with a very cellular
connective tissue. Young periosteum is present
on the surface of the repairing defect. The
coronal aspect of the defect demonstrates fragments
of exfoliating devitalized bone.

42 and 56 Day (Figures 32, 34, and 35)

The graft sites are filled with young
trabeculae of bone which are being actively
remodeled. The marrow spaces are rich in fibrous
tissue, and the walls of these spaces are lined
with fibroblastic appearing cells. Young
periosteum has formed on the surface of the
repairing graft. A new alveolar crest is present
which demonstrates active remodeling. Odontoclasts
are observed on the dentin surface adjacent to the
repairing defect, with active dentin resorption
occurring.

3. FROZEN TWO-WALLED GRAFT

a. EPITHELIUM

0 Day

Keratinized stratified squamous epithelium

demonstrats slight intercellular edema and acanthosis.

3 Day

Keratinized stratified squamous epithelium is seen demonstrating slight intercellular edema, acanthosis, dyskeratosis, and hyperplasis, as well as a polymorphonuclear leukocytic infiltration. The epithelium has migrated apically along the inner aspect of the flap separating it from the underlying fibrinopurulent exudate.

7 Day (Figure 20)

Keratinized stratified squamous epithelium demonstrating slight intercellular edema, acanthosis, and hyperplasia, can be seen. There is an apical migration of epithelium along the inner aspect of the flap, and in places the proliferating strand of epithelial cells is in contact with fragments of the bone graft. A polymorphonuclear leukocytic infiltrate is seen near the incision site.

14 Day (Figure 25)

Keratinized stratified squamous epithelium

with slight intercellular edema and a polymorpho-
nuclear leukocytic infiltration near the incision
site is present. Further apical migration of
epithelium is present which is in contact with
the young granulation tissue. The proliferation
surrounds some of the exfoliating graft fragments.

21 Day (Figure 28)

Keratinized stratified squamous epithelium
with intercellular edema and a decreased poly-
morphonuclear leukocytic infiltration near the
incision site is present. A strand of prolifer-
ating epithelium surrounded by chronic inflamma-
tory tissue extends apically into the connective
tissue to the level of the epithelial attachment.
Local areas of increased intercellular edema are
present within this strand of proliferated
epithelium.

28 Day

Keratinized stratified squamous epithelium
with slight intercellular edema is present. A
polymorphonuclear leukocytic infiltration is pre-
sent near the incision site to a lesser degree.

Proliferation and apical migration of the epithe-
lium is seen where it surrounds graft fragments
that are exfoliating from the defect site.

42 and 56 Day (Figure 37)

Keratinized stratified squamous epithelium
with only slight intercellular edema is present.
The epithelium is closely adapted to the tooth
surface and presents a normal epithelial attach-
ment.

b. CONNECTIVE TISSUE

0 Day

The full mucoperiosteal flap is completely
detached. The connective tissue is unremarkable in
appearance, and only slight vascular dilatation
is present.

3 Day

The connective tissue is characterized by
marked interstitial edema and vascular prolifera-
tion, collagen loss, and an acute inflammatory
infiltrate.

7 Day (Figure 20)

The connective tissue presents vascular

dilatation, interstitial edema, and subacute inflammatory infiltrate. Areas of collagen loss are apparent where the ground substance appears amorphous. Fibroblasts are proliferating and demonstrate large nuclei with single and double nucleoli. Young capillaries and fibroblasts are emanating from the connective tissue border to the adjacent graft area. There is attachment of the connective tissue to the underlying cortical plate by a well organized clot.

14 Day (Figure 23)

The connective tissue presents interstitial edema, vascular dilatation, and loss of collagen fibers. The inflammatory infiltrate consists primarily of lymphocytes and plasma cells, with an occasional polymorphonuclear leukocyte. Macrophages and proliferating fibroblasts are present, however the intensity of the inflammatory response began to decrease slightly.

21 Day (Figure 28)

The connective tissue presents only slight interstitial edema and appears in intimate contact

with the underlying graft particles and osseous
tissue because of the fibroblasts proliferating
from the connective tissue into the graft area.
Collagen fibers demonstrate definite organization.
Vascular dilatation is present and a chronic
inflammatory response is seen in the most coronal
part of the connective tissue where the overlying
epithelium is proliferating. An inflammatory
infiltrate of lymphocytes and plasma cells is
present in this coronal region.

<u>28 Day</u> (Figure 31)

Slight interstitial edema and vascular
dilatation is present, resembling the 21 day
specimen, and they show good orientation. The
inflammatory response present is one typical of
a chronic gingivitis coupled with the exfoliation
of graft fragments. The lamina propria in the
sulcular region, presumably due to these exfoliating
graft fragments, demonstrates increased interstitial
edema, vascular dilatation, loss of collagen, and a
subacute inflammatory cell infiltrate.

<u>42 and 56 Day</u> (Figures 33 and 37)

The connective tissue is repaired and the histologic observations are the typical inflammatory response found in chronic gingivitis. Well organized gingival fibers, particularly the circular group and the alveolar-gingival group of the gingival fiber apparatus, are evident. The lamina propria of the sulcular and col area presents slight interstitial edema, vascular dilatation, loss of collagen, and a subacute inflammatory cellular infiltrate. The 42 day specimen demonstrates devitalized graft fragments surrounded by an amorphous appearing fibrous connective tissue.

c. ALVEOLAR BONE

<u>0 Day</u>

The alveolar bone adjacent to the defect shows repair from the initial trauma of defect preparation. Basophilic reversal lines are seen indicating recent osteogenic activity, osteocytes are present in lacunae in the newly formed bone, and osteoid is present. Marrow spaces adjacent

to the defect present increased vascularity and
fibrous tissue proliferation when compared to
distant marrow spaces, and demonstrate osteoblastic
and osteoclastic remodeling simultaneously.

3 Day (Figure 17)

The alveolar bone is characterized by baso-
philic reversal lines indicating recent repair,
and the marrow spaces in close proximity to the
defect wall show an increase in the number of
fibroblasts and capillaries. The trabeculae
demonstrate both osteoblasts and osteoclasts on
their surface. Proliferating fibroblasts and
capillaries along the defect wall are emanating
into the graft site.

7 Day

The marrow spaces present further fibroblastic
activity, and both osteoclastic and osteoblastic
activity is observed as bone remodeling proceeds.
There is a reduction in the diameter of the
marrow spaces due to new osteoid apposition.

14 Day (Figure 23)

The alveolar wall of the defect presents

increased fibrous tissue proliferation with new
bone formation both toward the marrow spaces and
on the surface. New trabeculae of bone are
formed into the defect area which extend to the
graft fragments. Osteoclastic activity is present
along the defect wall.

21 Day (Figure 28)

The alveolar bone shows apposition on the
surface of the cortical plate and the crestal area.
The marrow spaces are reduced in size because of
new bone formation, and fewer osteoclasts are
seen. The dentinal surface of the tooth shows
repair by cellular cementum and cementoid apposi-
tion.

28 Day

The alveolar bone shows further reduction
in the diamater of the marrow spaces. The defect
is characterized by osteoblastic activity with
new trabeculae projecting into the graft area
from the adjacent alveolar walls. Cementoid ap-
position exists on the injured dentinal surface of
the tooth.

42 and 56 Day (Figures 37 and 38)

Continued osteoblastic activity is observed along the defect wall, and the alveolar crest and buccal surface show additional bone apposition and remodeling. Trabeculae of bone project from the walls of the defect to form a confluence with the remodeling graft fragments which have regenerated the defect. Cellular cementum and cementoid apposition exist on the dentinal surface of the tooth.

d. FROZEN CANCELLOUS BONE AND MARROW GRAFT

0 Day (Figure 16)

Technical difficulties made it impossible to obtain an ideal section demonstrating the frozen 0 day graft. The available histologic material, however, demonstrates bone graft fragments which appear de-vitalized. No osteocytes are seen occupying lacunae in these fragments. An extravasation of red blood cells is present.

3 Day (Figure 17)

The devitalized bone graft fragments are scattered throughout an area of an acute inflammatory infiltrate. Fragments more adjacent to

the alveolar bone are surrounded by proliferating
fibroblasts emanating from the adjacent marrow
cavities. Areas of fatty marrow are also noted.

7 Day (Figure 20)

The graft site presents a fibrinopurulent
exudate at the coronal aspect containing numerous
polymorphonuclear leukocytes and lymphocytes.
A few graft fragments are being exfoliated. The
graft fragments present empty lacunae and a
variation in staining, presumably due to necrosis.
A localized area of necrotic debris is present
in the coronal aspect of the defect site between
the graft material and the cementum. The defect
site is characterized by a proliferation of
fibroblasts which appear more extensive than the
7 day fresh graft. All bone fragments are
enveloped in proliferating connective tissue and
young capillaries, and some osteoclastic activity
can be seen on these fragments.

14 Day (Figures 23, 24, and 25)

A well organized cellular fibrous connective
tissue surrounds all graft fragments. Numerous

young fibroblasts and proliferating capillaries are present. At the coronal aspect of the defect some exfoliating bone fragments are surrounded by proliferating epithelium and a chronic inflammatory infiltrate. Osteoclasts are abundant in the graft area while at the same time osteoblasts and osteoid tissue are seen, with new bone trabeculae formation. Devitalized graft fragments demonstrate both bone resorption and apposition on their periphery.

21 Day (Figures 28 and 29).

The bone graft fragments are scattered throughout the young fibrous connective tissue in the repairing defect. Capillary proliferation is still abundant. Bone fragments are surrounded by osteoblasts and new osteoid tissue, demonstrating the incorporation of large graft fragments into new bone, and a dense cellular infiltrate on the cortical bone surface resembles young periosteum. Although osteoclasts are present they are fewer in number. Cementoid is deposited on the dentin surface of the tooth.

28 Day (Figure 31)

The graft site is filled with young trabeculae of bone in which more basophilic graft particles are scattered. Active bone remodeling is occurring but the number of osteoclasts has decreased. The marrow spaces are filled with a very cellular connective tissue, and young periosteum is present on the surface of the repairing defect. The coronal aspect of the defect presents fragments of exfoliating devitalized bone surrounded by chronic inflammatory tissue and necrotic debris. Cementoid apposition is present on the dentin surface of the tooth.

42 and 56 Day (Figures 33, 37, and 38)

The graft sites are filled with young trabeculae of bone which are being actively remodeled. The marrow spaces are rich in fibrous tissue, and the walls of these spaces are lined with cells. Young periosteum has formed on the surface of the repairing graft. A new alveolar crest is present which demonstrates active remodeling. Exfoliating graft fragments, however, are still present in

the coronal aspect of the repairing defect. Some of these fragments remain subjacent to well organized dentogingival fibers of the gingival fiber apparatus, and some show osteoid formation on their surface.

4. TWO-WALLED CONTROL

   a. EPITHELIUM

      <u>7, 21, and 42 Day</u>

        The epithelium demonstrates a histologic picture similar to that described for the epithelium under fresh and frozen bone grafts, with a similar sequential healing pattern. The keratinized stratified squamous epithelium presents slight intercellular edema, acanthosis, and a polymorphonuclear leukocytic infiltration in the col region. The epithelium becomes closely adapted to the tooth surface and demonstrates a normal epithelial attachment.

   b. CONNECTIVE TISSUE

      <u>7, 21, and 42 Day</u> (Figure 39)

        The connective tissue also demonstrates a histologic picture similar to that described

previously. The tissue presents interstitial
edema, vascular dilatation, fibroblast prolifer-
ation, and a chronic inflammatory infiltrate of
plasma cells and lymphocytes.

c. ALVEOLAR BONE

7 Day

The alveolar bone demonstrates repair from
the initial trauma of defect preparation. The
marrow spaces adjacent to the defect site present
an increase in connective tissue with active re-
modeling occurring evidenced by osteoclasts and
osteoblasts. Proliferating connective tissue
emanates into the defect site. New bone apposi-
tion is present on the buccal surface of the
cortical plate, and the crestal region demon-
strates osteoid and young trabeculae formation,
with active remodeling.

21 and 42 Day (Figure 39)

The marrow spaces adjacent to the defect site
demonstrate a slight increase in fibrous connective
tissue. New bone apposition is present on the
buccal surface of the cortical plate, and the
crestal region shows the formation of young
trabeculae with active remodeling.

d. DEFECT SITE

7 Day

The defect site is characterized by proliferation of young fibroblasts and capillaries from the adjacent marrow spaces and overlying connective tissue. Cementoid apposition was present on the dentin surface of the tooth.

21 Day (Figure 39)

The defect site is filled with young fibrous connective tissue with slight vascular dilatation. Cementoid is deposited on the dentin surface of the tooth.

42 Day

The defect site is filled with a mature fibrous connective tissue, with formation of new bone trabeculae occurring from the crest and the adjacent defect walls. A few small spicules of bone are seen within the defect site. Cellular cementum is present on the dentin surfaces.

CHAPTER V

DISCUSSION

The present study was most encouraging regarding autogenous bone and marrow transplants in periodontal osseous defects. Two-walled osseous defects in rhesus monkeys demonstrated repair by regeneration and reattachment following correction with fresh autogenous iliac transplants, frozen autogenous iliac transplants, and surgical curettage. The sequential healing phenomena was, in general, histologically similar between the fresh and frozen iliac transplants. The findings showed that the intrabony pocket may be eliminated by the induction of osteogenesis by the insertion of autogenous cancellous bone.

The control defects, following curettage, demonstrated similar events in healing but this series of events was significantly delayed when compared to the defects corrected

by bone grafting. In the absence of graft material, the defect healed by adaptation of epithelial attachment to the root surface with a decreased amount of new bone formation.

Although no differences was found between the osteogenic potential of fresh or frozen iliac transplants other significant difference did exist. At the time of defect correction the macroscopic appearance of the fresh bone core was one of viability and vascularity while the frozen bone core grossly appeared lighter in color and less viable, presumably due to the freezing, thawing, and deglyceralization. Similarly, the microscopic appearance of the fresh 0 day graft was one of vital tissue, with osteocytes in lacunae, and young fibrous connective tissue surrounding the trabeculae. The frozen bone graft, conversely, appeared devitalized, with empty lacunae and surrounding tissue disruption. It is possible that any one or all of the factors implicated in cryoinjury (Chapter II) may be responsible for this nonvital appearance of the frozen bone grafts, and they may in fact be non-vital. It should be noted however, that the terms vital and non-vital are only morphologic descriptions at the light microscope level, and are certainly not indicative of the histochemical or biomolecular activities that may be occurring.

Finally, a significant difference in the healing phenomena occurred on the dentin surface adjacent to the repairing defect, and this will be discussed further. The frozen grafts and controls demonstrated normal apposition of cementoid and cellular cementum, however the fresh grafts demonstrated odontoclastic root resorption occurring concomitantly with apposition of cementoid and cellular cementum nearby. Significantly, there was no evidence of odontoclastic activity in any frozen graft or control defect specimen.

The clinical and histologic findings demonstrated that the experimental defects created in this study simulate those of chronic periodontitis. These defects, then, showed an ability to be consistently created with a marked degree of similarity to one another, and were excellent experimental models of chronic periodontitis which could be utilized for further study. It must be emphasized, however, that artificially created periodontal defects, regardless of their appearance, are never identical in all respects to those defects which result from a natural disease process. Specifically, the complex and often unknown etiologic factors of natural periodontal disease are not present in the experimental animals, and therefore the repair processes proceed without

the influence of these complicating factors. The healing, then, is accomplished witout the presence of a significant variable factor, the natural etiology, and must be interpreted with this in mind at all times.

The sequential healing description of the gingival epithelium demonstrated its potential for swift and extensive regeneration. Mobilization of cells to colonize the surgical wound was achieved by two distinct processes; movement of epithelial cells into the wound, and provision of a sufficient number of epithelial cells for the regeneration by local mitotic division[69]. Because the margins of the mucoperiosteal flap could not be minutely approximated to the tooth surface, a small clot-filled area remained for the advancing epithelial cells to negotiate. These epithelial cells moved as a sheet with a free edge, and these migrating sheets of cells moved along the wound surface and beneath the clot, apparently digesting fibrin and damaged extracellular substance of connective tissue in the process. The ability to move across a solid substrate is an inherent property of epithelial cells[70]. It is possible that epithelial cells, like many other cells, possess lytic properties of their own, and this conceivably would enhance their

locomotive ability. Support for this concept has come from studies which have shown that a collagenase is produced in healing wounds, and migrating epithelial cells apparently possess high collagenolytic activity[69]. On occasions, the apically migrating epithelium came into direct contact with exfoliating graft fragments. When this occurred the fragments were enveloped in epithelium, and this process appeared to facilitate their eventual extrusion[71].

Rapid regeneration and repair was also seen within the connective tissue of the mucoperiosteal flap, and the dominant cellular activity was demonstrated by fibroblasts and endothelial cells. The provision of new blood vessels for the wounded area in the early healing stages was provided by mitotic division of the endothelial cells of pre-existing capillaries, which, as a result, produced new sprouts. Restoration of the connective tissue continuity involved four processes; production of new fibroblasts, migration of these cells into the wounded zone, formation of new intercellular material, and remodelong. It was observed in this study, as it has been in others[72][32], that fibroplasia began in the loose perivascular connective tissue. This suggests that new fibroblasts did not originate from the

dense fibrous connective tissue along the wall of the wound, but instead from areas capable of supplying undifferentiated cells. Grillo, in fact, has stated that it is impossible to separate the proliferative response of the capillaries from that of the fibroblasts, and suggests that a fibroblast-capillary system may represent a primary reparative response in mammals[72].

There was no significant difference, sequentially or histologically, in the healing of the defects receiving either fresh or frozen osseous tissue. The initial blood clot which had formed was replaced by loose connective tissue that surrounded the graft fragments. Osseous regeneration followed, due to the presence of osteogenic or potentially osteogenic cells. It was demonstrated on a histologic level that the fresh and frozen osseous graft material appeared non-vital in the early healing stages (no osteocytes within the lacunae). Regardless of the degree of viability present at the time of implantation, it is most likely that necrosis of the bone fragments occurred shortly thereafter, due to lack of a vascular supply to the osteocytes within the grafted bone. It is felt, therefore, that the transplant does not itself proliferate and form new bone, but it may

cause cells of the defect site to differentiate into osteo-blasts and stimulate the growth of new trabeculae.   Because graft fragments were distinguished within the regenerated bone as late as 60 days following implantation, they acted as islands for surface osteogenesis to occur on, resulting in a confluence of new bone between graft and defect wall. Exfoliating necrotic graft fragments (sequestra) were equally observed in both fresh and frozen graft specimens, and were most likely related to either overpacking graft material into the defects or to the impossibility of utilizing a protective dressing following implantation.   Occasionally a sequestrum would become surrounded by new bone and appear as an involucrum, similar to that seen in acute osteomyelitis. This suggests that, even though the exfoliating fragments were coronal to the area of osseous regeneration, the poten-tial or stimulus for osteogenesis to occur was present, and did in fact occur on these isolated fragments.

The osteogenic cells have an inherent potential for producing bone, and are those cells of the endosteum, bone marrow, and the cambium layer of the periosteum of young bones[73].   It is the perivascular undifferentiated mesen-chymal cell which has the capacity to be induced in some

manner into becoming an osteogenic cell[74]. The origin of
these cells, then, remains a key factor in osseous regen-
eration. The histologic findings demonstrated connective
tissue cells and capillaries proliferating into the defect
from the periodontal ligament, from the overlying connective
tissue, and from the adjacent marrow spaces. It appeared
microscopically, however, that the majority of these cells
had their origin in the adjacent marrow spaces of the alve-
olar defect wall. The dramatic changes which occurred in
the adjacent alveolar bone, compared to areas further removed
from the defect, would support this concept.

Despite differences of opinion as to the origin of the
potential osteogenic cells, there is much agreement and evi-
dence as to how these undifferentiated cells become involved
in osteogenesis. This study demonstrated, as others have[9]
[31][32], that osteoclastic resorption of the osseous defect
walls and grafted bone fragments played a significant role
in the subsequent osteogenesis by which the defect underwent
regeneration. The 7 day specimens demonstrated this osteo-
clastic activity only, where as at 14 days both osteoclastic
and osteoblastic activity were present. It has been hypoth-
esized that the necrosis of the transplant may be the greatest

factor in the formation of new bone by the host[75]. It is
the initial osteoclastic resorption which in some way induces
the undifferentiated connective tissue cells to differentiate
into osteoblasts, and thus facilitate repair. The biologic
mechanism by which this occurs may involve the release of an
inductive substance(s) from mature bone by osteoclastic
activity. Such a histochemical material may then be free
to effect the differentiation of the undifferentiated cells
into osteoblasts. Thus, the inductive power of the grafted
bone fragments was well demonstrated in this study. There
is no doubt that stimuli exist which induce undifferentiated
mesenchymal cells to take on the function of active osteo-
blasts, or possibly chondroblasts[76]. The appearance of a
chondroid-like material in the 56 day fresh graft specimen
possibly resulted from this same induction process, and it
is reasonable to assume that this chondroid-like material
repair would eventually be replaced by bone. This would
imply that at least some connective tissue cells possess the
genetic apparatus which controls the formation of the extra-
cellular substance of bone (and possibly cartilage), how-
ever a specific osteogenic stimulus is required to activate
this function. The nature of this stimulus (or perhaps

stimuli, because it is certainly possible that a number of different mechanisms may be involved) is of more than academic interest. However, as pointed out by McLean and Urist[27], no substance has yet been isolated that can be shown convincingly to have bone induction properties.

The osseous regeneration in the control defects, which lacked graft material, was slower and presented a different pattern of apposition. Although osseous regeneration eventually occurred, the osteoblastic activity was present only along the wall of the defect and at the adjacent alveolar crest. This pattern of bone apposition, then, would explain why the control defects demonstrated a slower repair, and places further emphasis on the osteogenic potential of the bone grafts.

A significant finding in this comparative healing study was that of external root resorption in the fresh grafts, while none was found in the frozen grafts or in the controls. Active odontoclasts were observed within lacunae along the dentin surface with variable distribution. The 21 day specimen demonstrated odontoclastic activity above the alveolar crest level, at the crest, and below the crest midway to the base of the repairing defect. Cementoid apposition on the

dentin surface had occurred apical to this. The 42 day day specimen demonstrated odontoclastic activity above and at the alveolar crest level, while cementoid apposition on dentin was apparent apical to the crest level.

Although root resorption has been attributed to many factors, reports recently have noted root resorption in human subjects treated with iliac transplants[4][77][78][79][80]. Other investigations have presented evidence that viable marrow may suppress cementogenesis[81][82][83]. It would appear desirable, from the results of this and other studies, to modify the iliac graft material in some way so that its bone induction properties would be retained while its odontoclastic activity would be curtailed. Storage techniques such as those utilized here may have this effect, for, root resorption has not been reported in the literature in clinical cases treated with frozen graft material. Perhaps in those cases demonstrating root resorption, viability was somehow maintained, and odontoclastic activity in some way was triggered. It is also possible that vital cells introduced into the defects inhibited cementogenesis and/or periodontal ligament formation. The grafting of frozen non-vital material assured that the subsequent granulation tissue

formation originated from the host site exclusively, and
normal cementogenesis and/or periodontal ligament formation
followed. It does appear that storage of hip marrow material
in proper media for a length of time decreases the possibil-
ity of root resorption[79].

A second possible factor related to root resorption is
the degree of root preparation prior to implantation. Root
resorption in this study was found to occur on dentin in
each instance, and tissue reattachment studies have indicated
that when cementum is removed by root planing, dentin resorp-
tion may precede cementogenesis[84][85]. Thus exposed dentin
itself, or in conjunction with osseous graft material, may
potentiate root resorption. This does not explain however,
the appearance of odontoclasts in the fresh grafts only in
this study, for, the control defects and the frozen grafts
all exhibited exposed dentin. It suggests, instead, that the
potential for odontoclastic stimulation is inherent in fresh
grafts only.

The findings presented in this study provide additional
information in regard to the clinical application of iliac
crest transplants in periodontal therapy. Because more
rapid osteogenesis and total regeneration occured in those

defects receiving autogenous grafts, it is conceivable that
in the grafted defects there was inhibition of the apical
migration of epithelial attachment, especially in the
early stages of healing, and therefore prevention of perio-
dontal pocket reformation. Other investigators concur with
this concept. The success of autogenous iliac transplants,
furthermore, widens the surgical armanentarium of the
periodontist. Instead of correcting periodontal osseous
defects with destructive techniques, such as osseous resec-
tion, a constructive approach can be undertaken to actually
increase the attachment apparatus of the diseased tissue.
This has always been the goal of periodontal therapy from
a purist point of view, but only recently has it been pos-
sible. The present study suggests, that the best results
in the treatment of periodontal osseous defects may be
achieved by utilizing frozen iliac transplants which do
not elicit odontoclasia, since root resorption must be
considered an undesirable result of therapy. For severely
involved osseous defects, the possibility of sequential
implant procedures to achieve a more ideal result is
apparent. If each procedure gains additional coronal bone

apposition, then this approach is certainly feasible. Furthermore, when clinical application of iliac crest transplants becomes predictable, the preservation and storage of autogenous graft material for future use in the same patient would appear to offer definite advantages in the planning and execution of the desired surgical techniques. At our present level of knowledge, freezing would appear to be the banking method of choice for long-term preservation.

It should be realized, however, that until further research yields better understanding, we must remain guarded regarding the nature of repair and long term prognosis of implant cases. Although bone can be successfully regenerated, it is only part of the triad of the attachment apparatus. Reconstruction of new cementum, periodontal ligament, and supporting alveolar bone must be achieved simultaneously, so the problem is not one of repairing bony deformities but rather one of formation of a new attachment apparatus. We must also realize that, although animal studies are encouraging, in the last analysis only human material will offer sufficient insight to be valid.

CHAPTER VI

CONCLUSIONS

1.   The experimental defects employed in this study on rhesus monkeys simulated those of chronic periodontitis, and therefore represented excellent experimental models for healing studies.

2.   The two-walled chronic periodontal osseous defects corrected by fresh and frozen iliac transplants demonstrated a more rapid osteogenesis when compared to similar defects corrected by curettage alone.   This accelerated repair may function to inhibit the apical migration of the epithelial attachment and thereby prevent reformation of periodontal pockets.

3.   Histologically the sequential healing events of fresh and frozen iliac transplants demonstrated no significant differences.

4.   Fresh iliac transplant specimens elicited odon-
toclastic activity on the external surface of the dentin,
while no external root resorption was observed with
frozen iliac transplant or control specimens.

5.   Iliac transplants have been utilized successfully
to restore the attachment apparatus in primates, however
caution must be observed when extrapolating findings
from animal studies to the human subject.

CHAPTER VII

SUMMARY

A study was undertaken to examine histologically the sequential healing phenomena in chronic two-walled periodontal osseous defects after being corrected by fresh and frozen autogenous iliac transplants.

Three adult rhesus monkeys served as the experimental models and provided 19 specimens from 0 to 56 days post-operatively. Sixteen of these served as graft specimens, and three served as control specimens in which the defects were corrected by curettage only.

The two types of graft material were procured and utilized as follows: 1) seven days prior to their implantation cores of cancellous bone from the iliac crest were glycerolized, and subjected to controlled freezing to -197^0C. for grafting into the distal osseous defect;

2) at the time of implantation fresh cores of cancellous bone taken from the contralateral iliac crest were grafted into the mesial osseous defect.

Histologic sections from each specimen were stained with H and E, a description of the sequential healing events was recorded, and the similarities and variations of repair were discussed.

The fresh and frozen autogenous bone grafts induced a more rapid osseous regeneration than the controls, and differences were not observed in the healing events between the fresh and frozen grafts.

Fresh iliac transplant specimens demonstrated odontoclastic activity on the external dentin surface.

CHAPTER VIII

ILLUSTRATIONS

Fig. 1. Preoperative appearance of the experimental area. Note the slight marginal and papillary gingivitis.

Fig. 2. Experimentally created two-walled osseous
defects (d) mesial and distal to the first molar. A control
defect (c) has been created distal to the second molar.

Fig. 3. Wooden irritants (arrows) stabilized in position by sutures.

Fig. 4.   7 day postoperative illustration of irritants still in position.   Note the inflammed gingiva.

Fig. 5.   30 day postoperative illustration.   Note the
persistance of marginal and papillary inflammation.

Fig. 6. Exposed iliac crest.

Fig. 7. Iliac crest following the removal of one
core of cancellous bone and marrow.
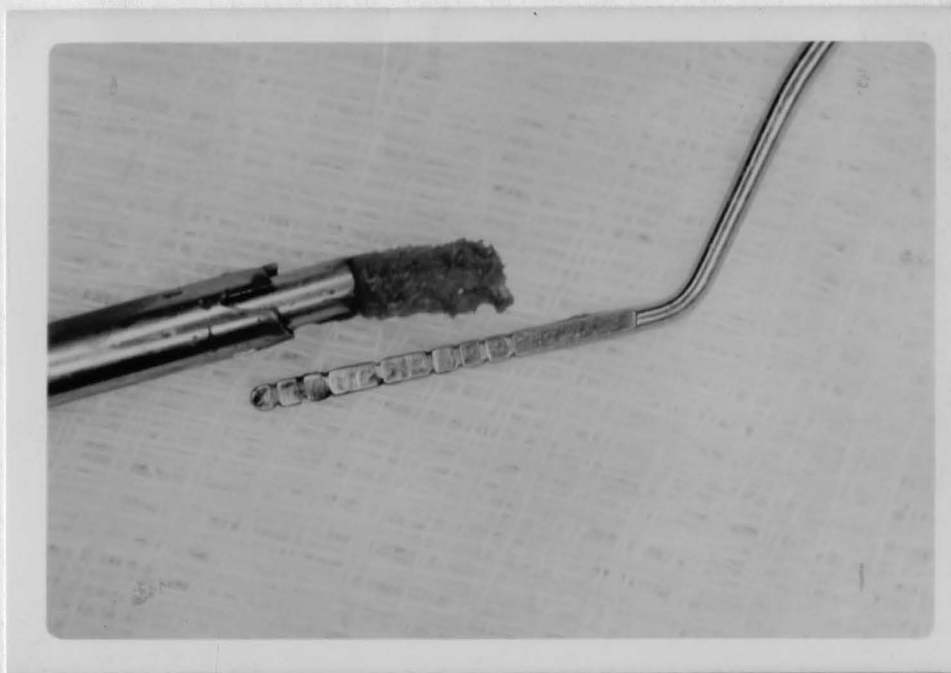
Fig. 8. Illustration of one core of iliac crest cancellous bone and marrow. By comparison to the periodontal probe the core size is approximately 5mm. by 2mm.

Fig. 9. Two-walled osseous defects (d) 30 days after their creation. Note the changes in the osseous topography resulting from osteoclastic resorption initiated by the irritants that were introduced 30 days previously.
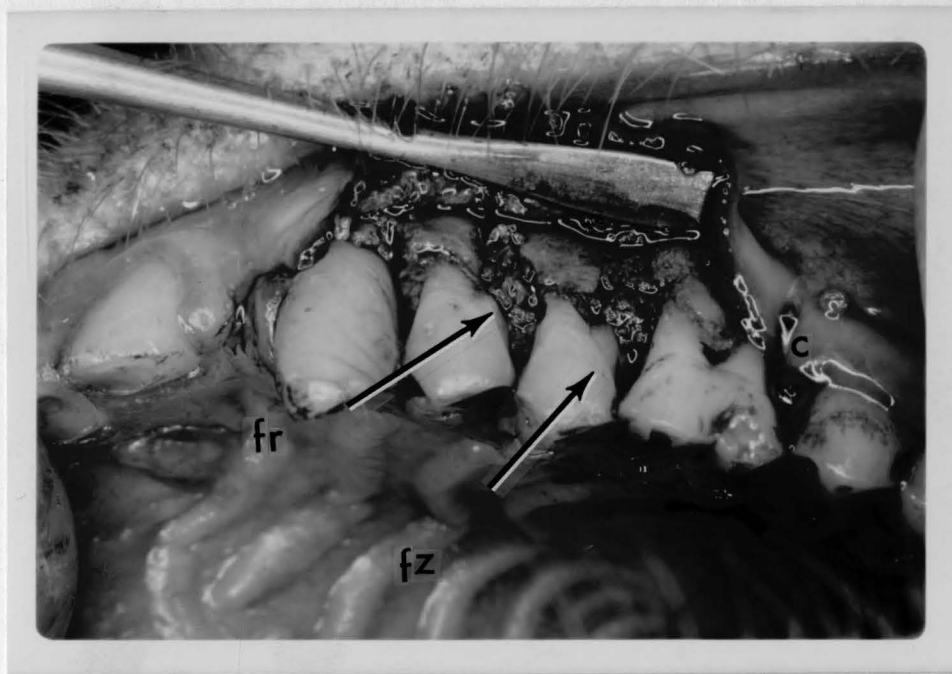
Fig. 10. Illustration of iliac crest transplants
in place. The fresh graft (fr) has been placed mesial to
the second bicuspid, the frozen (fz) graft distal to the
second bicuspid, and the control (c) defect distal to the
first molar has been sutured closed following curettement.

Fig. 11. Mucoperiosteal flap reapposed and sutured to assure coverage of the graft areas by soft tissue.

Fig. 12. 7 days postsurgically. Note the marginal
and papillary inflammation.

Fig. 13. 42 days postsurgically. Note the improved appearance of the gingival tissues.
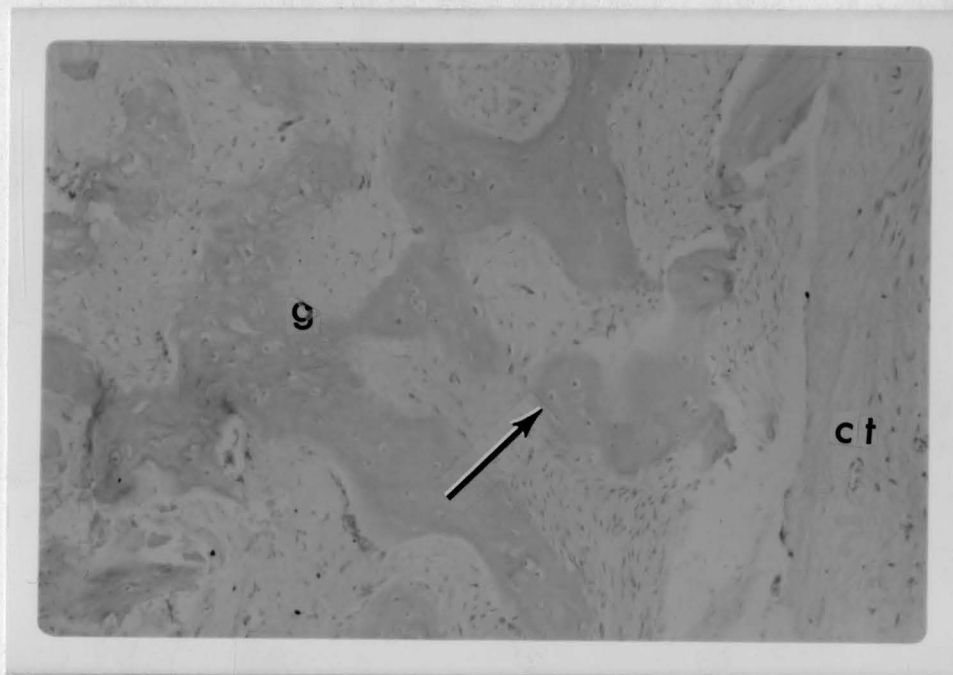
Fig. 14. 0 day fresh graft, 100X. Graft(g)-connective tissue (ct) interphase. Note the viable appearance of the fresh graft material and the osteocytes (arrow) within the lacunae.
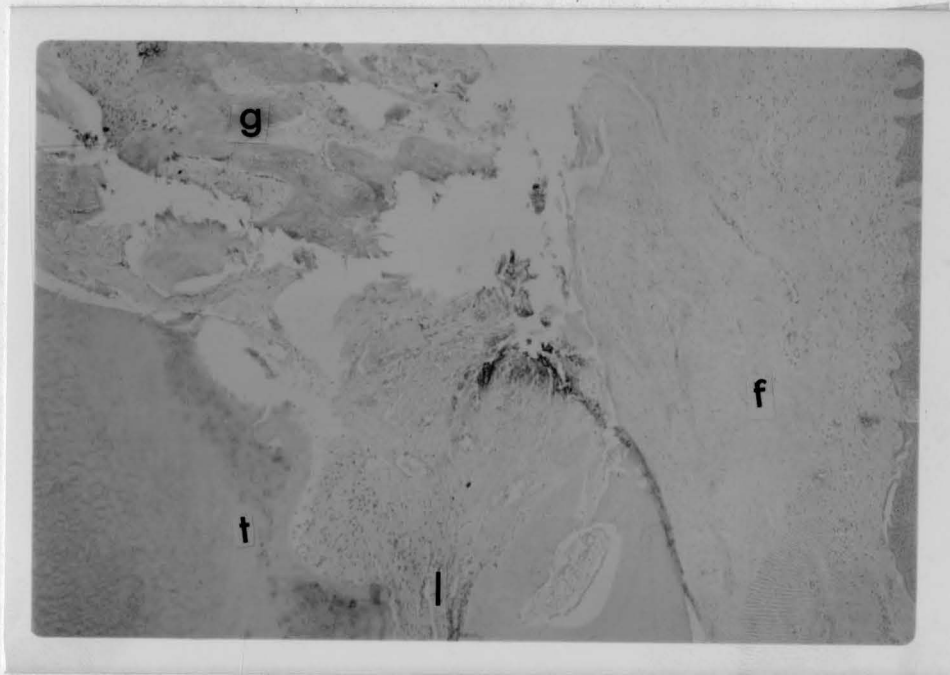
Fig. 15. 0 day fresh graft, 40X. Note the base of
the defect and the close contact of the graft (g) to the
tooth (t), periodontal ligament (l), and overlying flap (f).
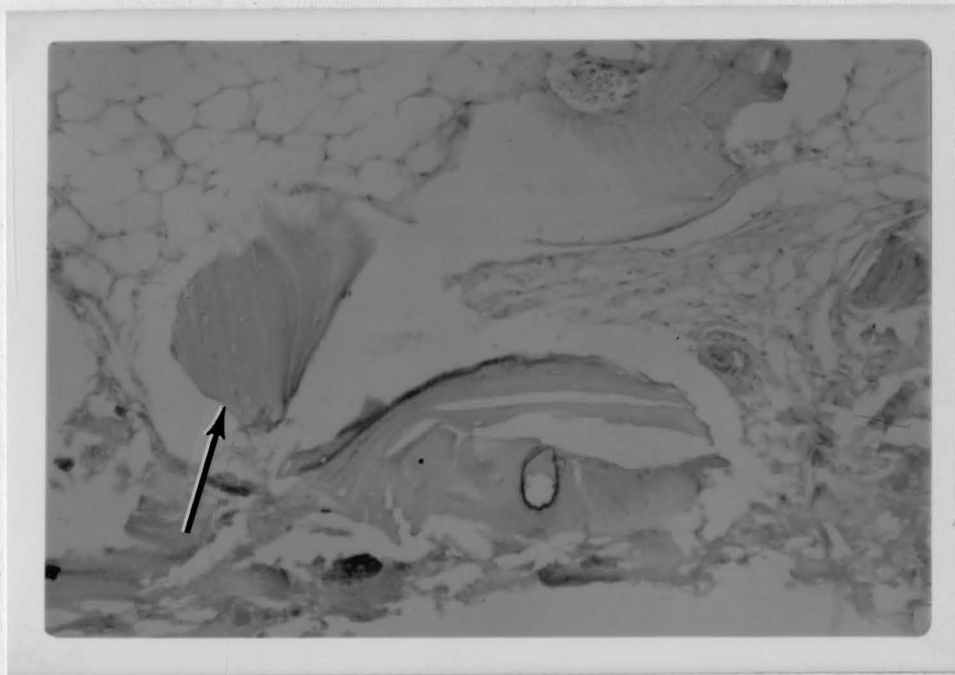
Fig. 16. 0 day frozen graft, 100X. Note the non-vital
bone fragments with empty lacunae (arrow), surrounding
tissue disruption, and presence of fatty marrow.
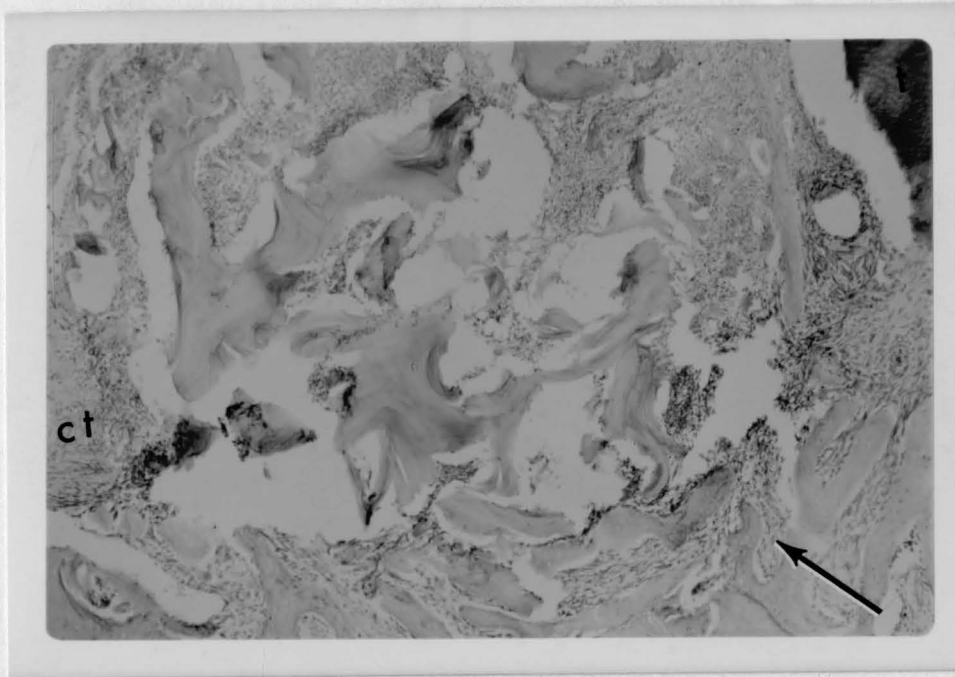
Fig. 17. 3 day frozen graft, 40X. The graft is seen
between the connective tissue (ct) and the tooth (t).
Note the proliferation of fibroblasts from adjacent marrow
spaces (arrow) at the base of the defect, and the acute
inflammatory infiltrate surrounding the non-vital appearing
graft fragments.
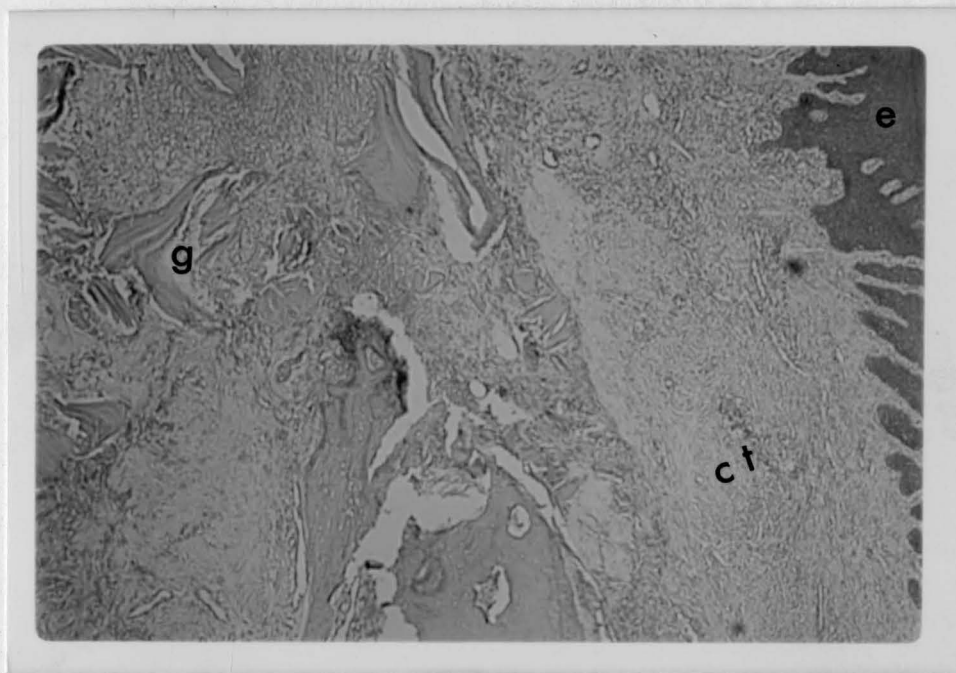
Fig. 18. 7 day fresh graft, 40X. Note the graft fragments (g), are becoming surrounded by proliferating connective tissue. The connective tissue (ct) shows vascular dilatation and interstitial edema, and the epithelium (e) shows intercellular edema, acanthosis, and hyperplasia.
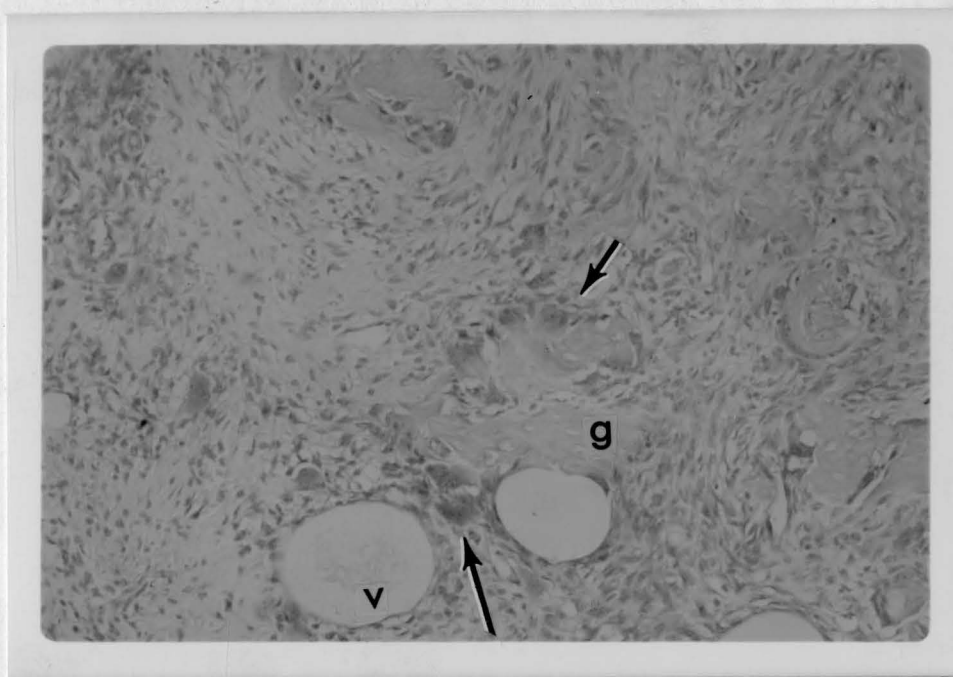
Fig. 19. 7 day fresh graft, 100X. Note the envelopment of graft fragments (g) by proliferating young fibrous connective tissue, the vascular dilatation (v), and the osteoclastic activity (arrow).
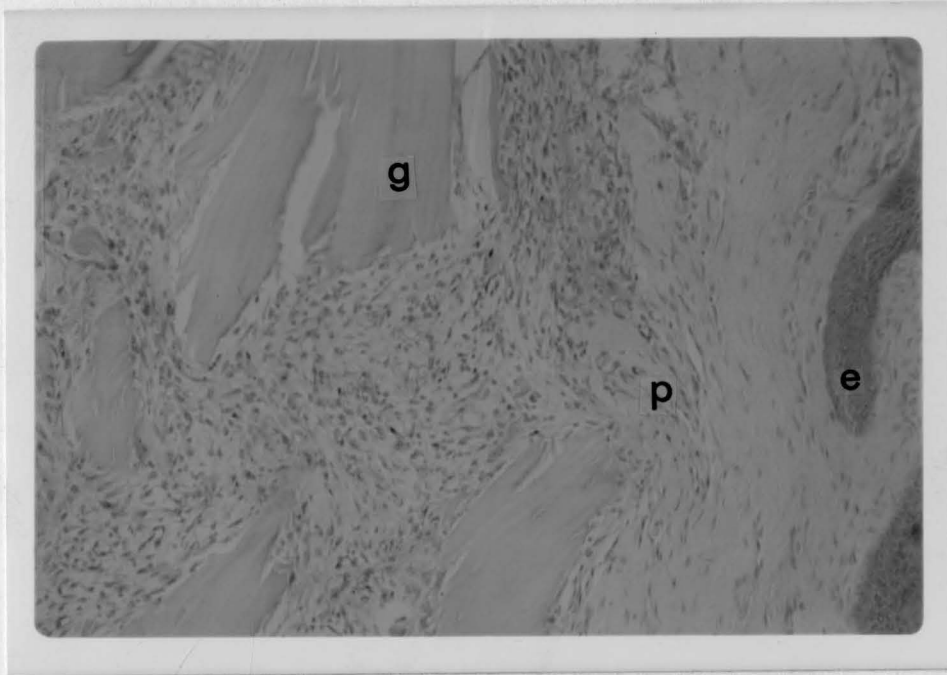
Fig. 20. 7 day frozen graft, 100X. Note the proliferation of young fibrous connective tissue and capillaries(p), the non-vital appearance of the graft fragments (g), and epithelial hyperplasia (e).

Fig. 21. 14 day fresh graft, 40X. Note the apical migration of the epithelium surrounding some of the exfoliating graft fragments (arrow), the osteoclastic activity, and the osteoblastic activity with formation of osteoid on graft fragments as well as young trabeculae (yt).
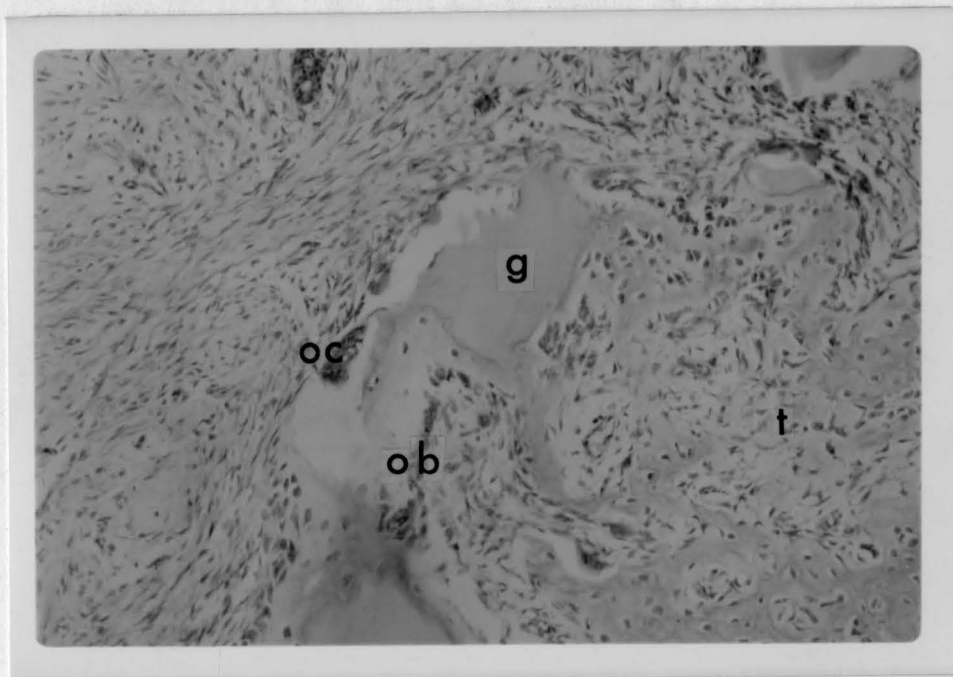
Fig. 22. 14 day fresh graft, 100X. Note the osteo-
clastic (oc), and osteoblastic (ob), activity surrounding
the graft fragment (g), and the new trabeculae formation(t).

Fig. 23. 14 day frozen graft, 40X. Base of graft
with connective tissue on the left and alveolar wall on
the right. Note the incorporation of graft fragments (g)
in the regenerating osseous defect, and the active
remodeling.
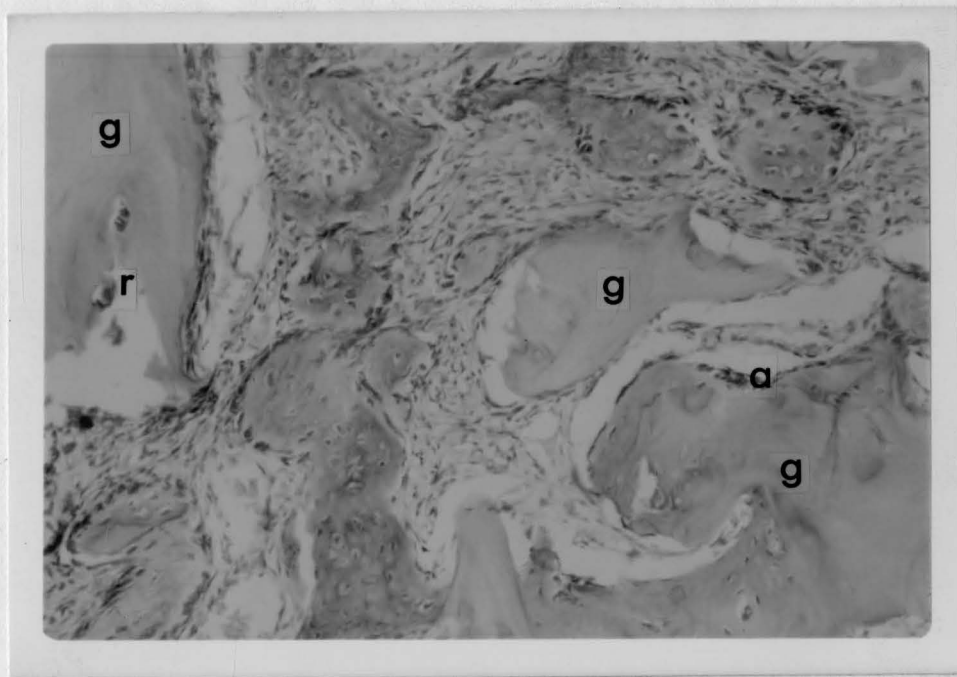
<u>Fig. 24.</u> 14 day frozen graft, 100X. Center of graft
area. Note the incorporation of graft fragments(g), and
the active bone resorption(r), and apposition(a) on their
periphery.
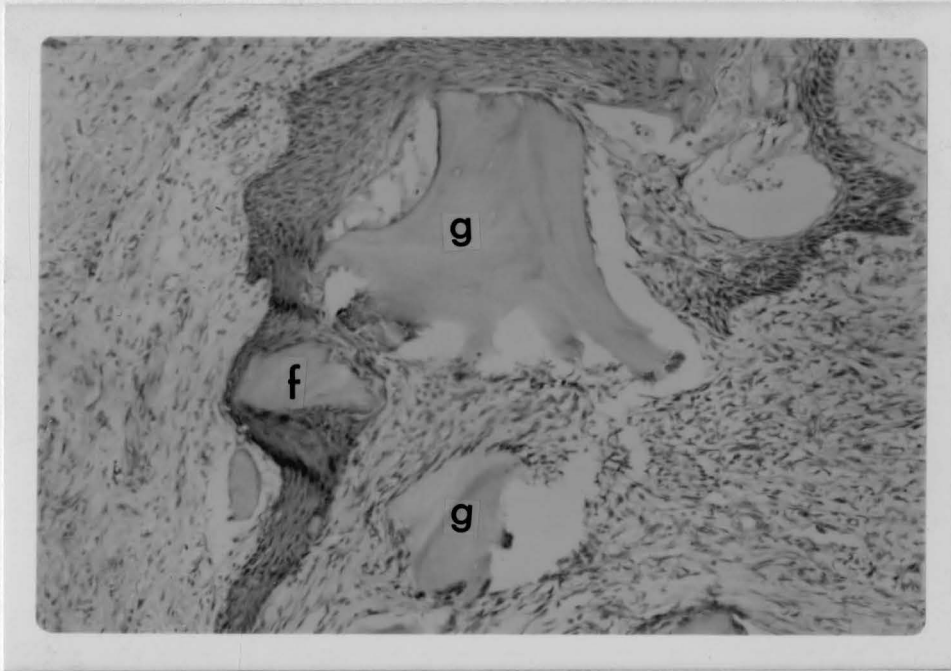
Fig. 25. 14 day frozen graft, 100X. Note the exfoli-
ating graft fragments (g) which exhibit both osteoblastic
and osteoclastic activity on their periphery, and
complete epithelial envelopment of one fragment(f).
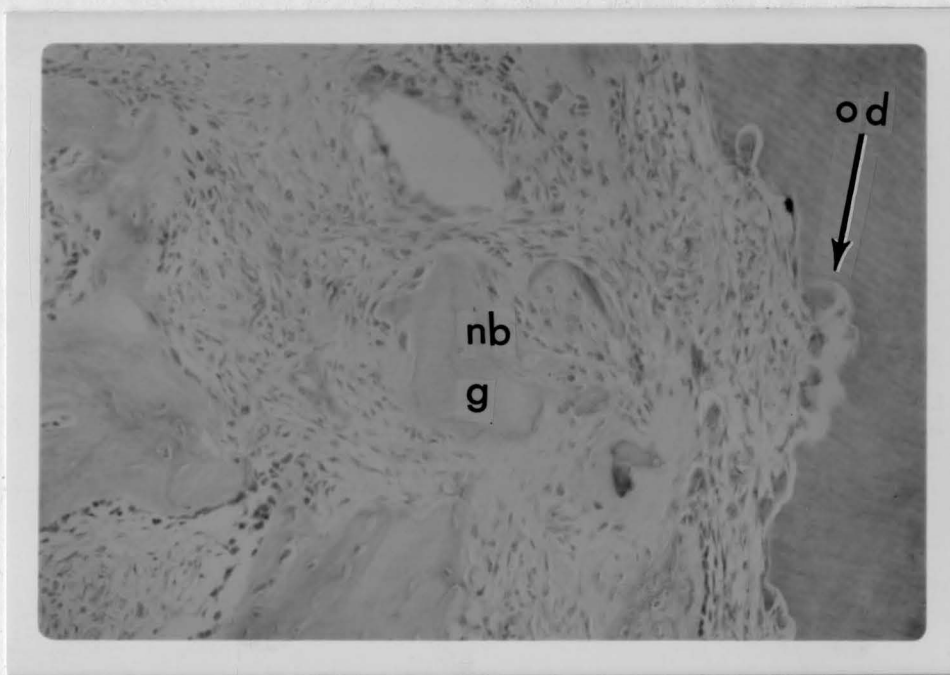
Fig. 26. 21 day fresh graft, 100X. Note the incorp-
oration of non-vital graft fragments(g) into new bone
formation(nb), and active odontoclasts(od) on the dentin
surface.
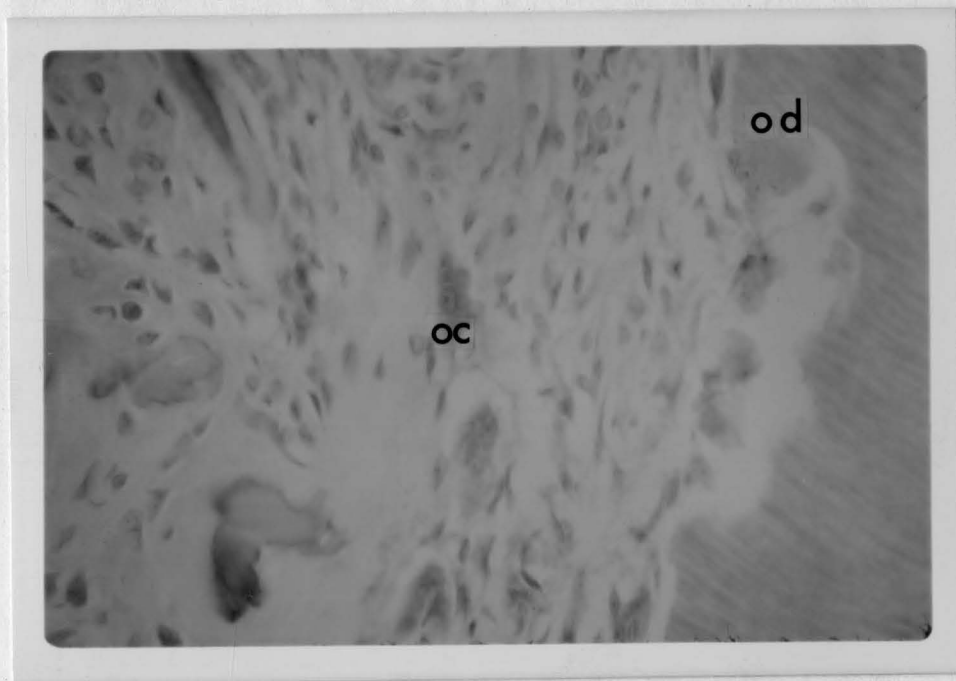
Fig. 27. 21 day fresh graft, 250X. Note the identical appearance of the multinucleated odontoclasts(od) and osteoclasts(oc).
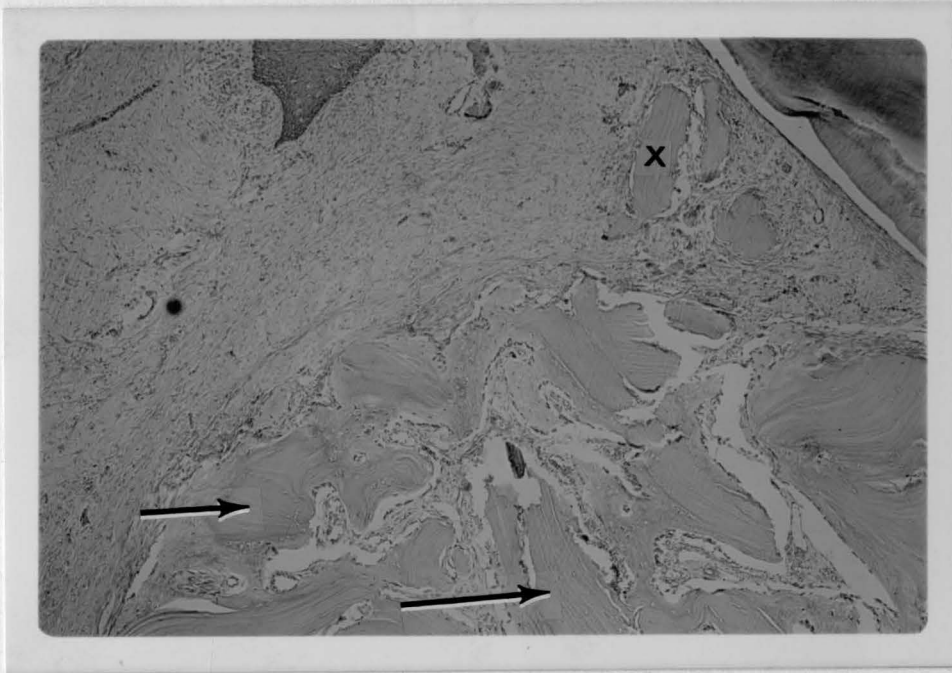
Fig. 28.   21 day frozen graft, 40X.   Note the incorp-
oration of non-vital graft fragments into the regenerating
alveolar crest(arrows), and the exfoliation of some
fragments(x).
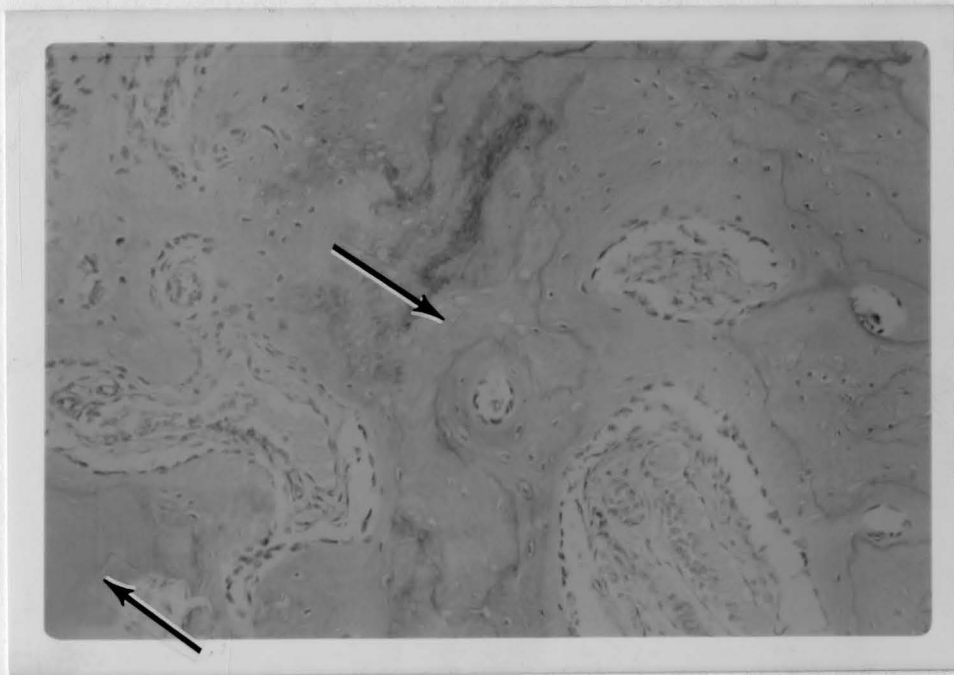
Fig. 29. 21 day frozen graft, 100X. Note the graft
fragments which have been completely enveloped in new bone
(arrow), and the reduction in diameter of marrow cavities
by bone apposition.

Fig. 30. 28 day fresh graft, 100X. Note the exfol-
iating graft fragments(g) and the epithelial proliferation
(ep), as well as what appears to be an epithelial pearl(p).
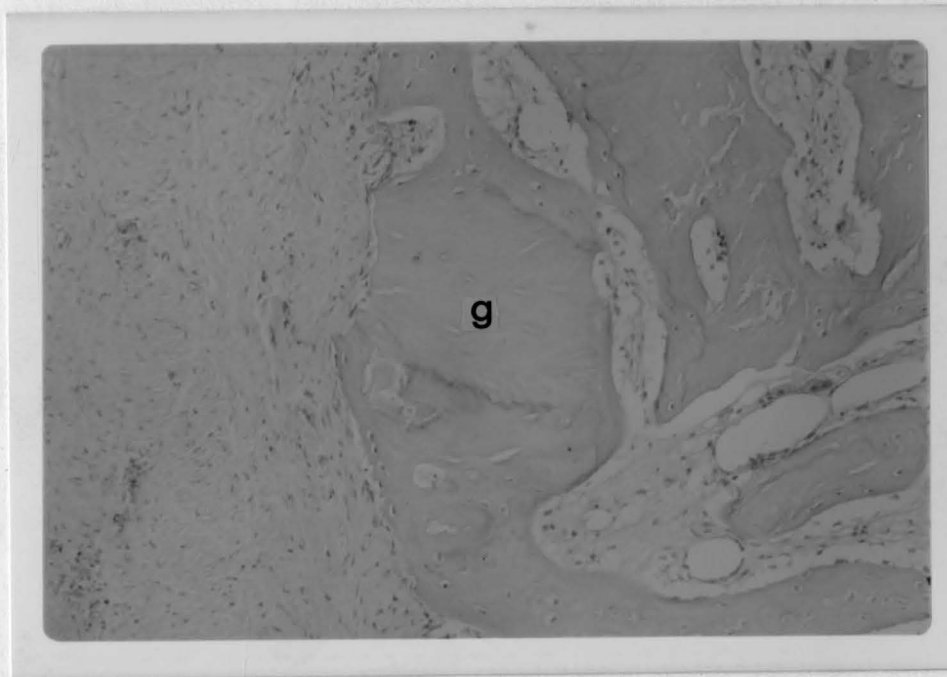
Fig. 31. 28 day frozen graft, 100X. Connective
tissue-graft interphase. Note the large non-vital graft
fragment(g) incorporated within the reformed bone.

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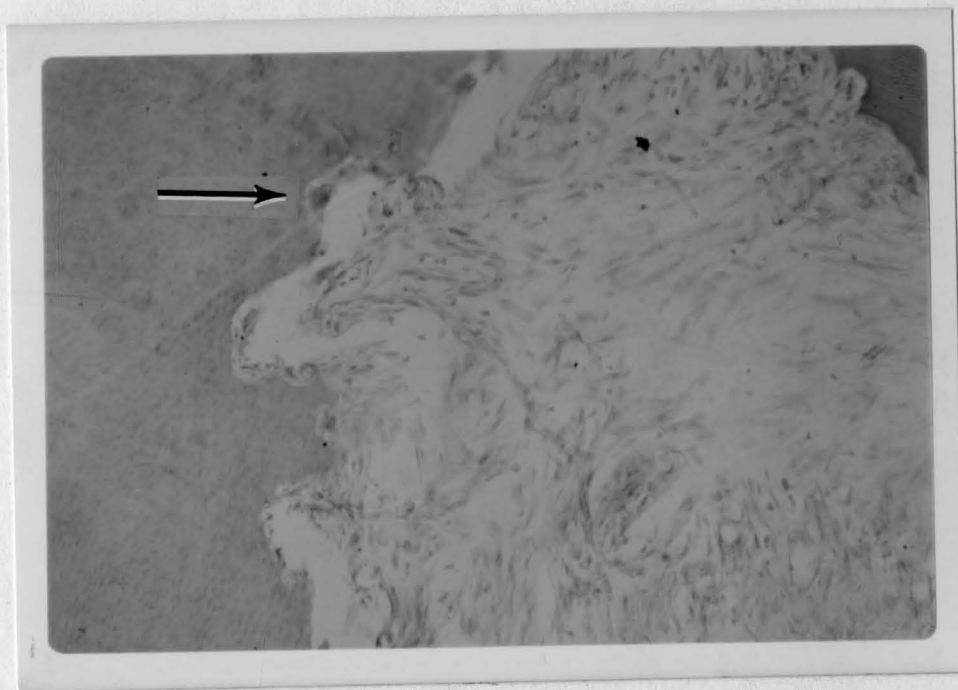
Fig. 32. 42 day fresh graft, 100X. Note the active
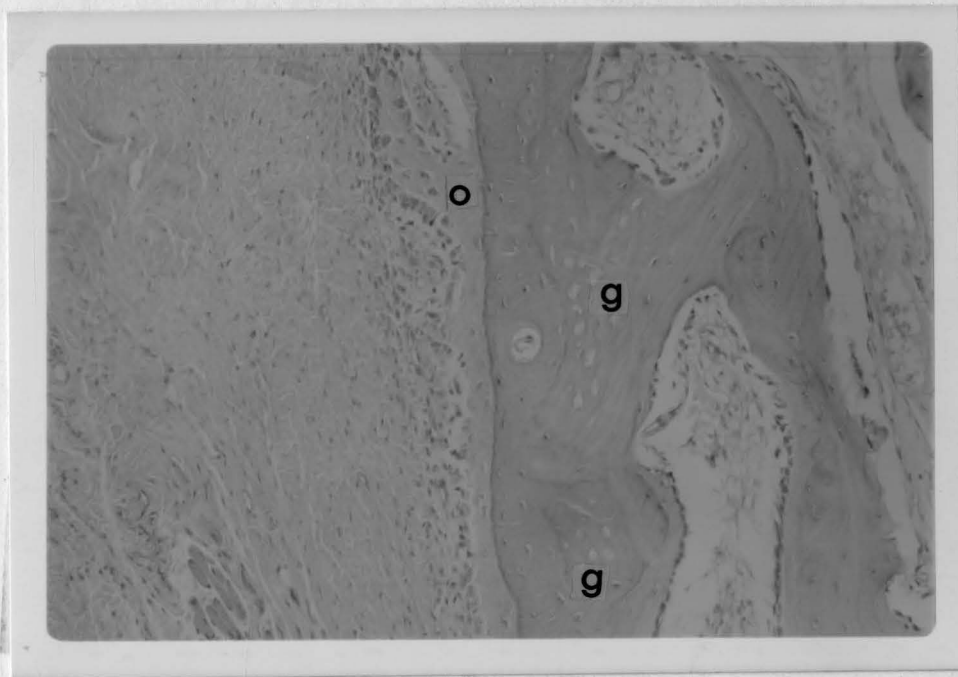odontoclastic activity on the dentin surface(arrow).

<u>Fig. 33.</u> 42 day frozen graft, 100X. Connective
tissue-graft interphase. Note the apposition of surface
osteoid(o), as well as incorporated graft fragments(g)
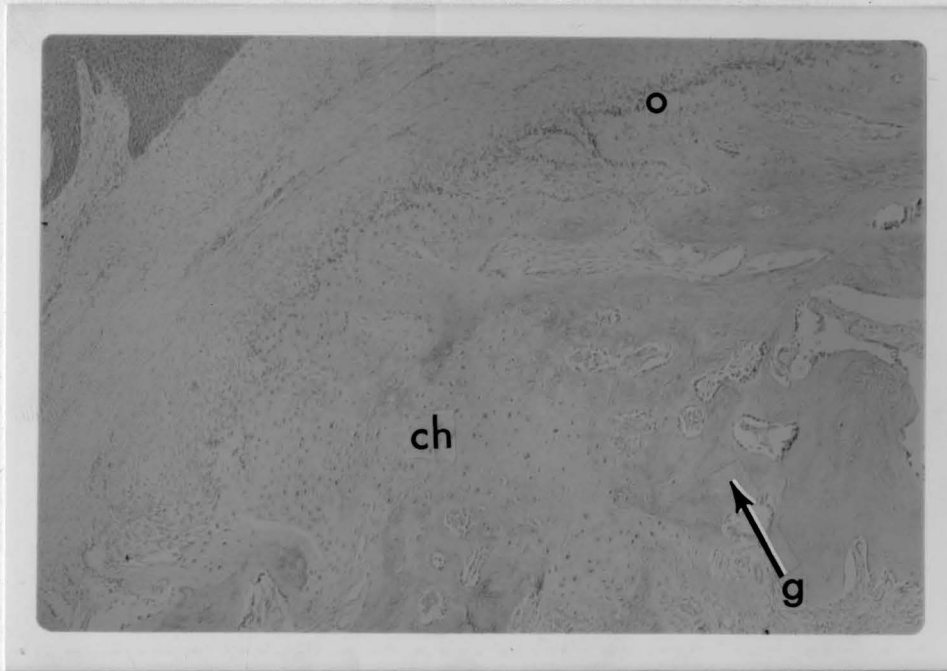still visible and non-vital in appearance.

Fig. 34. 56 day fresh graft, 40X. Note the osteoid
at the alveolar crest(o), as well as the graft fragments(g)
which have been incorporated within the reformed bone
and are still visible, and a chondroid-like material(ch)
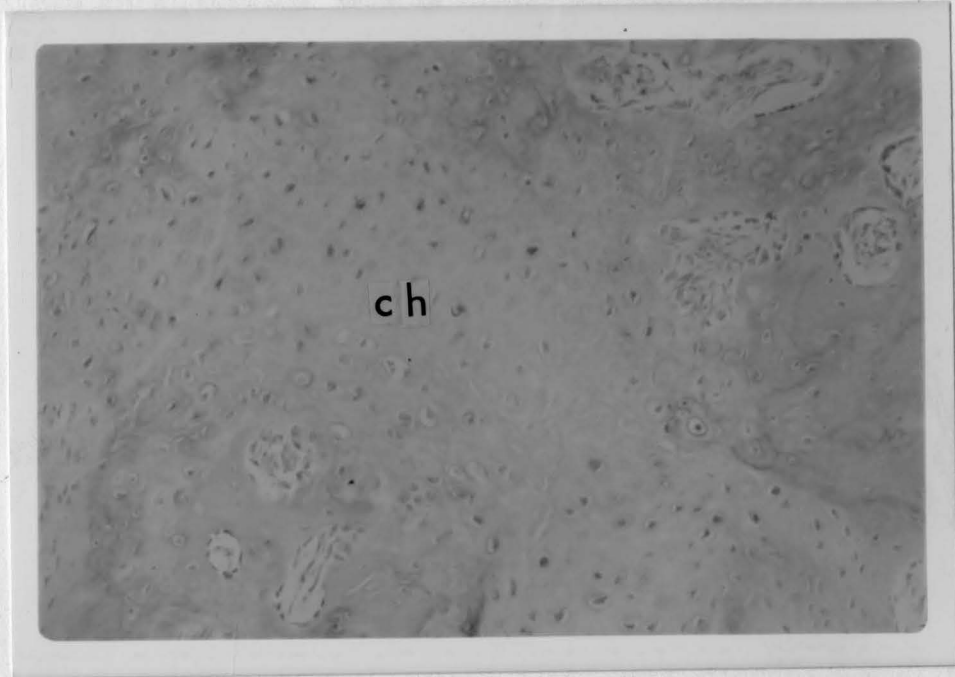in the repair area.

Fig. 35. 56 day fresh graft, 100X. Note the chondroid-
like material(ch) within the reformed bone, and that it
appears to be undergoing ossification.

Fig. 36. 56 day fresh graft, 40X. Note the slight
interstitial edema, vascular dilatation, epithelial
proliferation(arrow), and chronic inflammatory cellular
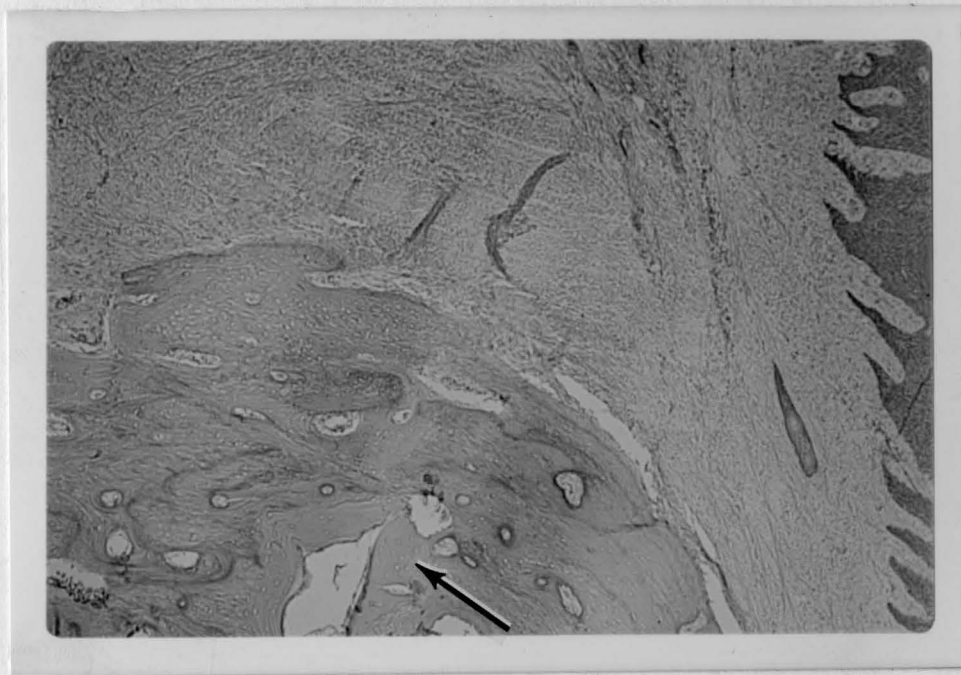infiltrate in the col region.

Fig. 37. 56 day frozen graft, 40X. Note that the incorporated graft fragments in the regenerated crest are still visible(arrow).
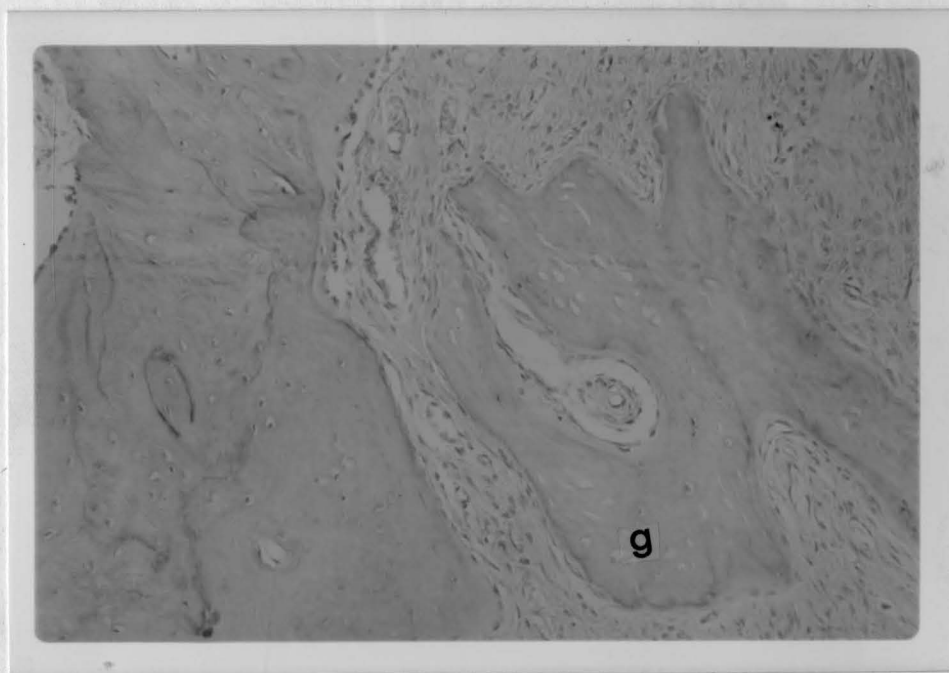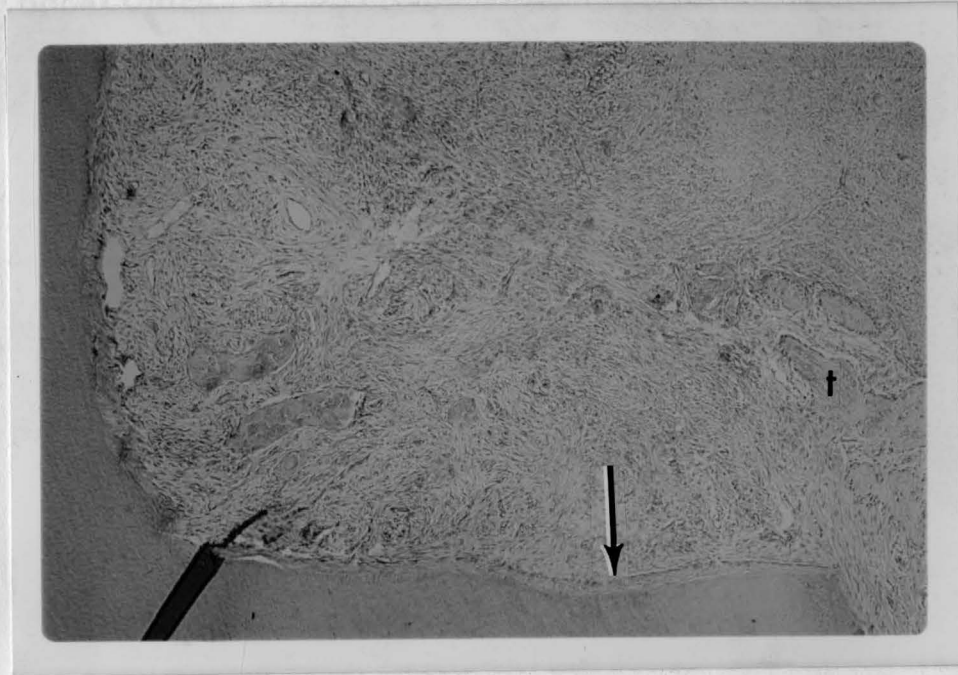
Fig. 38. 56 day frozen graft, 100X. Note that osseous
remodeling and incorporated graft fragments(g) are still
evident, and the recent reduction in the diameter of the
marrow cavity by bone apposition.

Fig. 39. 21 day control, 40X. Note the fibrous
connective tissue that fills the osseous defect, the
cementoid apposition(arrow), and the young trabeculae(t),
at the alveolar crest.

REFERENCES

1. Schallhorn, R. G.: Eradication of bifurcation defects utilizing frozen autogenous hip marrow implants. Perio. Abstracts, 15:101, 1967.

2. Schallhorn, R. G.: The use of autogenous hip marrow biopsy implants for bony crater defects. J. Periodontol., 39:145, 1968.

3. Seibert, J.: Reconstructive periodontal surgery: Case report. J. Periodontol., 41:113, 1970.

4. Schallhorn, R. G., Hiatt, W. H., and Boyce, W.: Iliac transplants in periodontal therapy. J. Periodontol., 41:566, 1970.

5. Hegedus, Z.: The rebuilding of the alveolar processes by bone transplantation. Dent. Cosmos, 65:736, 1923.

6. Beube, F. E. and Silvers, H. F.: Influence of devitalized heterogeneous bonepowder on regeneration of alveolar and maxillary bone of dogs. J. Dent. Res., 14:15, 1934.

7. Beube, F. E. and Silvers, H. F.: Further studies on bone regeneration with the use of boiled heterogeneous bone. J. Periodontol., 7:17, 1936.

8. Murry, C. R.: The modern conception of bone formation and its relation to surgery. J. Dent. Res., 11:839, 1938.

9. Beube, F. E.: Factors in the repair of alveolar bone and cementum. Oral Surg. Oral Med. Oral Path., 21:379, 1949.

10. Cross, W. G.: Heterogenous bone graft, Dent. Practitioner. 5:429, 1955.

11. Cross, W. G.: Bone grafts in periodontal disease. Dent. Practitioner, 6:98, 1955.

12. Cross, W. G.: Bone implants in periodontal disease-a further study. J. Periodontol., 28:184, 1957.

13. Cross, W. G.: The histology of a bone implant. Dent. Practitioner and Dent. Record, 8:26, 1957.

14. Cross, W. G.: The use of bone implants in the treatment of periodontal pockets. Dent. Clinics N. America, 107-115, Mar. 1960.

15. Linghorne, W. J., and O'Connell, D. C.: Studies in the regeneration and reattachment of supporting structures of the teeth-regeneration of alveolar process. J. Dent. Res., 30:604, 1951.

16. Stallard, R., and Randall, W.: Effect of calf bone on rate of bone regeneration in tibia of guinea pigs. J. Periodontol., 30:348, 1959.

17. Radentz, W., and Collings, K. C.: The implantation of plaster of Paris in the alveolar process of dogs. J. Periodontol., 36:357, 1965.

18. Forsberg, H.: Transplantation of Os Purum and bone chips in the surgical treatment of periodontal disease. Acta Odont. Scandinav., 13:235, 1956.

19. Schaffer, E. M.: Cartilage grafts in human periodontal pockets. J. Periodontol., 29:176, 1958.

20. Levander, G.: An experimental study of the role of the bone marrow in regeneration. Acta Chir. Scand., 83:545, 1940.

21. Goldhaber, P.: Bone induction across a millipore filter in vivo. J. Dent. Res., 40:703, 1961.

22. Bruns, P.: Ueber transplantation von knochenmark. Arch. Klin. Chir., 26:661, 1881.

23. Ham, A. W., and Harris, W. R.: Repair and transplantation of bone. Biochemistry and Physiology of Bone. Edited by G. H. Bourne. Academic Press, New York, 1966.

24. Bloom, W. A.: A note on osteogenesis by myeloid reticular cells. J. Infect. Dis., 107:11, 1960.

25. Burwell, R. G.: Studies in the transplantation of bone. VII. The fresh composite homograph-autograft of cancellous bone. J. Bone and Joint Surg., 48-B:532, 1964.

26. Cushing, M.: Review of Literature: autogenous red marrow grafts: their potential for induction of osteogenesis. J. Periodontol., 40:492, 1969.

27. McLean, F. C. and Urist, M. R.: Bone. University of Chicago Press, Chicago, 1968.

28. Nabers, C.L. and O'Leary, T. J.: Autogenous bone transplants in the treatment of osseous defects. J. Periodontol., 36:5, 1965.

29. Nabers, C. L. and O'Leary, T. J.: Autogenous bone grafts: Case report. J. Am. Soc. Periodontol., 5:251, 1967.

30. Robinson, R. E.: Osseous coagulum for bone induction. J. Periodontol.-Periodontics, 40:503, 1969.

31. Rivault, A. F., Toto, R. D., Levy, S., and Gargiulo, A. W.: Autogenous bone grafts: osseous coagulum and osseous retrograde procedures in primates. J. Periodontol., 42:787, 1971.

32. Coverly, L. K.: Osseous coagulum: A histologic study. M. S. Thesis, Loyola University, 1972.

33. Rosenberg, M. M.: Free osseous tissue autografts as a predictable procedure. J. Periodontol., 42:195, 1971.

34. Burwell, R.: Studies in the transplantation of bone VIII. Treated composite homograft-autografts of cancellous bone: An analysis of inductive mechanisms in bone transplantation. J. Bone and Joint Surg., 48:532, 1966.

35. Kramer, G.: Evaluation of the variations in approach to treatment of the periodontal osseous lesion. Presented at the 5th annual meeting of the Amer. Soc. of Periodontists, June, 1966.

36. Boyne, P. and Luke, A.: Acceptance of bone homografts combined with autogenous marrow. J. Dent. Res., 47 (Supplement):110, 1968.

37. Richter, H., Sugg, W., and Boyne, P.: Stimulation of osteogenesis in the dog mandible by autogenous bone marrow transplants. J. Oral Surg., 26:392, 1968.

38. Boyne, P.: Use of marrow grafting in regeneration of the mandibular body. J. Dent. Res., 48 (Supplement):96, 1969.

39. Boyne, P. and Yeager, J.: Evaluation of the osteogenic potential of frozen marrow. J. Dent. Res., 48 (supplement):96, 1969.

40. Small, G. and Hayward, J.: Osteogenic induction with bone marrow. J. Dent. Res., 48 (Supplement):69, 1969.

41. Yeager, J. and Boyne, P.: Use of bone homografts and autogenous marrow in restoration of edentulous alveolar ridges. J. Oral Surg., 27:185, 1969.

42. Newman, M. G. and Boyne, P. J.: The effect of calcified bone matrix on the osteogenic potential of hematopoietic marrow. J. Oral Surg., 32:506, 1971.

43. DeBruyer, P. P. H. and Kabisch, W. T.: Bone formation by fresh and frozen, autogenous and homogenous transplants of bone, bone marrow, and periosteum. Amer. J. of Anatomy, 96:375, 1955.

44. Polge, P., Smith, A., and Parkes, J.: Rivival of spermatozoa after vitrification and dehydration at low temperatures. Nature, London, 164:666, 1949.

45. Smith, A. and Polge, P.: Storage of bull spermatozoa at low temperatures. Vet. Rec., 62:115, 1970.

46. Smith, A.: Use of glycerol for preservation of living cells at low temperatures. Proc. Royal Soc. Medicine, 47:57, 1954.

47. Pegg, D.: Freezing of bone marrow for clinical use. Cryobiology, 1:64, 1964.

48. Pegg, D.: In vitro assessment of cell viability in human bone marrow preserved at -79°C. J. Appl. Physiol., 19:123, 1964.

49. Pegg, D.: Cytology of human bone marrow subjected to prolonged storage at -79°C. J. Appl. Physiol., 19:301, 1963.

50. Malinin, T. I.: Long term storage of bone marrow cells at liquid nitrogen and dry ice temperatures. Cryobiology, 7:65, 1970.

51. Rubinstein, A. and Trobaugh, F.: Ultrastructural changes in frozen hematopoetic tissue, Abstract. XIII International Congress of Hematology, Munich, Germany, Aug., 1970.

52. Barkin, M. and Newman, R.: Ultrastructure of bone Marrow prior to and after programmed freezing. Oral Surg., Mar., 1972.

53. Kromer, H.: Behandling av de infraossose lommer. Norske Tannlaegeforenings Tidende, 66:564, 1956.

54. Ashwood-Smith, M. J.: Preservation of mouse bone marrow at -79°C. with DMSO. Nature, 190: 1204, 1961.

55. Barchi, R. L. et al.: Ultrastructural changes in the mouse of frozen-preserved canine ileum. Cryobiology, 6:211, 1969-1970.

56. Maryman, H. T.: Review of Biological Freezing. In Cryobiology. H. T. Meryman, ed., pp. 1-114, Academic Press, N. Y., 1966.

57. Smith, A. U.: Current Trends in Cryobiology. A. U. Smith, ed., pp. 139-153, Plenum Press, N. Y., London, 1970.

58. Sherman, J. K.: Pre-treatment with protective substances as a factor in freeze-thaw survival. Cryobiology, 1:298, 1965.

59. Meryman, H. T.: Cryoprotective agents. Cryobiology, 8:173, 1971.

60. Farant, J., et al.: Preservation of structure and function of an organized tissue after freezing and thawing. Proc. Royal Soc. Medicine (London), B168:293, 1967.

61. Meryman, H. T.: Osmotic stress as a mechanism of freezing injury. Cryobiology, 8:489, 1971.

62. Litvan, G. G.: Mechanism of cryoinjury in biological systems. Cryobiology, 9:182, 1972.

63. Persidsky, M. D.: Lysosomes as primary targets of cryoinjury. Cryobiology, 8:482, 1971.

64. Steere, R. L.: Freeze-etching and direct observation
    of freezing damage. Cryobiology, 6:137,
    1969.

65. Mazur, P.: Kinetics of water loss from cells at
    subzero temperatures and the likelihood
    of intracellular freezing. J. Gen. Physiol,
    47:347, 1963.

66. Lovelock, J. E.: Denaturation of lipid-protein complex-
    es as a cause of damage by freezing.
    Proc. Royal Soc. Medicine (London), B147:
    427, 1957.

67. Wolstenholme, G. E. and O'Connor, M.: The Frozen Cell.
    pp. 51-63, J. and A. Churchill, London,
    1970.

68. Dragoo, M. R. and Irwin, R. K.: A method of procuring
    cancellous iliac bone utilizing a trephine
    needle. J. Periodontol., 43:82, 1972.

69. Abercrombie, M.: Biology of the Periodontium. Edited
    by A. H. Melcher and W. H. Bowen. Academic
    Press, London-New York, 1969.

70. Schmidt, A. J.: Cellular Biology of Vertebrate
    Regeneration and Repair. University of
    Chicago Press, Chicago, 1968.

71. Tieche, R. W.: Oral Pathology. McGraw-Hill Book Co.,
    New York, 1965.

72. Grillo, H. C.: Biology of the Periodontium. Edited
    by A. H. Melcher and W. H. Bowen. Academic
    Press, London-New York, 1969.

73. Urist, M. R. and McLean, F. C.: Osteogenic potency
    and new bone formation by induction in
    transplants to the anterior chamber of the
    eye. J. Bone and Joint Surg., 34A:443,
    1952.

74. Trueta, J.: The role of the vessels in osteogenesis.
    J. Bone and Joint Surg., 45B:402, 1963.

75. Haggerty, P. C. and Maeda, I.: Autogenous bone grafts:
    A revolution in the treatment of vertical
    bone defects. J. Periodontol., 42:626,
    1971.

76. Melcher, A. H.: Biology of the Periodontium. Edited
    by A. H. Melcher and W. H. Bowen. Academic
    Press, London-New York, 1969.

77. Rhodes, P.: Reconstructive periodontal surgery. The
    iliac crest bone marrow autograft. Presented
    at the 55th Annual Meeting, Amer. Acad.
    of Periodontology, Philadelphia, Pennsyl-
    vania, Oct., 1969.

78. Schallhorn, R. G.: Additional observations on hip
    marrow implants. Presented at the Spring
    Meeting, Amer. Acad. of Periodontology,
    New Orleans, Louisiana, May, 1969.

79. Burnette, E. W.: Fate of an iliac crest graft. J. Perio-
    dontol., 43:88, 1972.

80. Seibert, J.: Personal Communication.

81. Morris, M. L.: Implantation of human dentin and cemen-
    tum with autogenous red marrow in the rat.
    J. Dent. Res., 49 (Supplement): 180, 1969.

82. Morris, M. L.: The implantation of human dentin and
    cementum with autogenous bone and red
    marrow into the subcutaneous tissues of the
    rat. J. Periodontol.-Periodontics, 40-259,
    1969.

83. Morris, M. L.: A study of the inductive properties of
    the organic matrix of dentin and cementum.
    J. Periodontol., 43:10, 1972.

84. Hiatt, W., Stallard, R., Butler, E. and Badgett, B.:
    Repair following mucoperiosteal flap
    surgery with full gingival retention. J.
    Periodontol., 39:11, 1968.

85. Stallard, R. and Hiatt, W.: The induction of new bone
    and cementum formation. I. Retention of
    mineralized fragments within the flap.
    J. Periodontol., 39:273, 1968.

APPROVAL SHEET

This thesis, submitted by Don M. Morris, has been read and approved by three members of the faculty of the Department of Oral Biology.

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

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

May 17, 1973
Date

Alice McAllister
Signature of Advisor