A HISTOPATHOLOGIC STUDY OF

AUTOLOGOUS ORTHOTOPIC PULP GRAFTS IN DOGS

BY

BUCKNER S. BURCH, D.D.S.

A THESIS

Presented to the Department of Oral Pathology
and the Graduate Education Committee of the
University of Oregon Dental School
in Partial Fulfillment
of the Requirements for the Degree of
Master of Science
July, 1965

APPROVED

[redacted]

Professor of Dentistry

[redacted]

David B. Mahler, Ph.D.
Chairman, Graduate Education Committee

ACKNOWLEDGEMENTS

The author is indebted to Dr. Norman H. Rickles for his advice and criticism during the preparation and completion of this work and to Dr. Robert J. Bruckner and Dr. Stanley W. Jacob for their most helpful suggestions and comments.

The author wishes to thank Elda Harvey for her capable technical assistance which greatly aided in the completion of this work.

This investigation was carried out during the tenure of Graduate Training Grant 2 TI DE 29-06 from the National Institute of Dental Research, United States Public Health Service.

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF PLATES

LIST OF PLATES

INTRODUCTION

The field of transplantation arose out of the need to replace defective or missing tissue and thus is especially applicable to dentistry. It has been estimated that more than one third of the children in the United States will become dental cripples through the loss of one or more permanent first molar teeth before they reach adulthood. (46) This early loss of teeth can largely be attributed to uncontrolled caries and subsequent pulp infection. The definitive treatment of teeth with diseased pulps is currently limited to tooth extraction or partial or complete pulp removal and the appropriate adjuvant treatment. An alternate approach to this problem would be the removal of the pathologically involved portions of the dental pulp and their replacement with healthy, viable pulp tissue. This pulp tissue could be obtained from extracted teeth that had been malposed or impacted or from teeth extracted for orthodontic reasons. The following study is a preliminary investigation of the problem of pulp transplantation and will consist of a histopathologic evaluation of autologous orthotopic pulp grafts in dogs.

Since there have been no reports in the literature dealing specifically with the transplantation of pulp tissue from one tooth to another the review of the literature has necessarily dealt with related areas. The areas most concerned with the problem are the basic concepts of transplantation and the transplantation of teeth.

REVIEW OF THE LITERATURE

Basic Concepts of Transplantation

History of transplantation. The literature of antiquity shows tissue transplantation being practiced in ancient times. (35) The Ebers Papyrus (1500 B.C.) reveals that tissue grafting was utilized by the Egyptians as far back as 3500 B.C. The earliest references to the art were principally concerned with the correction of noses damaged by the ravages of syphilis and the avenging knife of the conquering enemy or the outraged husband.

The progress of tissue transplantation, like that of science as a whole, followed the rise and fall of empires. The first century found Rome a medical center with tissue transplantation flourishing as a fine art. Galen (130-210 A.D.) more than anyone else was responsible for the advanced reparative surgery practiced during the greatness of Imperial Rome. He gave instructions regarding the repair of defects about the ears, nose and mouth and advised the approximation of wounds by sutures. During the Dark Ages all science declined and what was known of transplantation was forgotten. The art of transplantation fared little better during the Middle Ages. Gasparo Tagliacozzi (1546-1599) of Bologna is generally considered the father of plastic surgery as it is known today. He is given credit for the use of skin flaps from the arm.

The history of tissue transplantation includes many notable advances. However the last twenty years stand out as the "golden era".

One of the major milestones in tissue transplantation was the book entitled "The Biologic Basis of Individuality" published in 1945. (59) In it Leo Loeb tried to systematically analyze the mechanisms involved in homograft rejection. Although many of the concepts presented have been altered by more recent investigations it ranks as a scholarly classic in the field. (25)

Transplantation terminology. Before embarking on a discussion of the transplantation of tissues it is necessary to classify the various types of grafts or transplants. Transplanted tissue may be classified according to the relationship between the donor and host, anatomical position and viability.

Many tissues of man and experimental animals can be excised and grafted to other positions in the same individual. These autografts re-establish their blood supply and function normally for the life of the individual.

Isografts are transplants between individuals of the same (or nearly the same) genetic background such as monozygotic twins and highly inbred laboratory animals. Isografts will normally survive for the lifetime of the host.

If normal tissue is transplanted between individuals of the same species but of a different genetic background the graft will be destroyed. Such transplants are homografts and may survive and function for a time, and with few exceptions inevitably end in failure. The process which destroys grafts between genetically different individuals is referred to as the homograft reaction. Transplants between members of different species are heterografts and like homografts are doomed to failure. These four kinds of grafts are described as autologous,

Table 1. Proposed Revisions of Transplantation Terminology (42)

Nouns			Adjectives		
Old Terms	Proposed New Terms	Alternative New Terms	Old Terms	Proposed New Terms	Alternative New Terms
Autograft	Autograft	-	Autologous	Autoplastic	-
Isograft	Syngeneic homograft or graft	Isogenic homograft or graft	Isologous	Syngeneic	Isogenic
Homograft	Allogeneic homograft or graft	-	Homologous	Allogeneic	-
Heterograft	Xenograft	Heterospecific graft Interspecific graft Heterograft	Heterologous	Xenogeneic	Heterospecific Heterologous

isologous, homologous and heterologous.

Transplants classified as to their anatomical position are orthotopic grafts and heterotopic grafts. An orthotopic graft is transplanted to an anatomically similar position, as skin to a prepared site in skin. Grafts placed in unnatural positions are heterotopic, such as a tooth bud transplanted to the brain.

The cells of a transplant may not have to survive for the graft to be considered successful. Thus, a homostatic transplant can function as a framework to be ultimately replaced by the host tissue. A freeze-dried artery is such a graft. In contrast the success of a homovital transplant depends on the maintenance of cellular viability. A kidney transplant is an example of a homovital graft.

Revised and more precise terminology has recently been advocated for transplantation research. (42) This terminology is starting to appear in the literature, especially European, but most American writers still use the established terminology as described in the previous paragraphs. Table 1 lists the established terms and proposed new terms.

Transplantation genes and antigens. It is generally accepted that homografts and heterografts are rejected by an immunologic reaction directed against certain substances residing in the graft. These substances are designated as transplantation antigens or histocompatibility factors. Transplantation antigens are determined by genes designated as histocompatibility genes. These are inherited by simple Mendelian laws and are generally considered to be dominant.

Essentially all the nucleated cells of an individual contain the same transplantation genes. Thus transplantation antigens are individual specific rather than organ or tissue specific.

The minimum number of histocompatibility loci has been estimated at fourteen in mice, (2) and twenty-three in man. (60) Histocompatibility factors are also linked to the Y and possibly the X chromosomes in certain strains and combinations of laboratory animals. (2) The number of possible genotypes in an entire species would be extremely large when all the alternative allelic forms are taken into consideration. One important histocompatibility "region" (H-2) in mice can be occupied by at least eighteen different alleles. (2)

Different loci produce antigens of different strength; therefore, certain histocompatibility genes play a more important role in tissue compatibility than do others. The strength of the rejection reaction is due to the combined effects of the various loci.

The exact nature of the transplantation antigens is unknown. It is believed that the antigens determined by at least one locus (H-2) in mice consists of a complex lipoprotein probably associated with the cell membrane and endoplasmic reticulum. (62)

Immune response. The immune response effecting the rejection of grafts is initiated by the passage of graft antigens to the regional lymph nodes via the draining lymphatics. After the transplantation antigens have entered the recipient's regional lymph nodes, specific antibody is produced which appears both attached to the proliferating host immune cells and in the serum. The activated lymphoid cells pass into the general circulation and may subsequently activate other lymphoid cells of the body. (61) In the blood stream the activated lymphoid cells travel to the transplantation site and by an unknown mechanism destroy the graft.

The primary agent responsible for this destruction is believed to

be attached to the invading lymphocytes, that is, a cell-bound antibody. The role of serum antibodies in graft rejection is unknown; however, it is quite possible that graft rejection is due to the combined effects of cell-bound and serum antibodies.

Once an animal has rejected a homograft with a first-set reaction the host develops a generalized altered reactivity to subsequent homografts from the same donor. If a second homograft of skin is applied during the first seven days following the first-set reaction, the graft does not take, and the process of rejection is referred to as a white graft reaction. (79) If the latent period after the first-set reaction is lengthened to ten to fifteen days, the second graft initially takes but then exhibits a second-set reaction which is more rapid and more violent than the first-set reaction. (78) The white graft and the second-set reactions are specific because they are directed only against those grafts which contain the same transplantation antigens as the original donor. (80)

Homograft reaction. Since skin is used extensively in homograft studies, it will be used as a model in describing the gross and histologic changes which occur in the homograft reaction. (24) Autografts and homografts of skin are grossly and microscopically identical for the first few postoperative days. By the second or third day they have acquired a blood supply through ingrowth of host capillaries and anastomoses of graft vessels with those of the host. Of the two, vascular anastomosis appears to play the more important role. When the blood supply is first established, the graft exhibits dilated vessels and a sluggish flow of blood which increases and becomes generalized by the fifth or sixth day. During this period the graft

becomes a healthy pink color. In the autograft the number of vessels increases up to the seventh or eighth day and the dilated vessels become less numerous. By the tenth day the vascular pattern of the autograft is similar to that of the surrounding normal skin. In contrast, by the end of the first postoperative week the homograft and graft bed are invaded with numerous lymphocytes and plasma cells and the graft vessels exhibit progressive dilation and diminished blood flow. The flow of blood ceases by the eighth or ninth day and hemorrhage occurs. The homograft grossly becomes swollen and cyanotic forming a brownish eschar by the fifteenth day. The final stage is reached in three weeks when the homograft is sloughed. Meanwhile the autograft has healed into place and cannot be distinguished from the surrounding host tissue.

The histological findings in the first-set, white graft and second-set reactions differ. The second-set reaction, like the first-set reaction, is associated with initial graft vascularization, but unlike the first-set reaction rapid vascular breakdown occurs. Gross signs of rejection are evident on the fourth or fifth postoperative day. (78) In contrast the white graft reaction is characterized by the complete absence of vascularization, the graft remaining white and opaque and gradually changing into a pale eschar. (79)

Homograft survival. As previously discussed, grafts will survive permanently if the host contains all the histocompatibility genes found in the graft. There are also certain circumstances, both natural and artificial, in which homograft survival is prolonged.

A homograft to the brain will survive permanently if the host has not been previously sensitized by grafts containing the same antigens

elsewhere in the body. The survival of homografts in the brain is felt to be due to the absence of an organized lymphatic system in the central nervous system. Thus there is no afferent pathway for the antigenic stimulus of the graft to reach the host's lymphoid tissue. (81) Orthotopic corneal grafts survive as long as there is no vascular invasion. (65) The cornea normally contains neither lymphatic nor blood vessels. Vascular and nerve homografts do not survive but are used clinically because they form a framework for the host's own cells. Homografts of cartilage survive for extended periods of time because of the physical protective action of the mucopolysaccharide matrix. This matrix tends to isolate the chondrocytes of the graft from the host. (38)

Homografts survive in other privileged sites other than the brain and have prolonged survivals when certain host conditions exist. The privileged sites include the anterior chamber of the eye (81) and the cheek pouch of the Syrian hamster. (8) Host conditions which favor prolonged homograft survival are chronic uremia (22), hypogammaglobulinemia (40), Hodgkin's disease (53), neonatal thymectomy (41) and extensive burns. (52)

The homograft reaction can be modified by agents which decrease the general immunological response of an animal. This occurs with total body irradiation, cortisone, ACTH and drugs cytotoxic to the lymphoid tissue.

Acquired immunologic tolerance. Acquired immunologic tolerance is the state in which an otherwise immunologically competent host fails to react against a specific antigen. In 1945 Owen reported that dizygotic cattle twins often contained a mixture of two distinct red

blood cell types, its own and that of its twin. (77) This state of red blood cell chimerism was the consequence of prenatal exchange of hematopoietic precursors through vascular anastomosis between the placentas and the subsequent establishment and functioning of the transferred cells in the new host. Erythrocyte chimerism appears to be rare in animals other than cattle. However, it has been demonstrated in man on at least three occasions. (9, 23, 74)

In 1951 Anderson and co-workers demonstrated the acceptance of reciprocal skin homografts between dizygotic cattle twins that were erythrocyte chimeras. (3) Billingham and associates successfully recreated this state of diminsished resistance to foreign tissues by injecting mouse fetuses with cells from a foreign strain. (6) These mice, when adult, tolerated skin grafts from the donors of the injected cells. This observation gave rise to the concept of acquired immunological tolerance and has greatly broadened the approach to tissue transplantation. It has been subsequently shown that other animals can be made tolerant to homografts by using the same techniques.

The process of acquired immunologic tolerance can be explained by the Burnet theorem that if a young animal is confronted with an antigen before it is capable of responding by the formation of specific antibodies; the production of these antibodies in later life will be reduced or wholly suppressed. (16) The ability to abolish acquired immunologic tolerance by injecting adult isologous normal lymphoid cells into tolerant animals corroborates this theorem.

It was initially believed that immunologic tolerance could be produced only in young animals, but subsequent studies have proved otherwise. Homograft tolerance has been produced in mature animals

by repeated injections of intact spleen cells (13), splenic cell extracts (64) and by parabiosis for prolonged periods of time. (41)

The amount of antigen necessary to induce tolerance in the adult is far greater than in the newborn. A sufficient amount of antigen must be transported to the cells of the immune centers and this high level must be maintained for a sufficiently long period of time. (25)

The state of specific unresponsiveness to an antigen can also be produced to substances other than histocompatibility factors. These substances include serum proteins (85) and pneumococcal polysaccharides. (27)

Cells of certain organs are more effective in producing tolerance than others. Cells from lymph nodes and spleen seem most effective. Persistence of tolerance may not require the persistence of viable foreign cells. However, it does appear that the transplantation antigens must persist for maintenance of a tolerant state. (25)

Graft versus host reactions. When homologous lymphoid cells are used to induce tolerance in neonatal animals a runting syndrome is often observed. The features regularly associated with this syndrome are transient enlargement of the spleen and liver, late lymphoid atrophy, anemia, emaciation, failure to grow and often death. This phenomenon is referred to as runt disease and is the result of the homologous immunologically competent lymphoid cells reacting against the antigens of the host tissue. The severity of the graft versus host reaction is more or less directly related to the genetic disparity between the donor and host. (7)

Three prerequisites are essential for the graft to react against the host: 1) the graft must contain mature immunologically competent cells; 2) the host must contain antigens foreign to the graft; and 3) the host must tolerate the graft for a long enough period of time for the immune cells of the graft to grow and react against the host.

Transplantation of Teeth

History of tooth transplantation. The literature dealing with the history of tooth transplantation and reimplantation has been thoroughly reviewed. (20, 32) There is evidence that transplantation of teeth was practiced in ancient Egypt and later by the Etruscans, Greeks and Romans. Indian relics of North and South America reveal instances of tooth transplantation and replacement of teeth by stones.

Albulcasis de Condue (936-1013) is credited as being the first individual in the literature to advocate the replacement of missing or lost teeth. Pierre Fauchard in 1746 cited several instances in which he successfully reimplanted teeth which had previously been extracted because of pain. (26) He also discussed a homologous tooth transplant which was retained for six years.

John Hunter (1728-1793) popularized the replanting and transplanting of teeth during the middle and latter part of the eighteenth century. He advocated the transplantation of teeth from one individual to another.

Dentistry in colonial America consisted to a large extent of extracting and replanting teeth. Harvard Dental School and a dental school in Philadelphia were founded by men who were distinguished for their transplantation operations.

Reports of success varied greatly, some operators claiming one hundred percent success, while others indicated they had only partial success. The transplantation of teeth from one individual to another began to lose favor in this country as well as in Europe near the end of the eighteenth century when it was found that communicable diseases such as syphilis and tuberculosis were sometimes transmitted in this manner.

During the latter part of the nineteenth century clinical reports on transplantation of teeth became numerous. The majority of these reports considered such operations satisfactory.

The study of tooth transplantation can be divided into two categories, clinical and experimental. The vast majority of the literature in the area consists of clinical case reports with a notable paucity of papers representing fundamental research efforts.

Clinical transplantation. In reviewing the literature of tooth transplantation one is not impressed with its success as a technique for permanently replacing missing teeth. The universal failure of homologous human tooth transplants can be explained by our current concepts of transplantation immunology. The impermanence of fully developed autotransplanted teeth and reimplanted teeth may be related to the inadequate blood supply available through the small apical foramina. The majority of the latter are clinically successful for a maximum of two to four years. (32)

The autologous transplantation of tooth germs or unerupted teeth has recently received considerable attention. These procedures are generally more successful than those involving fully developed teeth. (19, 32, 36, 75, 91) Nordenram, after thoroughly evaluating sixty

unerupted third molars that were transplanted to the first molar site, reported that 73% were successful. (75) The maximum period of observation for this series was seven years.

The task of evaluating the results of tooth transplantation experiments is especially difficult since the criteria for measuring success varies extensively between the investigators. The points of greatest variance in measuring success are the length of survival and the relative importance of maintaining a normal vitality response. This latter point will be subsequently discussed.

It is generally believed that tooth transplantation follows the same rules governing the transplantation of other tissues, (33, 83) with autotransplantation of partially developed teeth enjoying a reasonable degree of success and homotransplantation of teeth resulting in inevitable failure. (1 , 19, 47) Some workers have reported homotransplanted teeth which were retained and clinically successful for several years, (21, 67) but there are no reports in the literature of permanently surviving homologous tooth transplants. (83)

The rejection of retained homotransplanted teeth is a slow insidious process characterized by various forms of calcified material being formed along the pulp walls, slow root resorption, loosening and, in the absence of surgical intervention, eventual exfoliation.

Experimental transplantation. Experimental transplantation of teeth and tooth germs may be divided into *in vivo* and *in vitro* studies. The majority of the *in vitro* studies with tooth germs have been concerned with the survival, differentiation and organization of tooth germs and the relationship of epithelial tissues to mesenchymal tissues. (39, 45, 55, 56) However, some transplantation-oriented *in vitro*

studies also have been undertaken. (57) Most of the *in vivo* investigations have been done with experimental animals because of the inherent difficulties in recovering human teeth following clinically successful transplantation.

While Yumikura had previously carried out *in vitro* investigations with teeth (32) Glasstone was the first to report on the actual cultivation of whole tooth buds. (39) She showed that tooth buds removed from the body retained their capacity for producing developing tooth components in a normal histologic manner. This work demonstrated the organizing influence of the ameloblasts on the dental papilla. An interesting finding of this and other studies was that the enamel and dentin of tooth buds grown in tissue culture did not calcify. (56, 58)

It has been shown that rat tooth buds can be maintained in tissue culture for at least twenty days. (56) At the end of this period they had reached a level of development equivalent to five days *in vivo*. The enamel matrix and predentin did not calcify and the amount of matrix formed was quantitatively equivalent to that which occurs before the tissues are calcified *in vivo*.

In vivo studies of tooth transplantation dates back to the time of John Hunter (1728-1793) and perhaps earlier. (32) He transplanted a freshly extracted human tooth to an incision in a cock's comb. Several months later, after sacrificing the cock and injecting the head with dye, he demonstrated patent blood vessels within the pulp of the transplanted tooth.

Teeth and tooth germs have been transplanted to many different sites including the abdominal musculature (49); the brain (29); the

anterior chamber of the eye (28); the sacrolumbar fascia (76); the marrow cavity of the tibia (89); the chorio-allantoic membrane (10); and subcutaneous tissue. (48) Intact tooth germs generally retained their form with regular differentiation of the odontoblasts and ameloblasts and produced histologically normal tubular dentin and enamel.

The transplantation of tooth germ elements has also been investigated. (49, 73) Isolated enamel-forming tissues were found to produce no organizing effect on the host tissue. The ameloblasts lost their characteristic form and reverted to stratified squamous epithelium. In transplanted intact pulp tissue the odontoblasts differentiated and/or survived and produced a modified tubular dentin. When pulp tissue free of odontoblasts was transplanted, the stellate-shaped pulp cells were easily seen, but no dentin or calcified material was found. These findings indicated that enamel formation did not occur without the influence of odontoblasts and that ameloblasts were not necessary for dentin formation. (73)

Fleming (28) found that homologous and heterologous tooth germ transplants to the anterior chamber of the eye survived and developed for prolonged periods of time when the embryonic tissue was transplanted prior to calcification. When calcification had occurred prior to transplantation the graft was usually destroyed. He also demonstrated that homologous subcutaneous tooth bud transplants survived better when the hosts were periodically injected with serum or tissue extract from the donors. (34)

Lefkowitz was the first investigator to describe the microscopic findings in the rejection of homologous tooth bud transplants. (57) He found that homologous tooth bud transplants in rats exhibited the

usual homograft reaction. The first evidence of rejection was seen at the end of the first week and was characterized by a dense infiltrate of lymphocytes in the dental sac. Within two weeks there was a more extensive lymphocytic infiltrate and by three weeks there was degeneration of the enamel organ and dental papilla. Specimens taken at three weeks showed that none of the soft tissue elements of the transplant survived and that the formed enamel matrix and dentin were being resorbed. Tooth buds which had been cultured previous to transplantation in a medium containing the host's serum fared better. At the end of the first week the buds were vascularized as were the uncultured buds, but unlike the latter there was no lymphocytic infiltrate. At the end of six weeks, when the study was terminated, there was no evidence of rejection. Development of the transplanted cultured buds progressed to root formation with a thin layer of cementum covering the gingival third of the root. Mature dentin formed but the enamel remained in the matrix state. The major difference between the cultured six-week graft and the normal odontogenic pattern was the lack of enamel maturation and the decreased growth rate.

In rhesus monkeys treated with 6-Mercaptopurine the pulpal tissue of homologous incisor transplants retained a normal appearance for three to six weeks with elaboration of secondary dentin, while the pulpal tissue in untreated homologous teeth underwent a rapid change to acellular fibrous connective tissue. (70) At eight to twelve weeks the homologous transplants in the treated animals underwent similar changes. However, there was no round cell infiltration or necrosis noted in any of the specimens.

Several studies have been concerned with the storage of tooth buds by freezing. Tooth buds which had been pretreated with glycerol, frozen, and subsequently thawed and transplanted fared much better than those which were not pretreated with glycerol. (18, 76, 93) Similar results were achieved with tooth buds which had been frozen prior to placement in tissue culture. (37) These studies have shown that tooth germs can be frozen to an extremely low temperature and still remain viable. However, the survival rate for pretreated frozen transplanted teeth is about one-half that of unfrozen controls. (93)

Many investigators have published accounts of their work in this area, but these studies were usually limited to morphologic observation of autologous, homologous, and heterologous tooth or tooth germ transplants. It was not until 1962, nearly two hundred years after Hunter's transplantation studies, that one of the basic questions in tooth transplantation was investigated. That is, do teeth contain histocompatibility factors capable of eliciting an immunologic response in the host? Guralnick and Shulman were the first to investigate this problem and they demonstrated that teeth and/or their periodontal membranes contained transplantation antigens. (44) This was accomplished by showing that subcutaneous tooth transplants in rabbits caused an accelerated rejection of subsequent skin homografts from the same donor. A logical sequel to this work would be the determination of the individual role of the tooth's calcified and soft tissue components in the process of homograft sensitization.

Pulp reaction to tooth transplantation. A number of reports have described the histologic findings in transplanted human (51, 67, 68)

and animal teeth. (1 , 19, 36, 75) The monkey has been one of the most popular experimental animals because of the functional and anatomic similarity between its masticatory apparatus and that of man's. (1 , 36, 75) This similarity makes it possible to perform transplantation studies using techniques identical to those used in humans. Because of the inherent difficulties in recovering human teeth at regular intervals after transplantation it is difficult to determine histopathologically the sequence of events taking place. However, since the histologic changes seen in the available transplanted human teeth and animal teeth are similar, the findings in the latter are helpful in understanding the process as it occurs in man.

As with skin, revascularization had not occurred in the transplanted unerupted third molars during the first two or three postoperative days. (75) During this interval the nutrients and waste products were transported between the graft and host by diffusion through the organizing blood clot. This method of interchange has been demonstrated with autoradiograms. (75) In the initial period following transplantation Hertwig's root sheath was discernible although it had deviated in position. At thirty days the sheath was difficult to detect.

The pulp tissue showed progressive histological changes with the least evidence of alteration in the apical portion and the most notable changes in the coronal area, the region most distal to the source of potential diffusion. (36, 75) In some studies the pulpal tissue was free of mass necrosis or autolysis. (36) The range of reactions varied from complete necrosis to a nearly normal appearance. (19)

Four days following transplantation in regions contiguous to the

tissues of the host, the odontoblasts were still identifiable along the lower border of the root ends and in the vicinity of Hertwig's epithelial root sheath. The pulp in the roots retained the characteristic appearance of young pulp, while histologic changes were evident in regions remote from the vascular tissue of the host. Passing upwards from the apex to the crown of the tooth, the odontoblasts in some regions had disappeared whereas in others they were distorted, although still identifiable through their positions and arrangement.

The pulpal tissue in the upper chamber and horns was stained much more lightly than elsewhere and contained areas of hemorrhage. The cells in this and other areas of the coronal pulp exhibited pyknosis and karyorrhexis.

At the end of four weeks the pulp was relatively healthy, although certain significant changes were occurring. Bone-like tissue or osteodentin started to develop in the pulp tissue of both the root and the crown. This tissue tended to cover the entire wall of preoperatively formed dentin. The pulp showed different characteristics at different levels. In the apical and lower canal regions it was comparable to normal pulp in cellular and intercellular character. There was clear evidence of recanalization of the original ruptured vessels. The more remote portions of the pulp tissue contained extensive amounts of dense fibrous connective tissue. Occasionally, odontoblasts and narrow zones of dentin were evident on the pulpal surface of the osteodentin. (36, 51, 75)

Experiments carried out over a longer period of time, from several months to two years, revealed that practically all the pulp chamber was

filled in with osteodentin. Also, in many teeth there was a spike-like ingrowth of periapical bone which extended deeply into the pulp. (36, 75) The connective tissue surrounding this spike of periapical bone showed transformation into a fibrous structure similar to periodontal membrane with some fiber bundles linking the bone with the pulp canal walls.

The roots, if not fully formed at the time of transplantation, were distorted and failed to achieve their normal length. Evidence of resorption on the pulpal surface was not common and when it did occur was usually repaired with dentin. In contrast, cementum resorption was usually found. (19)

The formation of bone-like tissue or osteodentin in the pulp of transplanted teeth is a common phenomenon. The process can be followed with roentgenograms or histologic studies and occurs in homologous, autologous and heterologous tooth and tooth bud transplants. The withdrawal of vitamin A (92) and vitamin C (11) from the diets of experimental animals results in the deposition of a similar hard tissue in the pulp. The derivation of the cells responsible for the formation of the osteodentin has not been established. Wolbach feels that the cells may be dedifferentiated odontoblasts (92) but this theory has been challenged by Fleming who believes that the "osteoblastic" cells are differentiated from immature pulp cells. However, since dentin and osteodentin are formed in heterotopically transplanted pulp tissue containing odontoblasts and not in heterotopically transplanted pulp tissue free of odontoblasts (89) Wolbach's theory may have some merit.

Sorg, working with replanted hamster teeth, demonstrated an apparent inverse relationship between the quantity of pulpal nerve regeneration and the amount of osteodentin produced. (87) This relationship seemed to hold true even in teeth exhibiting adequate revascularization. He hypothesized that odontoblasts deprived of an adequate nerve supply may dedifferentiate and produce osteodentin.

The etiology of this pulpal response is even more poorly understood than the pathogenesis. Both trauma and interference with the pulpal blood supply may play a role in this phenomenon. Fleming has shown that tooth germs with undamaged pulps had a better blood supply and exhibited less tendency to form osteodentin than tooth buds that had been damaged during transplantation. (31) Another interesting finding is that osteodentin production is most prominent in the tissues farthest away from the incoming blood supply. These observations may be significant in light of the increasing evidence that a decrease in local circulation enhances bone formation. (43)

Autoradiographic and microradiographic studies using transplanted monkey teeth showd that the osteodentin was poorly calcified but had a high radioactive calcium uptake. When the transplanted teeth were compared to the control teeth by these methods there was no appreciable difference other than that of the osteodentin. (75)

The survival of the pulp of transplanted teeth (47), as does the survival of any transplanted tissue (66), depends on the early re-establishment of circulation. The first stages of circulation are characterized by an end-to-end anastomosis between the blood vessels of the graft and the host. (24) The proximity of the blood vessels of transplant and host may have some bearing on the following observations

with transplanted teeth: transplants not involving socket preparation are generally more successful than those in which extensive procedures have been done (71, 72); the failure of teeth transplanted in areas where the roots will project into large boney defects created by infections or cysts. (19)

The question of pulp response and nerve regeneration following tooth transplantation has been discussed in the literature but still remains unresolved. The return of sensation has been reported in both homologous (67) and autologous tooth transplants. (17, 19) However, sensation in transplanted teeth appears to be the exception rather than the rule. (33) It is generally conceded that vitality tests, mechanical, thermal or electrical, are probably not reliable indications of success or failure because the viability of the pulp is primarily dependent on the re-establishment of the vascular supply and nerve regeneration is variable. (47)

The fate of nerves in transplanted (75) and replanted (87) teeth of experimental animals has been described. Two days after transplantation there were signs of nerve degeneration and at seven to fourteen days no nerves were evident. Newly developing nerves were first observed in the apical region at thirty days and later further up in the coronal pulp chamber. The nerves tended to follow the blood vessels. (75) In replanted hamster teeth nerve regeneration occurred in only those teeth which contained histologically normal pulp. (87)

MATERIALS AND METHODS

Experimental Animals, Teeth and Time Intervals

Experimental animals. Seven dogs from two litters were used in this investigation. The animals had been kennel-raised and were a mixture of Samoyed, Labrador and Basenji. They ranged from eight to ten months of age at the time of the transplantation procedure and from ten to eleven months at the time of sacrifice. Two males and one female from the same litter were used in the nine-week postoperative time interval, and four males from another litter were used in the three- and five-week intervals. After the transplantation operation each animal was given one gram of tetracycline hydrochloride orally at seven day intervals. The drug was given to label the postoperatively formed dentin and not as an antibacterial agent. The animals ranged in weight from thirty-eight to forty-five pounds and were housed in steel cages and fed Purina Dog Chow.

Experimental and control teeth. The maxillary and mandibular canine teeth and the right mandibular first molars were utilized in this experiment. According to Miller these teeth are usually fully erupted by the fifth or sixth month. (69) The right maxillary and left mandibular canines were used as the host teeth, and the right mandibular and left maxillary canines as the controls. Each host tooth was treated identically, whereas each of the two control teeth served as a distinct type of control. The right mandibular canine

Table 2. Vital Statistics of Experimental Animals.

Kennel Number	Postoperative Time Interval (weeks)	Age at Operation (days)	Age at Sacrifice (days)	Sex
3416	9	244	307	Male
3422	9	264	327	Female
3420	9	271	328	Male
3059	5	293	328	Male
3057	5	301	336	Male
3056	3	304	324	Male
3058	3	308	329	Male

control was not disturbed (virgin control), and the left maxillary canine control was treated in a manner similar to the host teeth except that it did not receive any transplanted tissue (sham-operated control). The right mandibular first molar teeth were utilized as the donors of the pulp tissue.

Time intervals. The seven identically treated dogs were arranged into three postoperative time groups of three, five and nine weeks. At the end of each respective time period the experimental and control teeth were removed en bloc and the dog sacrificed. Two dogs were utilized in each of the three- and five-week periods and three in the nine-week period.

Surgical Procedure

Anesthesia. The surgical plane of anesthesia was initially produced by the injection of fifty milligrams of sodium pentobarbital solution for each five pounds of body weight into the cephalic vein on the dorsal surface of the foreleg. After anesthesia was attained an intravenous catheter was inserted into the cephalic vein and a three-way valve attached to the exposed hub of the catheter. One of the remaining two fittings on the valve was attached to a 20 ml. syringe containing anesthetic solution and the other to a 500 ml. bottle of physiologic saline by means of an intravenous tube set-up. A minimal flow of saline was constantly administered through the catheter to maintain patency; but, when indicated, the valve was turned and further anesthetic solution administered. The level of anesthesia was determined by the presence or absence of the eye lid reflex and the response to painful stimuli. Three to four additional doses of two milliliters

each were given during the six to seven hour operative procedure.

The induction and maintenance of anesthesia was uneventful except for one instance in which the dog stopped respiring. Resuscitative measures consisted of artificial respiration and intravenous administration of one milliliter of 1:1000 adrenalin chloride. Autonomous breathing was attained within ten minutes and the operation continued without further interruption. The dog's postoperative course was uneventful.

Preparation. After adequate anesthesia was attained and the three-way valve set-up completed, intra-oral x-ray films were made with ultraspeed dental occlusal x-ray film by exposing it with a dental x-ray unit with a long cone attachment. A modified occlusal technique included the two canine teeth in the maxilla in one film; another film demonstrated both mandibular canines. Films of the mandibular first molar teeth were made utilizing the right angle method.

Before opening the pulp chamber each experimental tooth was individually isolated with a rubber dam, scrubbed with surgical soap, rinsed with sterile saline and the tooth as well as the clamp and dam generously painted with tincture of Metaphen (nitromersol, Abbott). Sterile techniques were used throughout the remainder of the transplantation procedure.

Donor teeth. The pulp of the right mandibular first molar tooth in each animal was used as the donor tissue. After the tincture of Metaphen had dried, the mesial pulp chamber was opened using a 701 carbide bur in an air turbine propelled by carbon dioxide, with physiologic saline as a coolant. The teeth were opened by first cutting the

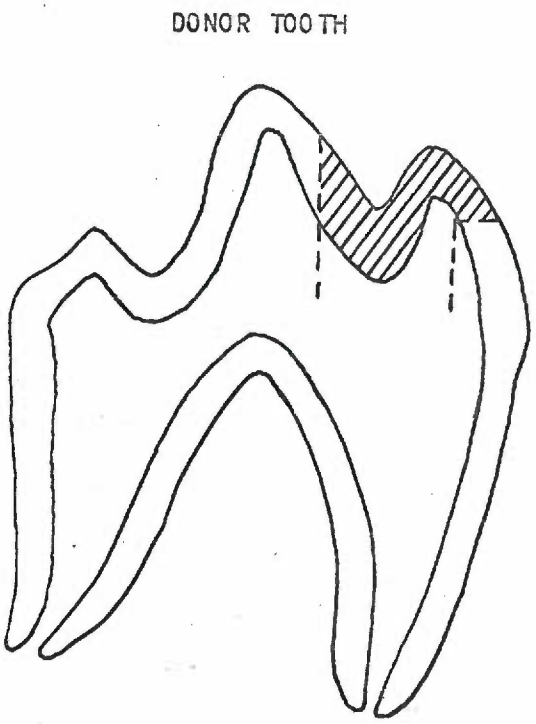


Figure 1. Diagram of the opened molar tooth.

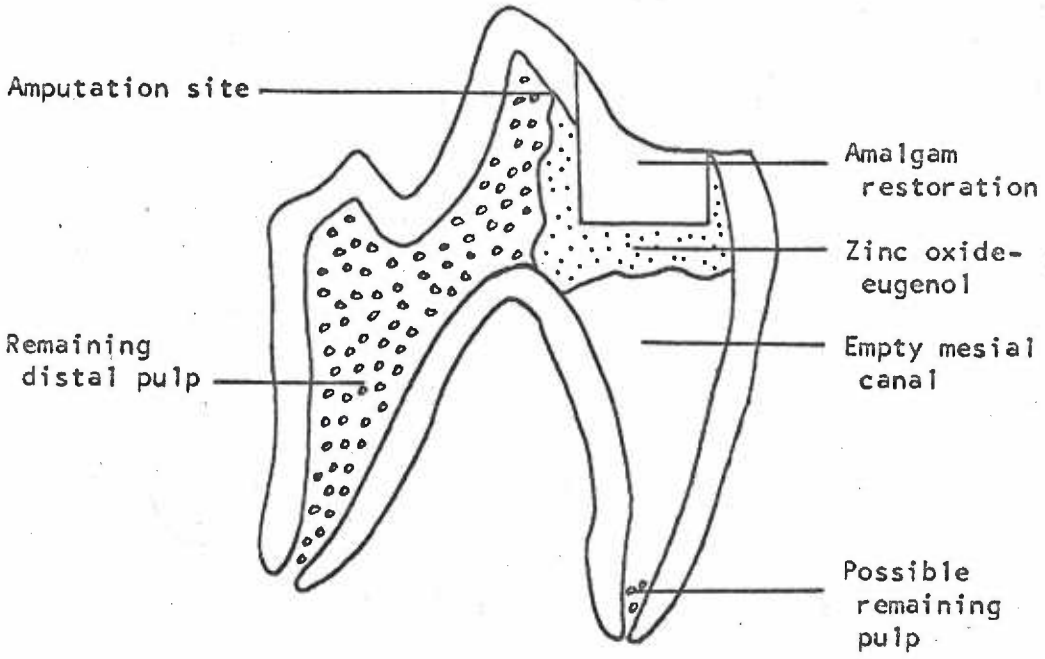


Figure 2. Diagram of the restored molar tooth.

mesial cusp flat at the level of the pulp horn and then preparing a wide occlusal opening into the pulp chamber (Fig. 1). After thorough cleansing of the preparation with sterile physiologic saline the pulp in the mesial canal was removed relatively intact with a coarse barbed broach and placed in a Petri dish containing about 2 ml. of sterile physiologic saline. (15, 84) The barbed broach was inserted into the mesial canal until resistance was met and then twisted one-half turn and withdrawn with the attached pulp. Bacteriologic cultures were taken of the empty canal and chamber with moistened paper points using thioglycollate and beef heart infusion broth as the media. The occlusal opening into the mesial pulp chamber was filled with zinc oxide-eugenol and a cavity preparation was made leaving a thick layer of this material for the base. Following this the tooth was restored with amalgam. (Fig. 2).

Host teeth. The right maxillary and left mandibular canines were used as the host teeth. The pulp chambers were entered by cutting the incisal cusp flat at the level of the pulp horn. Following this a cavity preparation was made with part of the floor consisting of a one to two millimeter ledge rimming the pulp canal (Fig. 3). The ledge provided support for the filling material preventing it from exerting pressure on the underlying pulp tissue. After thorough cleansing of the cavity preparation a segment of coronal pulp tissue approximately five millimeters long was removed from the canal using a small curette. A shallow circumferential groove was cut in the dentin with a 33 1/3 inverted cone bur in order to mark the level of the amputated pulp (Figs. 3 and 4). The walls of the canal from which the pulp was removed were thoroughly scraped with the curette in an attempt to

EXPERIMENTAL AND CONTROL TEETH

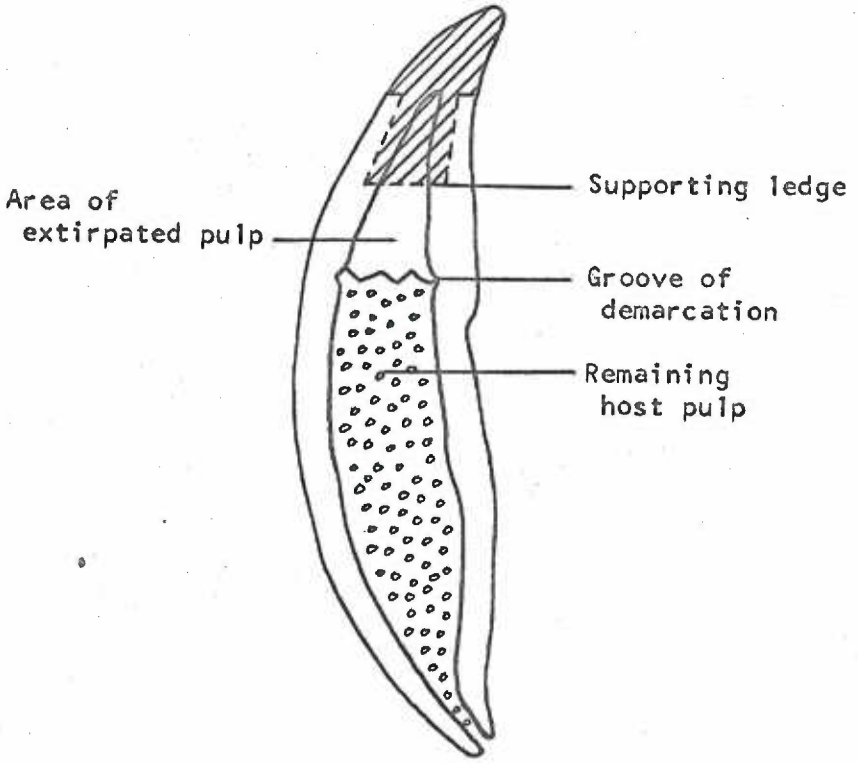


Figure 3. Diagram of the opened canine tooth.

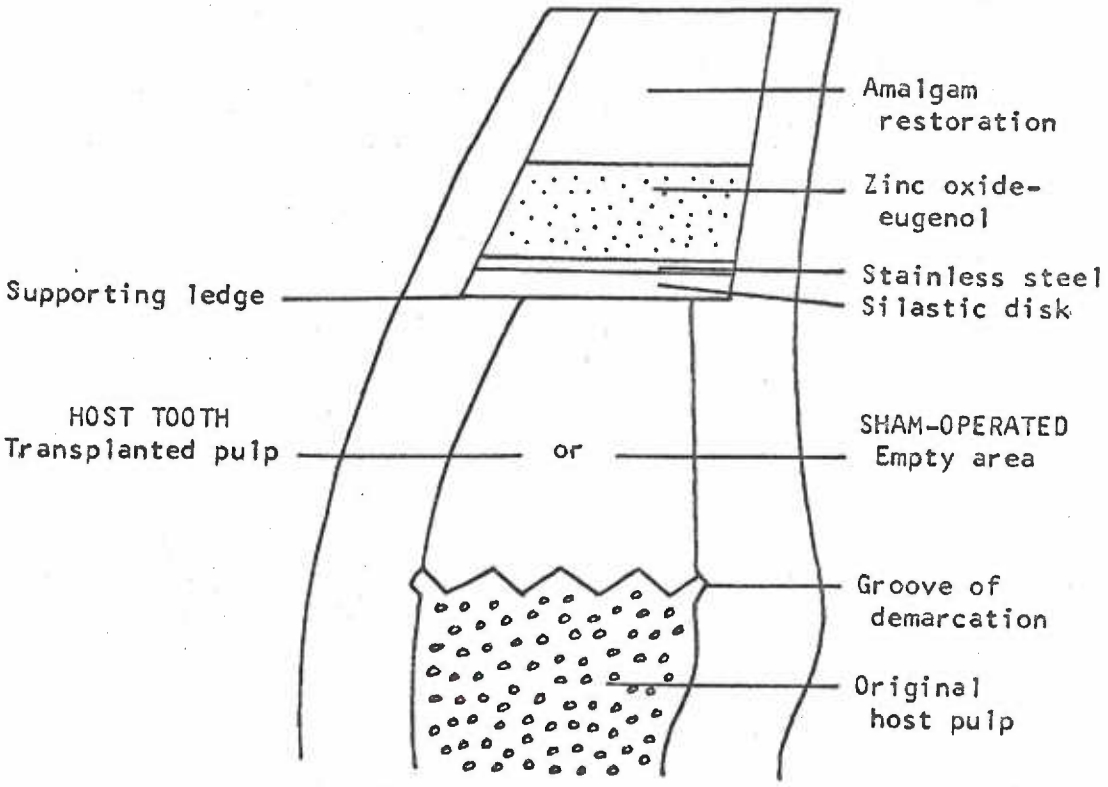


Figure 4. Diagram of the restored canine tooth.

remove any remaining fragments of pulp tissue and dentinal debris. Cultures were taken as in the donor teeth and a proper sized segment of donor pulp tissue then inserted into the vacant portion of the pulp canal. The tooth was then filled in the following manner (Fig. 4).

1. Silastic disk. (silicone elastomer, Dow-Corning)
2. Stainless steel disk.
3. Zinc oxide-eugenol.
4. Amalgam restoration.

At the end of the transplantation procedure the Petri dishes containing the pulp tissue were cultured and the remaining tissue fixed in 10% neutral formalin.

Control teeth. The right mandibular and left maxillary canines were used as the control teeth. The right mandibular canine was not disturbed (virgin control), and the left maxillary canine was treated in a manner identical to the host teeth except that no transplanted tissue was inserted; that is, it was sham-operated. These sham-operated teeth were restored leaving the extirpated portion of the pulp chamber vacant.

Postoperative course. The animals were examined bi-weekly and except for one instance of localized cellulitis there were no postoperative sequelae. The single case of cellulitis arose about two weeks after the transplantation procedure and lasted for one week. It occurred in the right maxillary canine area of an animal whose teeth were not decalcified after sacrifice (Kennel number 3416).

Removal of teeth. At the end of the respective time intervals the indicated dogs were anesthetized and x-ray films made of the experimental and control teeth. When adequate anesthesia had been attained

the teeth were surgically removed en bloc using an air turbine driven bur and bone chisels. Immediately after removal the teeth were sectioned transversely at the cervical line with a diamond disk under water coolant and placed in 10% neutral formalin. Following removal of the last experimental tooth the animals were sacrificed with an overdose of intravenous pentobarbital sodium solution. Several of the right mandibular canine teeth were inadvertently fractured during removal of the maxillary teeth.

Bacteriologic Cultures

When the transplantation operation had been completed cultures were taken of the Petri dishes containing the donor pulp. This was accomplished by submerging paper points in the saline for one minute and placing them in thioglycollate and beef heart infusion broth. These tubes along with those used to culture the pulp canals were incubated at 37°C for forty-eight hours. Gram stains were evaluated on material from the tubes demonstrating growth.

Histologic Preparation

Fixation. The teeth and their surrounding bone were fixed for five days in 10% neutral formalin which was agitated periodically and changed daily. (63) At the end of the fixation period the excess bone was removed with a diamond disk and water coolant. All the hard tissues were placed in decalcifying solution except those from one nine-week postoperative animal (Kennel number 3416). These undecalcified hard tissues were subsequently prepared for viewing with a fluorescent microscope. The fragments of donor pulp tissue remaining in the Petri dishes after transplantation were also fixed in 10%

neutral formalin solution and except for decalcification were processed and stained in a manner similar to the teeth.

Decalcification. The tissues were decalcified in 16% formic acid-3.4% sodium formate solution for two to three weeks. (54) During the decalcification procedure the solution was changed semi-weekly and constantly stirred. In order to determine when the tissue was adequately decalcified periodic x-ray films were taken, and the findings were compared with the examination of the gross tissue. In most instances the two methods agreed; however, the x-ray films gave a more objective means of evaluating the mineral loss and often resulted in the termination of the decalcification process sooner than if only the gross examination had been relied upon. The teeth were rubbery and translucent when adequately decalcified.

Processing, sectioning and staining. Following decalcification the tissues were washed in running water, dehydrated in increasing concentrations of ethyl alcohol, cleared in cedar oil and xylene and paraffin-embedded. (63) The coronal and root portions of each tooth were properly oriented and embedded in the same block of paraffin. The teeth were longitudinally sectioned with a microtome in a mesial-distal plane at a thickness of approximately eight microns. The total number of serial sections per tooth ranged from 350 to 500. Every tenth section was mounted and stained with hematoxylin and eosin. (63) After microscopically examining the stained slides, pertinent intermediate sections from each tooth were selected and stained with Masson's trichrome stain, Mallory's aniline blue collagen stain and the Brown and Brenn stain for bacteria in tissue. (63)

Undecalcified sections. Following fixation, the undecalcified teeth of the nine week postoperative animal (Kennel number 3416) were sectioned longitudinally with a diamond disk under a water coolant. One of the longitudinal halves was returned to the fixative, and the other longitudinal half was embedded in methyl methacrylate. (63) The enamel was removed with a diamond stone and water prior to embedding. After adequate curing, the blocks were cut into six to eight micron sections on a Jung microtome and mounted on slides. Some of the sections were stained with Ponta Chrome blue-black (Nutritional Biochemicals Corporation), hematoxylin and eosin, and in one instance with Weigert's tissue Gram stain. (63) The remaining unstained sections and those stained with Ponta Chrome blue-black were examined under a microscope illuminated with blue-violet light, while the hematoxylin and eosin and Weigert's tissue Gram stained sections were viewed with visible light. A Leitz Panphot microscope with an XB150 xenon bulb, BG12 exciting filter and OG1 eyepiece filter served for the fluorescent microscopic examination of the sections.

FINDINGS

The sequence of histopathological changes which occurs through the three time periods will be described separately for the host teeth, the sham-operated control and the donor teeth. The description will consist of a detailed picture of the teeth at the third postoperative week and of the subsequent changes and trends which become manifested in the later periods. Since there are no significant variations among the virgin control teeth as well as among the surplus donor pulp tissues, composite descriptions will be made of each.

The two-piece longitudinal sections of the teeth demonstrate the transverse cut made immediately after the removal of the tissue block. This cut is four to five millimeters below the amputation site in the pulp. The histologic description of the decalcified teeth is based on the hematoxylin and eosin stained sections supplemented by Masson's trichrome, Mallory's aniline blue and Brown and Brenn stained sections. Sections of undecalcified teeth used for tetracycline fluorescence studies will be described separately.

Virgin Control Teeth and Residual Donor Pulp

Virgin control teeth. (Figs. 5 to 14) Longitudinal sections of the teeth include the periodontal membrane, surrounding alveolar bone and, occasionally, gingiva. The root portion of the dentin is covered by cementum, and the coronal portion is bare. The dentin constitutes the greatest part of the tooth's calcified structure and consists of

the cytoplasmic processes of the odontoblasts embedded in an intercellular matrix. These odontoblastic processes are housed in regularly arranged canaliculi or tubules which extend from the pulp chamber toward the dentino-enamel or dentino-cemental junctions. Running more or less perpendicularly to the dentinal tubules are contour lines which probably represent differences in degree of matrix calcification. The innermost layer of dentin consists of a relatively uncalcified band of predentin which is intimately related to the underlying odontoblastic layer of pulp.

The pulp tissue encased within the dentin is made up of a peripheral odontoblastic layer and a core of primitive connective tissue. The morphology and the number of odontoblasts present in the layer ranges from one to five cells in thickness; however, it may be considerably thicker in the incisal region. Morphologically the odontoblasts vary from tall columnar to short cuboidal cells. The subodontoblastic pulp tissue does not exhibit a cell-poor zone, and in many instances it is more cellular than the core.

The most numerous cells of the pulp are the fibroblasts, and they assume either a stellate or a fusiform shape. Surrounding these connective tissue cells is an abundant ground substance and a few collagen fibers. The latter are most abundant in the apical region. In the narrow incisal area of the pulp chamber the odontoblasts are vacuolated, and the connective tissue assumes a homogeneous eosinophilic appearance (Figs. 11 and 12). This pulpal change begins approximately eight to nine millimeters above the transverse cut in the cervical region and extends throughout the remainder of the incisal pulp tissue. Within the central core are numerous arterioles,

metarterioles, capillaries and veins containing variable quantities of blood. In the more peripheral areas of the pulp the vascular elements are usually limited to small endothelial lined channels. Nerve trunks are often associated with the blood vessels and the two seem to decrease proportionately in size from the center of the chamber to the periphery. The pulp tissue also contains a few diffusely distributed macrophages, plasma cells and lymphocytes. One of the teeth contains a false denticle in the coronal pulp.

Several of the virgin control teeth were inadvertently fractured during the removal of the other teeth. This damage is characterized by fractured fragments of dentin, hemorrhage, necrosis and inflammation.

Residual donor pulp. (Figs. 15 to 18) The fragments of pulp tissue remaining in the Petri dishes generally appear normal with the exception of a minimal loss of fine morphological detail and focal areas of necrosis and hemorrhage. The areas of frank necrosis are limited to the peripheral regions of the specimens. Several pieces of pulp tissue contain regions in which the peripheral cells are polarized and probably represent areas of residual odontoblasts. Many small fragments of poorly preserved dentin accompanied the pieces of pulp tissue. The tissue also contains a moderate number of lymphocytes and macrophages and a few neutrophils. The blood vessels are free of red blood cells but often contain clumps of lymphocytes and neutrophils (Fig. 18).

Host Teeth

Three week period. (Figs. 19 to 25) The four host teeth in this time period show similar findings, the most striking being the apparent viability of the transplanted pulps. Subjacent to the disk of silicone

elastomer which was removed before sectioning, the pulp cavity contains a fairly well delineated one to two millimeter band of degenerating neutrophils, lymphocytes, macrophages, plasma cells and debris. The pulp tissue immediately underlying this area contains a mild to moderate inflammatory cell infiltrate. The infiltrate decreases and becomes focally distributed in the deeper tissues and is generally absent toward the radicular margin of the transplantation area. This pulpal infiltrate is principally of a chronic type and includes scattered multinucleated foreign body-type giant cells and numerous hemosiderin-laden macrophages.

Besides the presence of inflammatory cells the pulp in the transplantation area exhibits congested capillaries, fragments of dentin, hemorrhage, hemosiderin, intercellular and intracellular edema and focal areas of fibrosis. The areas of fibrosis are characterized by an increase in the number of fusiform-shaped fibroblasts and youthful collagen fibers at the expense of the stellate cells and amorphous ground substance. Some areas of transplanted pulp tissue show minimal inflammatory and reparative changes and appear nearly normal. Also within this same region are several large sclerotic blood vessels.

Within and just below the radicular limit of the transplantation area, as designated by the notch in the lateral dentinal wall, are numerous fragments of tubular dentin. These fragments have been covered with a postoperatively formed layer of dentin which is primarily atubular. Some of this atubular dentin or osteodentin contains embedded cells. The fragments were produced when the circumferential groove marking the junction between the graft and the host pulp was cut. Each mass of osteodentin contains several fragments of tubular dentin.

Some of these proliferating osteodentin masses become united either with similar masses or with the wall of the pulp chamber. The cells producing the postoperatively formed osteodentin vary markedly in character and range from columnar to flattened cuboidal cells. Some of the newly formed dentin associated with the columnar cells exhibits tubule formation. The giant cells in the area occasionally contain degenerating fragments of dentin (Fig. 22).

Two of the host teeth in this time interval contain a narrow well-delineated transverse band of extravasated red blood cells which traverses the pulp between the lateral walls of the root canal (Fig. 25). This band is situated approximately one millimeter below the dentin masses. In the other two teeth there are several areas of focal hemorrhage in the corresponding region. The areas of hemorrhage contain numerous hemosiderin-laden macrophages as well as hemosiderin pigment within the dentinal tubules. With the exception of the congested pulpal vessels the remaining pulp and periapical tissues are free of any histopathological changes.

The dentinal walls lining the area of the transplanted autologous pulp tissue were cut during the preparation of the circumferential groove. These cut surfaces are covered by a thin layer of postoperatively formed atubular dentin. Some of the cut tubules contain hemosiderin pigment. In one tooth the dentinal wall shows minute areas of resorption with associated multinucleated cells (Fig. 20). Beginning at the groove and extending apically, there is a thick layer of postoperatively formed tubular dentin which is separated from the preoperatively formed dentin by a prominent basophilic band. The basophilic band or calciotraumatic line tends to become less noticeable as the apex is approached.

Five-week period. (Figs. 26 to 30) The differences between the teeth in the three-week period and those in the five-week period reflect the continuing reparative process. The superficial band of degenerating inflammatory cells is narrower, measuring one millimeter or less in thickness. Within the underlying pulp tissue the inflammatory infiltrate is mild and focally distributed. It consists principally of lymphocytes and macrophages with many of the latter containing foamy cytoplasm or hemosiderin pigment.

The character of the connective tissue in the transplantation area varies within each tooth and between the teeth in the group. Some teeth contain large areas which are similar to normal pulp tissue, whereas in others there is extensive fibrosis. The quality of the pulpal connective tissue in the five-week period is generally not much different from that of the previous group; however, it does appear somewhat more fibrous.

The pulp tissue in the transplantation area contains several well-delineated eosinophilic masses of finely interwoven collagen fibers and a few nuclei (Fig. 27). These structures have a "cotton candy" or reticular appearance and are often associated with degenerating vessels. A few of the eosinophilic masses show vascular ingrowth. The degenerating blood vessels are less well preserved than in the previous period.

The cut surfaces of the dentinal walls enclosing the transplantation site are lined by a layer of osteodentin or tubular dentin which is thicker than that seen in the three-week period. Near the incisal area the walls are usually covered by osteodentin while in the deeper portions, still above the demarcation groove, tubular dentin formation predominates. Tubular dentin is also present on the periphery of the osteodentin which encompasses the original dentinal fragments. Well-

differentiated odontoblasts approximate the areas of tubular dentin production, while oval or cuboidal cells are associated with the osteodentin formation. A prominent calciotraumatic line separates the preoperatively and postoperatively formed dentin in both the crown and the root.

The hemorrhagic band noted just below the dentinal fragments in some of the three-week teeth is prominent in one of the teeth in this group, while in the remainder it is either minimal or absent.

Nine-week period. (Figs. 31 to 33) The main difference between the transplantation area of this and previous periods is the almost total resolution of the inflammatory process and the further deposition of dentin. With the exception of one tooth which contains a superficial focal accumulation of chronic inflammatory cells the infiltrate is minimal. Among the few remaining inflammatory cells, macrophages and multinucleated giant cells predominate, and many of the former contain hemosiderin. Although only a relatively few foreign body giant cells are seen, more are present in this time period than in previous ones. Some of these giant cells contain large vacuoles and are haphazardly arranged while others appear to be attempting to encircle amorphous material or are associated with fragments of dentin.

The connective tissue in the transplantation area is generally loose, cellular and relatively free of fibrosis. As in previous time periods it contains several large clear spaces bounded by a narrow band of increased tissue density (Fig. 31). In some instances the spaces appear to be large venous channels devoid of blood cells and in others to represent areas previously occupied by masses of dentin which were dislodged during sectioning. However, in most instances it is not possible to establish any of the above relationships, and for

lack of a better explanation, the term reticular atrophy may be applied.

There is a marked increase in the thickness of the postoperatively formed dentin on both the pulpal walls and on the pulpal masses of dentin. The layer of osteodentin formed initially after the transplantation procedure is overlaid by tubular dentin in all areas except the most incisal portion of the transplantation region.

One of the teeth exhibits an area of hemorrhage beneath the pulpal masses of dentin. The remainder of the radicular pulp and the periapical tissues are similar to those in the other time periods.

Sham-operated Control Teeth

Three-week period. (Figs. 34 to 35) The two teeth in which the incisal portions of the pulps were removed and the chambers left vacant appear similar to the host teeth in many ways. The space between the amputation site and the deepest portion of the filling is almost filled by the upward proliferation of the pre-existing pulpal tissue. In one of the teeth the most superior portion of the incisal pulp chamber contains a one millimeter band of necrosis, a large subjacent accumulation of acute and chronic inflammatory cells and pools of serum (Fig. 34). The underlying pulp tissue is fibrous and exhibits a mild chronic inflammatory infiltrate. By contrast, the incisal area of the other extirpated control tooth contains only a narrow band of inflammation and necrosis.

In both teeth fibrous connective tissue fills the pulpal amputation area and except for the abcess formation nearly extends to the region of the silicone elastomer. The regenerated connective tissue contains many clear spaces similar to those noted in the host. The cut dentinal

walls enclosing the transplantation area contain little or no post-operatively formed dentin. Some of the tubules in the region contain hemosiderin pigment. As in the host teeth numerous dentinal masses are present in the region; however, they appear in a more incisal position. Both sham-operated teeth also exhibit a calciotraumatic line, hemorrhagic band, vascular congestion and normal appearing radicular pulp and periapical tissues. The principal difference between the sham-operated control teeth and the host teeth is the greater amount of inflammation in the former.

Five-week period. (Figs. 36 to 40) As in the host teeth the primary difference between this and the three-week period is the decrease in inflammation and the increase in postoperatively formed dentin. Both teeth in this time period have a superficial band of degenerating inflammatory cells one millimeter or less in thickness. The underlying pulp tissue is free of abcess formation and contains only a minimal chronic inflammatory cell infiltrate. It is made up of loose fibrous connective tissue containing focal areas of fibrosis and has a pattern similar to that seen in the corresponding host teeth. However, the large sclerotic blood vessels seen in the host are absent. The pulp tissue in the operative site and surrounding the dentinal masses contains some hemorrhage, but there is no distinct hemorrhagic band. The quality, quantity and distribution of the dentin is similar to that in the five-week host teeth.

Nine-week period. (Figs. 41 to 43) The only significant difference between this and the five-week sham-operated teeth is the increased thickness of the tubular dentin on the walls of the pulp chamber and on the masses of osteodentin. The teeth also appeared

very similar to the host teeth in the corresponding time period. One of the sham-operated teeth contains one of the collagenous eosinophilic masses described in the host teeth (Fig. 41).

Donor Teeth

Three-week period. (Figs. 44 to 53) The mesial roots in both donor teeth, which were the source of the donor pulp tissue, show evidence of pulpal manipulation. The apical one-fourth to one-half of the canals are filled with an inflamed loose fibrous connective tissue. However, the extent of the inflammation and the character of the fibrous connective tissue varies in the two teeth. The canal containing the least amount of fibrous connective tissue exhibits an extensive infiltrate of neutrophils, foamy macrophages, lymphocytes, plasma cells, and Gram positive bacilli and cocci. The bacteria are most numerous in the middle third of the canal where they are concentrated along the dentinal walls (Fig. 45). Some of the bacteria have been phagocytized by the macrophages in the area.

The mesial canal in the other tooth is half filled with connective tissue and shows only a mild infiltrate of lymphocytes, plasma cells, macrophages and some hemorrhage. The more coronal portion of the mesial canal and pulp chamber is partially filled with necrotic debris, hemorrhage, inflammatory cells and an amorphous eosinophilic coagulum. The tissues approximating the dentinal walls of the mesial canal are free of odontoblasts, with the exception of the few remaining in the apical area.

A small area of chronic inflammation is present in the periapical tissues on the distal surface of the most severely inflamed mesial root (Fig. 48). It is similar to the tissue in the root canal in both

cell type and consistency and communicates with it through at least one apical foramen. Some of the macrophages in the periapical lesion contain phagocytized bacteria (Fig. 48A).

The amputation site at the distal coronal pulp is covered by an amorphous eosinophic coagulum, and is associated with a band-like infiltrate of chronic inflammatory cells which extends distally a short distance into the pulp. Except for the coronal amputation site there is no evidence of fibrosis. The tooth exhibiting the greatest inflammatory reaction in the mesial root also shows abcess formation in the occlusal pulp and focal resorption of the dentin lining the crown (Fig. 44) and mesial canal (Fig. 46). Both of these areas of resorption exhibit well-defined lacunae, but only those in the coronal dentin are associated with multinucleated giant cells.

The superior aspect of the distal coronal pulp contains extravasated red blood cells and pigment within the associated dentinal tubules. In the most severely inflamed tooth the area contains massive hemorrhage, while in the other tooth there are only small focal areas of hemorrhage. The pulp tissue in the distal canal and the associated periapical tissue appear normal.

Five-week period. (Figs. 54 to 58) As in the three-week period, the mesial roots of the donor teeth are only partially filled with viable tissue. In one tooth the apical half of the mesial root contains chronically inflamed loose fibrous connective tissue, while in the other tooth only a small portion of the canal is filled. In both instances the tissue extends occlusally more along the dentinal walls than in the center of the canal.

As in the three-week period, the mesial canal exhibiting the least amount of connective tissue proliferation also shows the most severe inflammation and a periapical lesion. The tissue and the canal above it contain numerous neutrophils, macrophages, lymphocytes and plasma cells. A similar but more severe inflammatory cell infiltrate is present in the area of bone destruction along the distal apical surface of this root (Fig. 55). The periapical lesion communicates with the pulp canal via several apical foramina.

This canal also exhibits an avascular focus of proliferating cells which appear to be young fibroblasts growing in a tissue culture-like pattern (Fig. 54). These cells are present near an area of inflammation and superior to the usual type of regenerating connective tissue in the canal.

The tissue in the canal showing the greatest amount of regeneration contains only a mild to moderate inflammatory infiltrate, and the associated periapical tissues are free of any pathological changes. The lower third of the canal is lined by postoperatively formed osteodentin, and the apical region by osteodentin or cementum. The connective tissue within the middle third of the canal contains an island of newly formed cementum or osteodentin (Fig. 56). Several finger-like projections of cementum extend into the canal in the apical region. The tissue in this region also contains an island of cementum which does not appear to be attached to the wall of the canal (Fig. 57). No dentinal fragments are seen similar to those found in the amputation areas of the host and sham-operated control teeth.

The pulp tissue adjacent to the amputation site exhibits a mild chronic inflammatory infiltrate and congested blood vessels. There is

no evidence of fibrosis except in the amputation area. The distal coronal pulp tissue exhibits a mild amount of hemorrhage and contains hemosiderin-laden macrophages. No pathological changes are evident in the tissues of the distal root or periapical area.

Nine week period. (Figs. 59 to 60) Only one of the donor teeth in this time interval is available for examination. It contains less evidence of inflammation and more extensive regeneration than do the teeth in the five-week period. The proliferating connective tissue occupies about three-quarters of the mesial canal and exhibits only a mild chronic inflammatory cell infiltrate. Osteodentin lines the walls next to the regenerated tissue in all except the apical region where tubular dentin is being formed by well-differentiated odontoblasts (Fig. 60). The occlusal pulp contains focal areas of mild chronic inflammation and the distal pulp contains a moderate amount of hemorrhage. There are no pathologic changes in the periapical tissues or distal radicular pulp.

Undecalcified Sections

Visible light. (Figs. 61 to 64) With the exception of the right maxillary canine the hematoxylin and eosin stained sections of the undecalcified teeth (Kennel number 3416) which were cut at eight microns on the Jung microtome exhibit the same findings as noted in the corresponding decalcified sections. The remaining pulp tissue in the root of the right maxillary canine (host tooth) is necrotic, and the periapical tissue contains an area of inflammation with proliferating epithelium. Although necrotic, the pulp is free of liquefaction degeneration and inflammatory cells and still shows the outline of the vascular channels (Fig. 61). There are several foci of Gram positive bacilli and cocci

in the apical portion of the pulp bordering the cystic space as well as in the middle of the root (Fig. 62).

Within the wide apical foramen is a clear space which is bounded laterally by dentin and apically by proliferating stratified squamous epithelium and masses of inflammatory cells. The epithelium lining the cystic space is undergoing degeneration in some areas and contains an extensive infiltrate of neutrophils, lymphocytes, macrophages and plasma cells (Fig. 63). The surrounding area of bone destruction contains a youthful fibrous connective tissue stroma with a similar but less intense inflammatory infiltrate. The infiltrate extends into the bone marrow spaces as well as a short distance incisally along the periodontal surface of the root.

The hard tissues contain cracks which run parallel to the direction of sectioning. These cracks are most prominent in the dentin, and in some areas it appears frayed. The soft tissue is well preserved and does not show this sectioning artifact.

The crown portion of the right maxillary canine tooth is devoid of pulp tissue and postoperatively formed osteodentin or dentin.

Blue-violet light. (Figs. 65 to 66) Examination of the undecalcified sections under blue-violet light reveals the presence of yellow fluorescent bands in the hard tissues. In bone the bands vary in thickness and orientation, whereas, in the dentin lining the pulp canal and the cementum the bands are oriented parallel to the long axis of the tooth and are fairly uniform in thickness and spacing. In the right maxillary canine tooth, in which the pulp is necrotic, fluorescent bands are evident in the cementum but not in the dentin.

In teeth other than the right maxillary canine the pulpal masses of osteodentin and dentin in the operative area contain a central core

free of fluorescence, which is surrounded by concentric fluorescent rings. The concentric rings tend to conform to the general outline of the central core and are closer together and narrower than the bands in the dentinal wall. A lesser amount of dentin or osteodentin is found enclosed by the concentric rings than is found in the central core.

There are seven discrete fluorescent bands in both the dentinal wall and the masses of dentin and osteodentin. The first of the seven doses of tetracycline hydrochloride was administered at the end of the second postoperative week, and the last dose was administered before sacrifice.

The unstained sections were superior to the Ponta Chrome blue-black counterstained sections in that the latter tended to mask the tetracycline fluorescence.

Bacteriologic Findings

Culture. Gram stains were done on the incubated culture media exhibiting evidence of growth. Cultures from two Petri dishes containing donor tissue and another culture from one maxillary right canine host tooth gave positive results. The stained smears showed the presence of mixed cultures of Gram positive bacilli and cocci. In several of the culture tubes the paper points started to untwist and fragment, mimicking bacterial growth. The two positive cultures from the Petri dishes probably reflect contamination of the dishes during handling of the pulp tissue prior to transplantation. The only tooth with a positive culture was the right maxillary canine which had manifested the postoperative sequelae (Kennel number 3416).

Tissue sections. The three-week donor molar with the least amount of tissue regeneration in the mesial canal (Kennel number 3056) and the right maxillary canine which manifested the postoperative sequelae (Kennel number 3416) are the only teeth to exhibit unequivocal bacteria in the specially stained tissue sections. In the molar tooth, which had a negative culture, Gram positive bacilli and cocci are present along the dentinal wall lining the middle third of the mesial canal and within phagocytes in the pulp and periapical lesion (Figs. 45 and 48). The right maxillary canine tooth contains several foci of Gram positive bacilli and cocci in the necrotic pulp (Fig. 62). Examination of the host teeth receiving the transplanted pulp tissue from the contaminated Petri dishes did not reveal the presence of any bacteria or unusual tissue response.

Radiographic Findings

Host and control teeth. There are diffuse radiopaque deposits in the operated pulp chambers of the canine teeth. These radiopaque areas are most prominent in the animals of the longest time interval. The virgin control teeth do not exhibit these changes. The periapical regions of the canine teeth are usually superimposed on the premolars, making interpretation of the areas impossible. Comparison of the preoperative x-ray films and the films taken at sacrifice reveal a general increase in dentin deposition and a narrowing of the apical foramina.

Donor teeth. As in the canine teeth the donor molars show an increase in dentin deposition during the postoperative period. This postoperatively formed dentin is limited to the distal occlusal pulp chamber and root canal. There is no evidence of any intrapulpal calcifications. The periodontal space in the apical area varies greatly in

thickness, making it difficult to interpret these areas. The donor tooth with the histologically small mesial periapical lesion (Kennel number 3056) gives an equivocal roentgenographic picture, whereas the tooth with the histologically large lesion (Kennel number 3059) reveals a definite area of periapical bone destruction.

DISCUSSION

Materials and Methods

Dogs were selected as the experimental animals because of their ready availability, relatively low cost, ease of maintenance, resistance to disease and tooth size. The large teeth simplified the instrumentation, transplantation and restoration of the teeth. The morphology of the canines made them suitable for the host teeth, and the abundant pulp tissue of the mandibular first molars made them desirable donor teeth. Sham-operated teeth with extirpated pulp chambers were utilized to evaluate the regenerative powers of the host pulp. The undisturbed teeth were used as normal controls.

Young dogs between eight to ten months of age were selected because their teeth were fully erupted and the apical foramina were still relatively wide open. The large apical openings would tend to insure a maximal blood supply to the pulp, and thus help to maintain optimal conditions for repair. The postoperative time intervals of three, five and nine weeks were selected to cover the span of inflammation and repair as well as to follow any trends which might occur. Two dogs were used in each time period to provide adquate material for study and to see if comparable findings occurred in different animals.

In order to decrease any variables associated with age and genetic background, the animals were selected from two litters born two months apart. It would have been desirable to eliminate the sex variable, but

since age and genetic background were considered more important, a compromise was made by the inclusion of one female. The marked similarity in ages at the time of sacrifice, ranging from 307 to 329 days, was fortuitous.

The Williams Air Orbit handpiece used in cutting the teeth has several desirable characteristics. It has air bearings, and thus requires no lubricant in the air supply or turbine head which might contaminate the operative area. After passing through the turbine most of the propellant is exhausted out the back of the handpiece rather than through the head as in many models. These features along with the use of a medical grade compressed carbon dioxide as the propellant helped decrease the likelihood of air borne contamination from the exhaust of the handpiece.

A preliminary investigation comparing pulps removed from teeth that were extracted and split open to pulps that were removed from the teeth in situ with barbed broaches did not reveal any significant histological differences. Pulps removed while the donor teeth were still in situ were used because the time interval between the loss of blood supply and the placement in saline is considerably less than with the other method.

A Petri dish containing a small amount of physiologic saline was used to store the donor pulp tissue prior to transplantation. Using skin, it has been shown that tissue stored in moist air has a much longer period of viability (15) and shows a smaller decrease in oxygen uptake (84) than if submerged in fluid or wrapped in a moist gauze.

Silastic (silicone elastomer, Dow Corning) stainless steel, zinc oxide-eugenol and amalgam were used in restoring the canine teeth.

A Silastic disk was applied directly over the operative area, as it has been shown to be well accepted by the host, eliciting little or no tissue reaction. (14, 82) The Silastic was cut into disks approximately one millimeter thick and thoroughly washed before autoclaving. (4) Due to the flexibility of the Silastic it was overlaid with a piece of stainless steel cut from matrix material. The matrix material was covered by a layer of zinc oxide-eugenol, which acted as a sealant as well as a thermal insulator for the overlying amalgam restoration.

Ten percent buffered neutral formalin solution (63) was used as the fixative agent because it penetrates rapidly, produces little distortion and protects against acid haematin formation. Acid haematin forms in blood rich tissues fixed in formalin solution with a pH below six. The haematin granules are dark brown or black and can usually be distinguished by being doubly refractile.

The teeth were sectioned transversely in the cervical area to enhance the penetration of the formalin into the pulp tissue. It has been shown that formalin entering an intact tooth through the apex penetrates eight to ten millimeters of the radicular pulp in the first ten minutes. (86) After this initial period there was only a minimal increase in formalin penetration during subsequent hours or days of fixation.

A 16% formic acid -3.4% sodium formate solution was used to decalcify the hard tissues. (54) This solution takes somewhat longer than with some other methods, but it has the advantage of preserving the nuclear stainability.

The histopathologic evaluation was based principally on the hematoxylin and eosin stained sections. However, selected sections of

each specimen were also stained by other methods. Mallory's aniline blue and Masson's trichrome stains were utilized to demonstrate the collagen fibrils and Brown and Brenn and Weigert's Gram stains to show bacteria in tissues. (63)

In the past few years it has been established that tetracycline antibiotics are incorporated into developing bone (88, 90) and teeth (5) at sites where the calcium salts are being deposited. The drug is bound into the tissue, which calcifies during the first few hours after the administration of the tetracycline. The mechanism of tetracycline fixation in calcifying tissues is not completely understood; however, it appears likely that these drugs are bound by the inorganic elements rather than the organic matrix. (88, 90)

The presence of these drugs in undecalcified hard tissues is easily detected by examination under ultraviolet or blue-violet light which causes yellow fluorescence of the tetracycline. When incorporated into teeth the presence of the drug will be revealed by distinct fluorescent bands in the hard tissues that were forming at the time of administration of the drug.

Each of the animals in this study were given one gram of tetracycline hydrochloride at weekly intervals during the postoperative course. The drug was administered to all the animals in order to standardize the experimental conditions. Tetracyclines are completely removed from tissues during decalcification; therefore, only the undecalcified teeth from the nine-week postoperative animal were examined for fluorescence. These sections were examined under a microscope illuminated with blue-violet light, and the sequential production of the postoperatively formed dentin as reflected by the incorporation of the fluorescent tetracycline was studied.

DISCUSSION (Continued)

Findings

The findings in this study illustrated the marked reparative capacity of the pulp tissue in young dog's teeth. The pulp tissue in the host and sham-operated teeth had proliferated and filled in the operative areas and also had resumed the production of dentin.

At the end of the three-week time period the extirpated areas of the sham-operated teeth were more severely inflamed and contained less regenerated connective tissue and postoperatively formed dentin than in the corresponding host tooth. However, in the five and nine week periods the host and sham-operated teeth appeared essentially the same. The only consistent histological finding distinguishing the two types of teeth in the latter time periods was the presence or absence of the sclerotic blood vessels and the eosinophilic fibrillar structures. These structures were routinely found in the host transplantation site and, with one exception, were absent in the extirpated area of the sham-operated teeth. The large sclerotic blood vessels are probably the degenerating vessels of the transplanted pulp tissue, and the associated fibrillar or "cotton candy" structures may represent the terminal stages of the vascular degeneration or a response of the surrounding tissue to the degenerating vessels.

The mesial extirpated canals of the donor teeth contained varying amounts of regenerated fibrous connective tissue as well as some post-operatively formed dentin. The regenerating tissue apparently arose from the remaining fragments of pulp in the canal and/or the periapical

tissue associated with the apical foramina. The amount of tissue proliferation in the mesial donor canal increased with time and appeared to be inversely related to the degree of inflammation. It would be interesting to leave similarly prepared teeth in situ for longer periods of time to see if the proliferating tissue would fill in the extirpated canal.

The proliferating connective tissue in the host, sham-operated and donor teeth had maintained its capacity for hard tissue formation. This was shown initially by the production of osteodentin and later, as further differentiation took place, by the production of tubular dentin. The ovoid or cuboidal cells associated with the osteodentin formation and the well-differentiated odontoblasts producing the tubular dentin may be derived from the remaining odontoblasts and predontoblasts or perhaps from the undifferentiated mesenchymal cells. However, the studies of Wolbach (92) and others would tend to discount the ability of the undifferentiated mesenchymal cells to differentiate and produce dentin.

The calciotraumatic line separating the preoperatively and post-operatively formed dentin in the crown and cervical portion of the root in the operated teeth has been found to occur in many different experimental procedures. These include the injection of fluoride, strontium, calciferol, parathyroid extract or the removal of the parathyroid glands or the kidneys. (50) The mechanism responsible for this phenomenon is not known, but it appears to be related to the effect of the metabolic disturbance caused by the reacting substance or by the operation.

The tetracycline incorporated into the hard tissues fluoresced a bright yellow when the undecalcified specimens were examined with a fluorescent microscope. The presence of the drug was characterized by fluorescent bands of various widths in the bone, cementum and post-operatively formed dentin lining the pulp chamber and the dentinal masses in the central pulp. Similar findings were observed in all the specimens except the host tooth with the necrotic pulp. The dentin in this tooth did not exhibit any fluorescence, thus indicating that there was no new dentin formed after the end of the second postoperative week when the first dose of tetracycline was administered. Since relatively healthy pulp tissue is essential for the production of dentin it would appear that the tooth's pulp was severely inflamed and/or necrotic by the time the drug was administered. The postoperative cellulitis which began at the end of the second postoperative week would tend to corroborate this point.

The experimental time intervals utilized did not permit the study of the initial inflammatory changes which took place in the operated areas. In the time periods observed the inflammatory processes were similar to those seen in connective tissues elsewhere in the body.

Bacteria were cultured from only one of the two teeth exhibiting unequivocal bacteria in the specially stained tissue sections. This finding would tend to question the significance of the negative cultures in the remainder of the teeth in this study. It is entirely possible that some of the teeth could have been initially contaminated with bacteria which were subsequently destroyed by the ensuing inflammatory reaction.

The positive culture was obtained from the nine-week host tooth with the completely necrotic pulp. This tooth also had an associated periapical lesion with proliferating epithelium. The other tooth histologically demonstrating bacteria was the three-week donor tooth with the small periapical lesion containing phagocytized bacteria. Bacteria could not be demonstrated culturally or histologically in the pulp or periapical tissues of the five-week donor molar with the large periapical lesion. It is interesting to contemplate whether this tooth might have initially contained bacteria which were subsequently destroyed or whether the canal and periapical lesion were always sterile as the culture indicated. The mesial extirpated canals of the donor teeth with the periapical lesions showed less tissue regeneration and more inflammation than the corresponding teeth without periapical lesions.

CONCLUSIONS

Autologous orthotopic pulp transplantation is a feasible procedure in young dogs. It is not clear from this investigation whether the transplant "took" or whether it was replaced by regenerating host pulp tissue.

Regeneration and differentiation of the pulpal connective tissue takes place in both the transplanted and sham-operated areas. Osteodentin and tubular dentin were produced by the regenerating pulp tissue.

The long range "success" of transplanted pulp tissue requires further study. The single instance of slight internal resorption in one of the transplantation areas must be evaluated further.

Bacterial contamination is a potential hazard in this procedure. However, only one of the twenty-one operated canine teeth and one of the seven donor molar teeth exhibited bacteria. Other teeth may have been contaminated but were able to overcome the bacterial invaders.

Regeneration of pulp tissue occurs in donor teeth. Extrapolation of these and the preceding findings to human pulp would be speculative, but the findings do indicate the desirability of clinical investigations.

SUMMARY

The study utilized seven dogs at three time periods. Two dogs were used at each of the three and five week intervals, and three at the nine week interval.

The four canine teeth in each animal were used as the experimental and control teeth, and the right mandibular molars as the donors of the transplanted pulp tissue.

A portion of the coronal pulp tissue was removed from three of the canine teeth. Two of these teeth became the hosts for the transplanted tissue, and the extirpated area in the third was left vacant, that is, sham-operated. The fourth canine remained undisturbed. The donor pulp tissue was obtained from the mesial canal of the right mandibular molar.

After completion of the operative procedure, bacterial cultures were taken and the teeth restored. The donor molar was filled with an initial layer of zinc oxide-eugenol which was overlaid with an amalgam restoration. The canines, which had been prepared with a pressure-absorbing ledge, were filled in the following manner: 1) Silastic disk, 2) stainless steel disk, 3) zinc oxide-eugenol, and 4) amalgam.

At the end of their respective time intervals the animals were sacrificed and the teeth removed en bloc. The tissues of six dogs were decalcified, stained and examined with a microscope illuminated with visible light. The tissues of the remaining nine week postoperative

animal were sectioned without prior decalcification after removal of the enamel, then stained and examined under both visible and blue-violet light.

The previously empty crowns of the host teeth were found to be filled with youthful appearing pulp tissue, and in the later time periods this pulp tissue showed decreasing amounts of inflammation and increasing amounts of osteodentin and dentin. The transplantation areas in the three week host teeth were filled with moderately inflamed loose fibrous connective tissue which contained fragments of tubular dentin surrounded by a layer of osteodentin. A thin layer of osteodentin also covered the cut surface of the dentinal walls lining the transplantation area. The superficial portion of the transplantation area subjacent to the floor of the restoration contained a one to two millimeter band of degenerating inflammatory cells. The radicular and periapical tissues were normal. Subsequent time periods showed relatively few sclerotic blood vessels and fibrillar eosinophilic structures in the transplantation area, decreased inflammation and increased dentin formation together with abundant pulp-like tissue. The dentin formed in the five and nine week intervals was principally tubular dentin.

The main differences between the three week sham-operated teeth and host teeth is the increased inflammation and lack of osteodentin formation on the wall of the operative area in the former. Sham-operated teeth in subsequent time intervals were nearly identical in appearance to the corresponding host teeth except for the absence of the sclerotic blood vessels and eosinophilic structures in all except one of the former.

The amount of proliferated tissue in the extirpated mesial canals of the donor teeth increased with time. The maximum level of proliferation was reached in the nine week period with three quarters of the canal filled. Canals exhibiting the least amount of tissue regeneration contained the most extensive inflammatory cell infiltrate and periapical lesions. One of the canals and the associated periapical lesion contained bacteria.

The undecalcified teeth examined under blue-violet light demonstrated seven distinct fluorescent bands in the postoperatively formed dentin. These corresponded to the seven weekly doses of tetracycline administered after the operation. The host tooth with a positive culture had a necrotic pulp containing bacteria as well as a periapical lesion with proliferating epithelium. The dentin of this tooth did not contain any evidence of fluorescence probably because the pulp became necrotic shortly after transplantation and could, therefore, form no dentin when the tetracycline was administered.

BIBLIOGRAPHY

1. Agnew, R. G., Fong, C. C., Giles, W. S. and Grant, T. S. Intra-oral transplantation of teeth. J. Dent. Res. 38:654 July-Aug. 1959.

2. Amos, B. Transplantation antigens in mouse, rat and man. Progr. Med. Gen. 3:106-143 1964.

3. Anderson, D., Billingham, R. E., Lampkin, G. H. and Medawar, P. B. The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity. 5:379 1951.

4. Bassett, C. A. and Campbell, J. B. Keeping Silastic sterile. The Bulletin. 2:5 1960.

5. Bevelander, G., Rolle, G. K. and Cohlan, S. Q. The effect of the administration of tetracycline on the development of teeth. J. Dent. Res. 40:1020-1024 Sept.-Oct. 1961.

6. Billingham, R. E., Brent, L. and Medawar, P. B. 'Actively acquired tolerance' of foreign cells. Nature. 172:603-606 Oct. 3, 1953.

7. Billingham, R. E., Brown, J. B., Defendi, V., Silvers, W. K. and Steinmuller, D. Quantitative studies on the induction of tolerance of homologous tissues and runt disease in the rat. Ann. N. Y. Acad. Sci. 87:457-471 May 31, 1960.

8. Billingham, R. E. and Silvers, W. K. Skin transplants and the hamster. Sci. Amer. 208:118-127 Jan. 1963.

9. Booth, P. B., Plaut, G., James, J. D., Ikin, E. W., Moores, P., Sanger, R. and Race, R. R. Blood chimerism in a pair of twins. Brit. Med. J. 1:1456-1458 June 22, 1957.

10. Borea, G. Transplantation of tooth germ into chick embryos. Dent. Abs. 8:360 June 1963.

11. Boyle, P. E., Bessey, O. A. and Howe, P. R. Rate of dentin formation in incisor teeth of guinea pigs on normal and on ascorbic acid-deficient diets. Arch. Path. 30:90-107 July 1940.

12. Boyne, P. J. and Miller, C. W. A study of tooth development by tetracycline induced fluorescence. J. Dent. Res. 40:1079 Sept.-Oct. 1961.

13. Brent, L. and Gowland, G. Induction of tolerance of skin homografts in immunologically competent mice. Nature. 196:1298-1301 Dec. 29, 1962.

14. Brown, J. B., Ohlwiler, D. A. and Fryer, M. P. Investigation of and use of dimethyl siloxanes, halogenated carbons and polyvinyl alcohol as subcutaneous prostheses. Ann. Surg. 152:534-547 Sept. 1960.

15. Buchan, A. C. Experimental studies on the storage of skin: The viability of human skin stored above freezing point. Brit. J. Plast. Surg. 11:206-233 1958-59.

16. Burnet, F. M. and Fenner, F. J. The production of antibodies. New York, Macmillan, 1949.

17. Clark, H. B., Tam, J. C. and Mitchell, D. F. Transplantation of developing teeth. J. Dent. Res. 33:653 Oct. 1954.

18. Costich, E. R., Avery, J. K. and Mackenzie, R. S. A study of methods of storage of tooth transplants. J. Dent. Res. 39:726 July-Aug. 1960.

19. Costich, E. R. Basic problems of regeneration and transplantation. Dent. Clin. N. Amer. July 1962. p. 513-526.

20. Costich, E. R., Haley, E. W. and Hoek, R. B. Plantation of teeth: A review of the literature. New York Dent. J. 29:3-13 Jan. 1963.

21. Cserepfalvi, M. Clinical report of homotransplantation. J. Amer. Dent. Ass. 67:35-40 July 1963.

22. Dammin, G. J., Couch, N. P. and Murray, J. E. Prolonged survival of skin homografts in uremic patients. Ann. N. Y. Acad. Sci. 64:967-976 Mar. 22, 1957.

23. Dunsford, I., Bowley, C. C., Hutchison, A. M., Thompson, J. S., Sanger, R. and Race, R. R. A human blood-group chimera. Brit. Med. J. 2:81 July 11, 1953.

24. Edgerton, M. T., Peterson, H. A. and Edgerton, P. J. The homograft rejection mechanism. Arch. Surg. 74:238-244 Feb. 1957.

25. Eichwald, E. J. Tissue transplantation. Advances Biol. Med. Phys. 9:93-205 1963.

26. Fauchard, P. The surgeon dentist. Translated from the second edition, 1746, by L. Lindsay. London, Butterworth and Co., 1946, p. 140-145.

27. Felton, L. D., Kauffmann, G., Prescott, B. and Ottinger, B. Studies on the mechanism of the immunological paralysis induced in mice by pneumococcal polysaccharides. J. Immun. 74:17-26 Jan. 1955.

28. Fleming, H. S. Homologous and heterologous intraocular growth of transplanted tooth germs. J. Dent. Res. 31:166-188 Apr. 1952.

29. Fleming, H. S. Early influence of methylcholanthrene on transplanted tooth germs: A histopathogenic study. J. Dent. Res. 31:398-411 June 1952.

30. Fleming, H. S. The osteogenic tendency of dentin after formation in tooth germ transplants. Oral Surg. 6:1315-1324 Oct. 1953.

31. Fleming, H. S. Observations concerning the dental pulp in tooth germ transplants. Oral Surg. 8:198-205 Feb. 1955.

32. Fleming, H. S. Transplantation of teeth. Transplantation of tissues, ed. by L. A. Peer, vol. II. Baltimore, Williams and Wilkins, 1959, p. 271-299.

33. Fleming, H. S. Factors involved in transplantation of teeth. Dent. Clin. N. Amer. July 1962. p. 527-536.

34. Fleming, H. S. Heterotransplantation of tooth germs. Int. Ass. Dent. Res. 41:81 Mar. 1963.

35. Fomon, S. Cosmetic surgery. Philadelphia, J. B. Lippincott, 1960, p. 161-170.

36. Fong, C. C. and Agnew, R. G. Transplantation of teeth: Clinical and experimental studies. J. Amer. Dent. Ass. 56:77-86 Jan. 1958.

37. Gerstner, R. and Butcher, E. O. Preservation and storage of tooth germs. J. Dent. Res. 37:35-36 Feb. 1958.

38. Gibson, T. Tissue repair and tissue transplantation. Mod. Trends Plast. Surg. 1:1-16 1964.

39. Glasstone, S. The development of tooth germs in vitro. J. Anat. 70:260-266 Jan. 1936.

40. Good, R. A., Varco, R. L., Aust, J. B. and Zak, S. J. Transplantation studies in patients with agammaglobulinemia. Ann. N. Y. Acad. Sci. 64:882-928 Mar. 22, 1957.

41. Good, R. A., Martinez, C. and Gabrielsen, A. E. Progress toward transplantation of tissues in man. Advances Pediat. 13:93-127 1964.

42. Gorer, P. A., Loutit, J. F. and Micklem, H. S. Proposed revision of 'transplantese'. Nature. 189:1024-1025 Mar. 25, 1961.

43. Gorham, L. W. and West, W. T. Circulatory changes in osteolytic and osteoblastic reactions. Arch. Path. 77:673-680 Dec. 1964.

44. Guralnick, W. C. and Shulman, L. B. Tooth transplantation. Dent. Clin. N. Amer. July 1962. p. 499-511.

45. Hahn, W. E. The capacity of developing tooth germ elements for self-differentiation when transplanted. J. Dent. Res. 20:5-19 Feb. 1941.

46. Hale, M. L. Autogenous transplants. Oral Surg. 9:76-83 Jan. 1956.

47. Hale, M. L. Autogenous dental transplants. Dent. Clin. N. Amer. Nov. 1959. p. 749-754.

48. Hoffman, R. L. Formation of periodontal tissues around subcutaneously transplanted hamster molars. J. Dent. Res. 38:682 July-Aug. 1959.

49. Huggins, C. B., McCarroll, H. R. and Dahlberg, A. A. Transplantation of tooth germ elements and the experimental heterotopic formation of dentin and enamel. J. Exper. Med. 60:199-210 Aug. 1934.

50. Jenkins, G. N. The physiology of the mouth, ed. 2. Oxford, Blackwell, 1960, p. 149-152.

51. Jump, E. B. Histologic changes in five transplanted human third molars. J. Dent. Res. 38:667 July-Aug. 1959.

52. Kay, G. D. Prolonged survival of a skin homograft in a patient with very extensive burns. Ann. N. Y. Acad. Sci. 64:767-774 Mar. 22, 1957.

53. Kelly, W. D., Lamb, D. L., Varco, R. L. and Good, R. A. An investigation of Hodgkin's disease with respect to the problem of homotransplantation. Ann. N. Y. Acad. Sci. 87:187-202 May 31, 1960.

54. Kristensen, H. K. An improved method of decalcification. Stain Techn. 23:151-154 July, 1948.

55. Lefkowitz, W., Bodecker, C. F. and Mardfin, D. F. Odontogenesis of the rat molar. J. Dent. Res. 32:749-772 Dec. 1953.

56. Lefkowitz, E. and Swayne, P. Normal development of tooth buds cultured in vitro. J. Dent. Res. 37:1100-1114 Nov.-Dec. 1958.

57. Lefkowitz, W. Homotransplantation of cultured tooth buds. Dent. Clin. N. Amer. July 1962. p. 537-554.

58. Lefkowitz, W. Contributions of tissue culture to dentistry. Proc. Inst. Med. Chicago. 24:127-131 Sept. 1962.

59. Loeb, L. The biological basis of individuality. Springfield, Charles C. Thomas, 1945.

60. Longmire, W. P., Stone, H. B., Daniel, A. S. and Goon, C. D. Report of clinical experiences with homografts. Plast. Reconstr. Surg. 2:419-426 Sept. 1947.

61. Mannick, J. A. Transfer of "adoptive" immunity to homografts by RNA: A preliminary report. Surgery. 56:249-255 July 1964.

62. Manson, L. A., Foschi, G. V. and Palm, J. In vivo and in vitro studies of histocompatibility antigens isolated from a cultured mouse cell line. Proc. Nat. Acad. Sci. 48:1816-1822 Oct. 1962.

63. Manual of histologic and special staining technics, ed. 2. New York, McGraw-Hill, 1960.

64. Martinez, C. Smith, J. M., Blaese, M. and Good, R. A. Production of immunological tolerance in mice after repeated injection of disrupted spleen cells. J. Exper. Med. 118:743-758 Nov. 1963.

65. Maumenee, A. E. The immune concept: Its relation to corneal homotransplantation. Ann. N. Y. Acad. Sci. 59:453-461 Jan. 24, 1955.

66. Medawar, P. B. Zoologic laws of transplantation. Transplantation of tissues, ed. by L. A. Peer, vol. II. Baltimore, Williams and Wilkins, 1959, p. 41-69.

67. Mezrow, R. R. Homologous viable tooth transplantation. Oral Surg. 17:375-388 Mar. 1964.

68. Miller, H. M. Transplantation and reimplantation of teeth. Oral Surg. 9:84-95 Jan. 1956.

69. Miller, M. E. Guide to the dissection of the dog, ed. 3, Ithaca, Edwards Brothers, 1955, p. 33.

70. Morris, M. E. Pulp changes in homologous and autologous incisor transplants in the Rhesus monkey associated with the use of 6-MP. Int. Ass. Dent. Res. 41:88 Mar. 1963.

71. Myers, H. I. and Flanagan, V. D. Preliminary evaluation of tooth transplantation technics in the hamster. J. Dent. Res. 34:715 Oct. 1955.

72. Myers, H. I. and Flanagan, V. D. A comparison of results obtained from transplantation and replantation experiments using Syrian hamster teeth. Anat. Rec. 130:497-513 Mar. 1958.

73. Nagai, I. and Yoshioka, W. The behavior of transplanted tooth germ differentiation and development in mice. J. Dent. Res. 41:504 May-June 1962.

74. Nicholas, J. W., Jenkins, W. J. and Marsh, W. L. Human blood chimeras: A study of surviving twins. Brit. Med. J. 1:1458-1460 June 22, 1957.

75. Nordenram, A. Autotransplantation of teeth. Acta. Odont. Scand. 21:1-76 suppl. 33 1963.

76. Obersztyn, A. and Zaleski, M. Transplantation of vital and non-vital permanent tooth germs in dogs. Dent. Abs. 7:115-116 Feb. 1962.

77. Owen, R. D. Immunogenetic consequences of vascular anastomoses between bovine twins. Science. 102:400-401 Oct. 19, 1945.

78. Rapaport, F. T. and Converse, J. M. Observations on immunological manifestations of the homograft rejection phenomenon in man: The recall flare. Ann. N. Y. Acad. Sci. 64:836-841 Mar. 22, 1957.

79. Rapaport, F. T. and Converse, J. M. The immune response to multiple-set homografts: An experimental study in man. Ann. Surg. 147:273-280 Mar. 1958.

80. Rapaport, F. T., Thomas, L., Converse, J. M. and Lawrence, H. S. The specificity of skin homograft rejection in man. Ann. N. Y. Acad. Sci. 87:217-222 May 31, 1960.

81. Rogers, B. O. Transplantation of skin. Transplantation of tissues, ed. by L. A. Peer, vol. II. Baltimore, Williams and Wilkins, 1959, p. 73-134.

82. Schilder, H. and Amsterdam, M. Inflammatory potential of root canal medicaments. Oral Surg. 12:211-221 Feb. 1959.

83. Shulman, L. B. The transplantation antigenicity of tooth homografts. Oral Surg. 17:389-394 Mar. 1964.

84. Skoog, T. An experimental and clinical investigation of the effect of low temperature on the viability of excised skin. Plast. Reconstr. Surg. 14:403-416 Dec. 1954.

85. Smith, R. T. and Bridges, R. A. Immunological unresponsiveness in rabbits produced by neonatal injection of defined antigens. J. Exp. Med. 108:227-250 Aug. 1958.

86. Sorenson, F. M. and Gatewood, D. C. Personal communication.

87. Sorg, W. R. Nerve regeneration in replanted hamster teeth. J. Dent. Res. 39:1222-1231 Nov.-Dec. 1960.

88. Steendijk, R. Studies on the mechanism of the fixation of the tetracyclines to bone. Bone and tooth symposium, ed. by H. J. J. Blackwood. New York, Macmillan, 1964, p. 49-63.

89. Sutro, C. J. and Pomerantz, L. Transplantation of tooth germ elements to marrow cavities of tibias of kittens. Arch. Path. 28:199-206 Aug. 1939.

90. Urist, M. R. and Ibsen, K. H. Chemical reactivity of mineralized tissue with oxytetracycline. Arch. Path. 76:484-496 Nov. 1963.

91. Voeker, C. J. Autogenous dental transplant. J. Missouri Dent. Ass. 43:13-16 Feb. 1963.

92. Wolbach, S. B. and Howe, P. R. The incisor teeth of albino rats and guinea pigs in vitamin A deficiency and repair. Amer. Jour. Path. 9:275-293 May 1933.

93. Yoshioka, W. and Gonzales, F. Survival of mouse tooth germs after freezing in liquid nitrogen as demonstrated by differentiation of isologous transplants. J. Dent. Res. 38:983-993 Sept.-Oct. 1959.

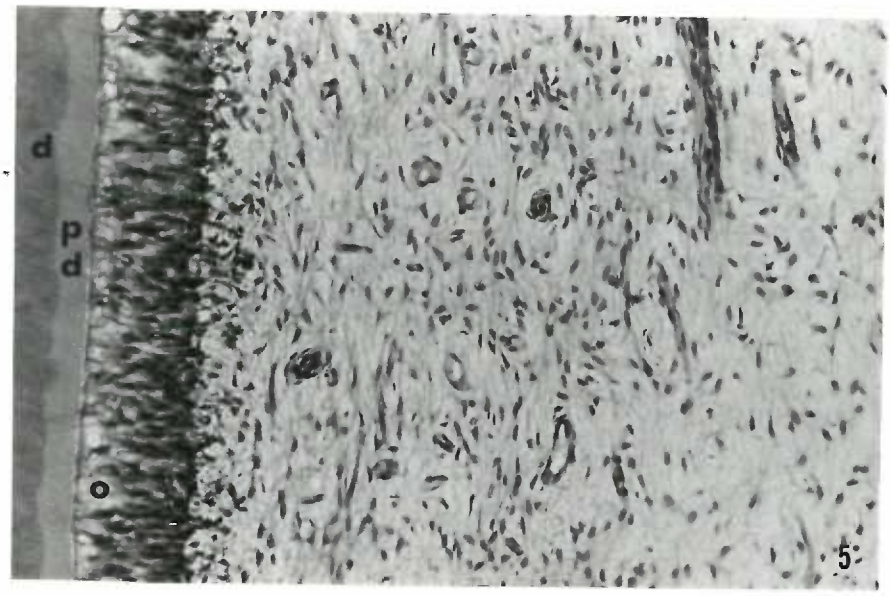
Plate 1

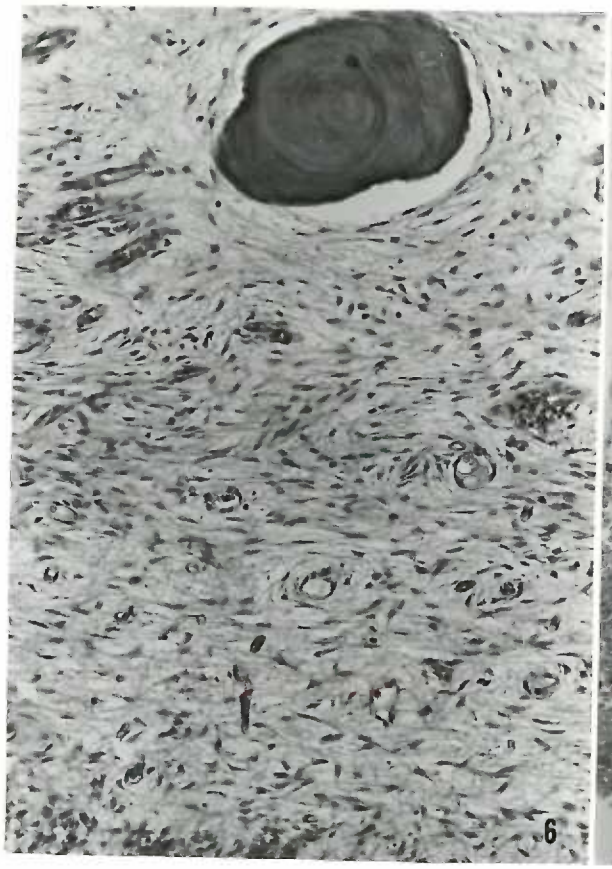
Virgin Control Teeth

Figure 5. Essentially normal pulp. Dentin (d), predentin (pd), odontoblastic layer (o) and pulp tissue in middle third of root. (H & E, 150x, enlarged three times)

Figure 6. Essentially normal pulp. False denticle in incisal portion of pulp tissue. (H & E, 150x, enlarged three times)

Figure 7. Normal pulp. Vascular channels in radicular portion of pulp. (H & E, 150x, enlarged three times)


d
p
d
o
5


6

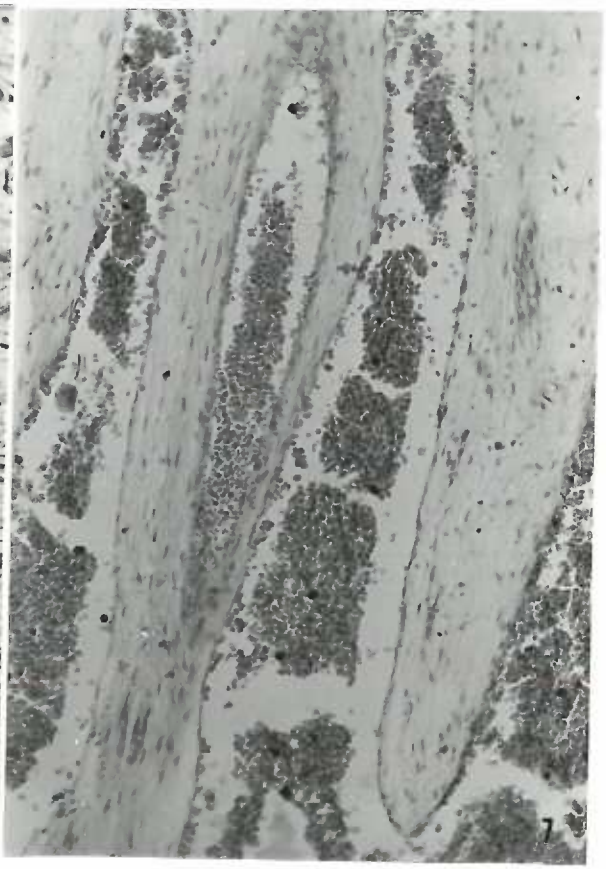

7

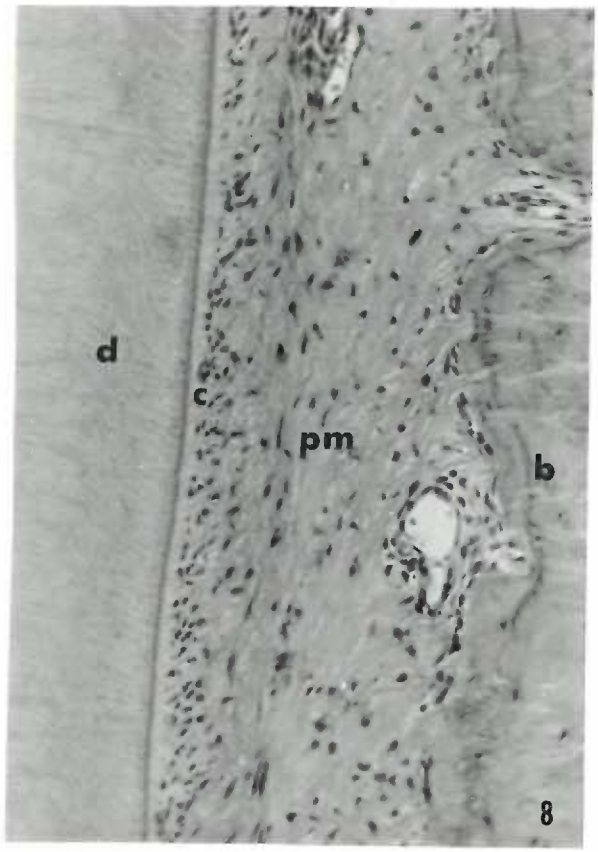
Plate 2

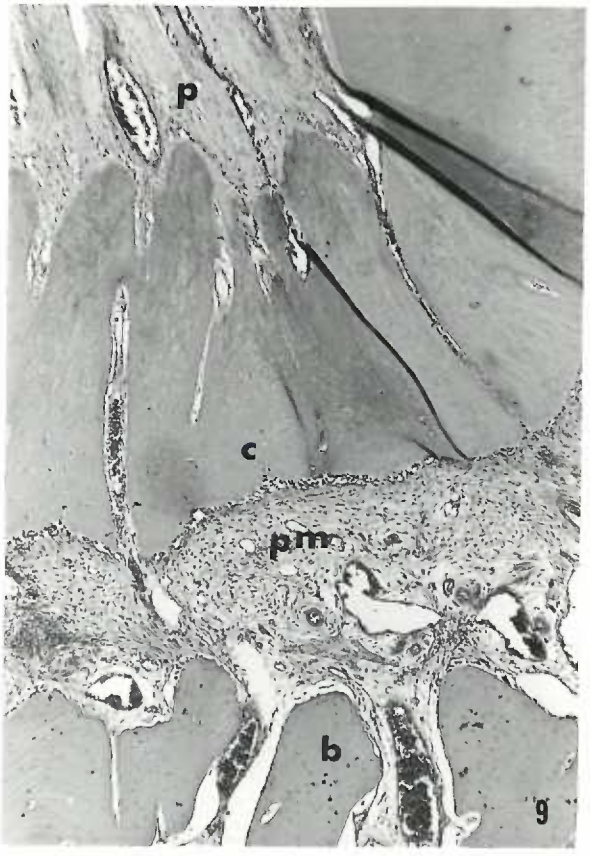
Virgin Control Teeth

Figure 8. Normal structures. Dentin (d), cementum (c), periodontal membrane (pm) and alveolar bone (b) in lower third of root. (H & E, 150x, enlarged three times)

Figure 9. Normal structures. Pulp (p), cementum (c), periodontal membrane (pm) and alveolar bone (b) in apical region. (H & E, 44x, enlarged three times)

Figure 10. Essentially normal bone marrow in periapical region with a few inflammatory cells which are either idiopathic or possibly due to intermittent pressures on tooth while operating on other teeth. (H & E, 300x, enlarged three times)


d
c
pm
b
8


p
c
pm
b
9

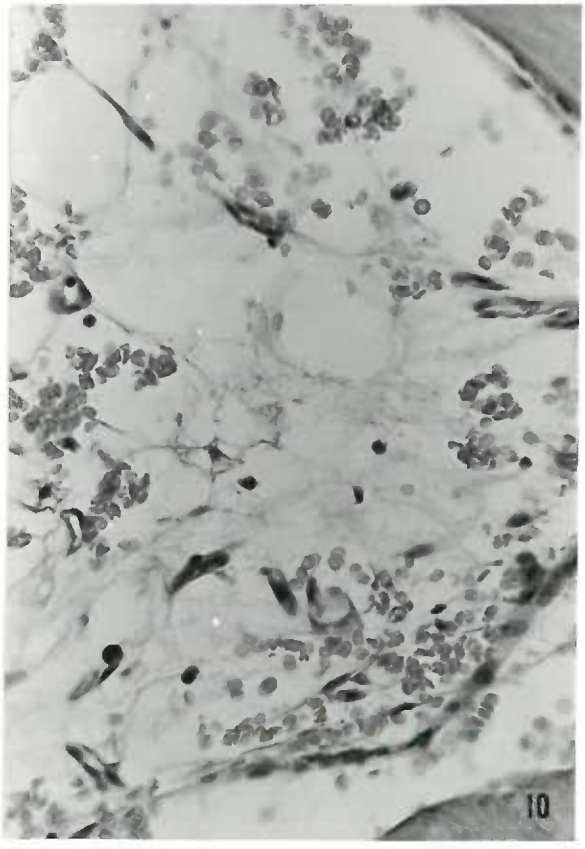
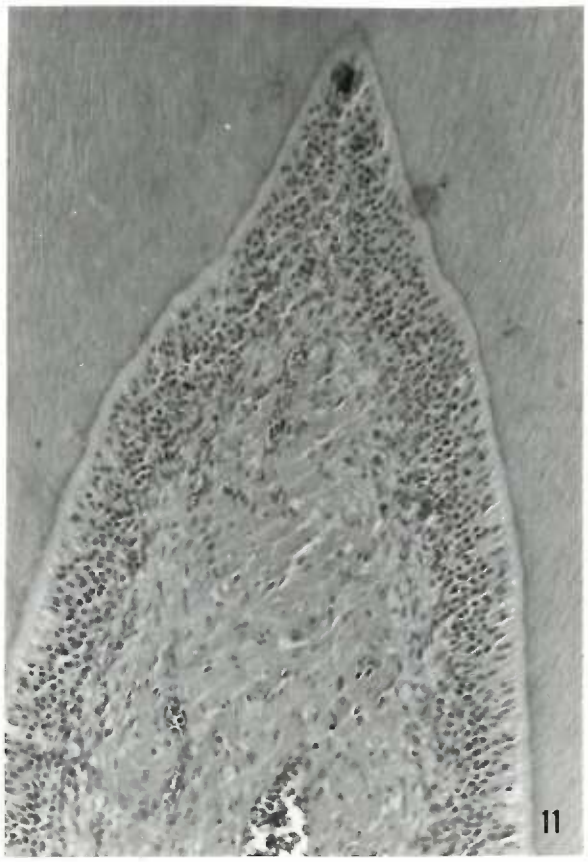

10

Plate 3

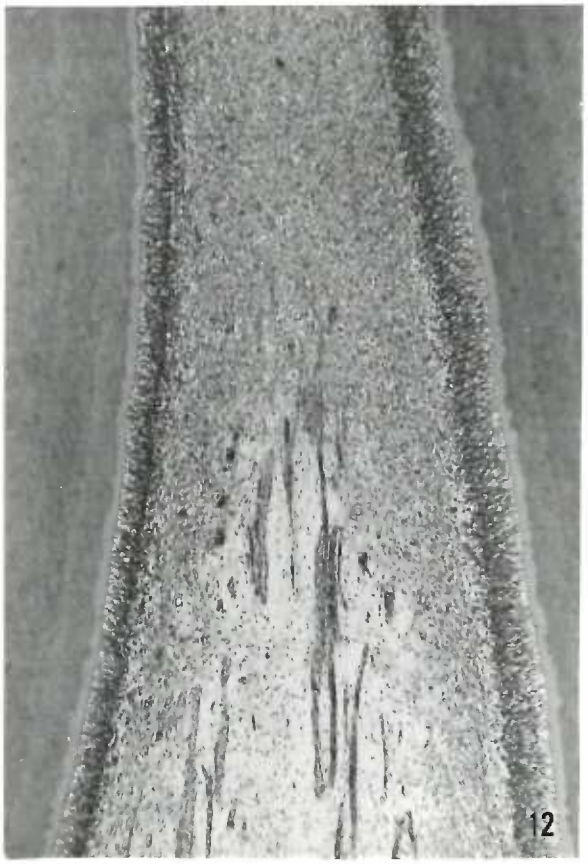
Virgin Control Teeth

Figure 11. Homogeneous eosinophilic pulp tissue in narrow incisal region. This early degenerative change may be due to lack of fixation. (H & E, 150x, enlarged three times)

Figure 12. Incisal pulp, approximately 10 millimeters above the transverse section showing the transition between normal and homogeneous eosinophilic pulp tissue. (H & E, 44x enlarged three times)



11



12

Plate 4

Virgin Control Teeth

Figure 13. Hemorrhage, inflammation and necrosis in crown fractured inadvertently during operations involving the other teeth. (H & E, 44x, enlarged three times)

Figure 14. Higher power view of hemorrhage and inflammation in fractured crown. (H & E, 150x, enlarged three times)


13

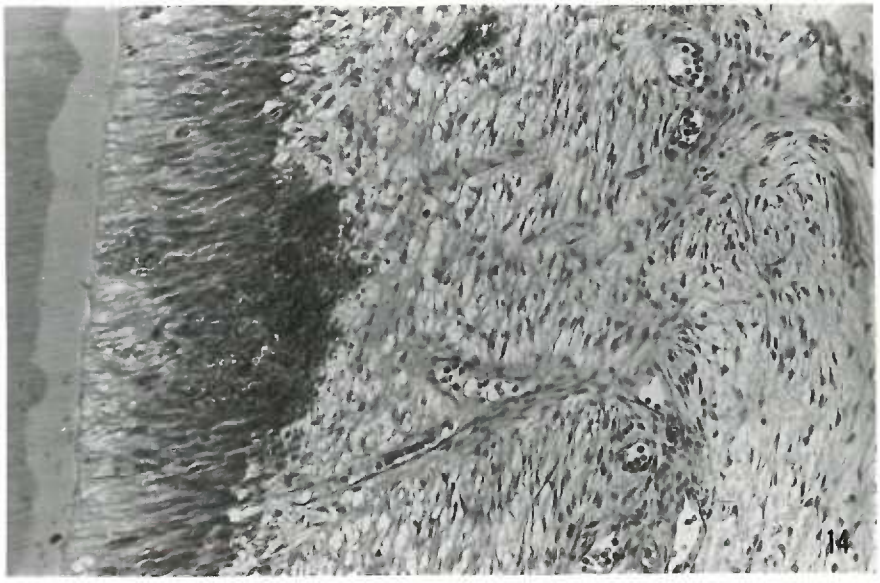

14

Plate 5

Residual Donor Pulp Tissue

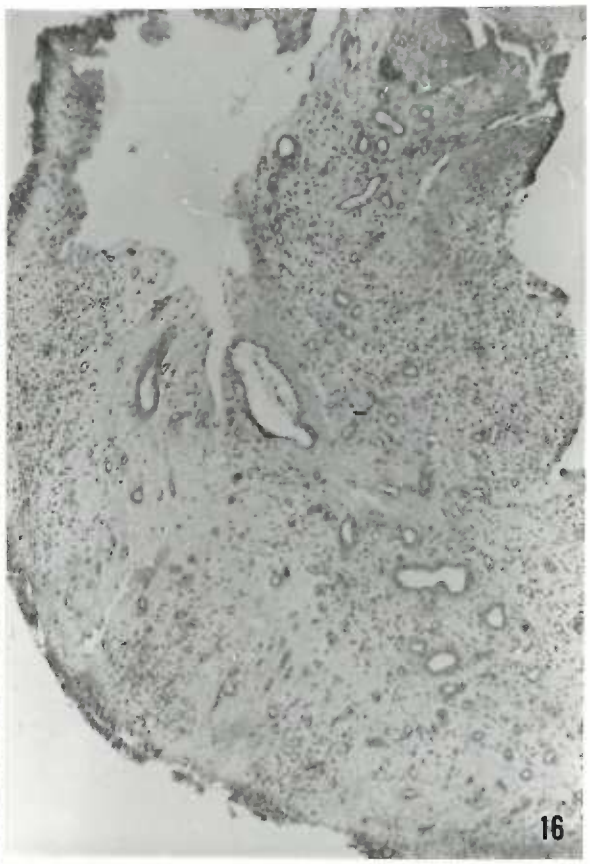
Figure 15. Fairly well preserved donor pulp tissue, after standing in physiologic saline for six to seven hours. (H & E, 44x, enlarged three times)

Figure 16. Poorly preserved donor pulp tissue with necrosis and inflammation. (H & E, 44x, enlarged three times)

Figure 17. Degenerating cells, presumably odontoblasts, on the periphery of a fragment of residual donor pulp tissue. (H & E, 300x, enlarged three times)

Figure 18. Plasma cells, lymphocytes and neutrophils in residual donor pulp tissue. Blood vessels are devoid of red cells, but contain marginated neutrophils and lymphocytes. (H & E, 300x, enlarged three times)


15


16


17

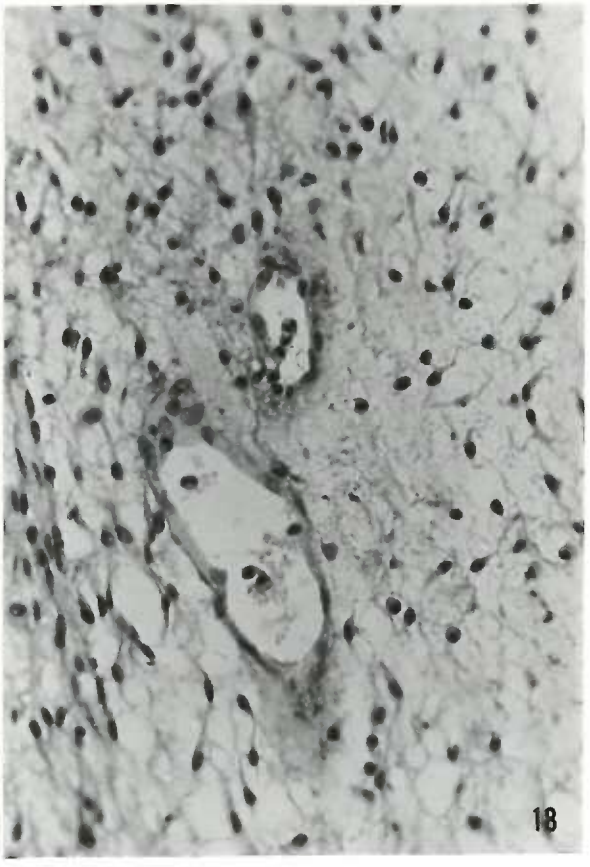

18

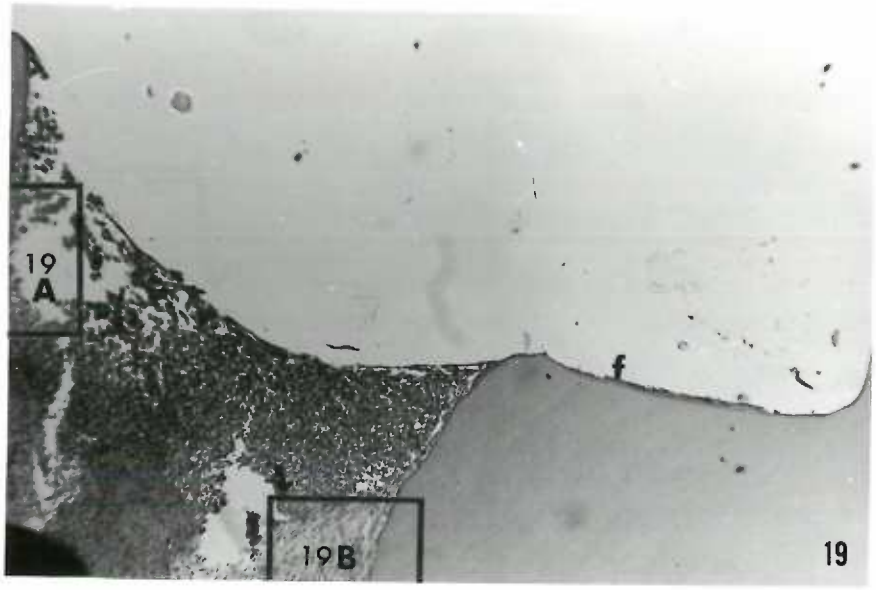
Plate 6

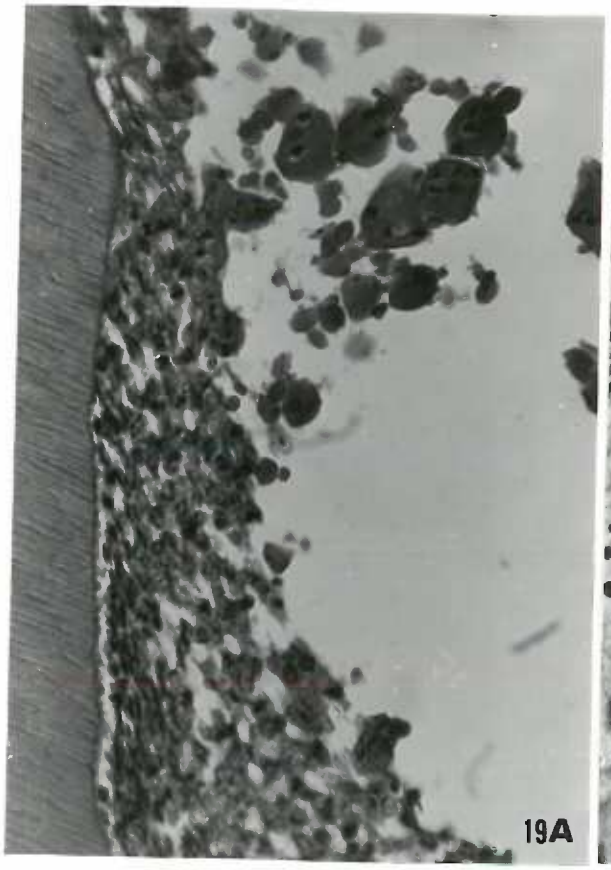
Three Week Host Teeth

Figure 19. Floor of cavity preparation (f) and superficial band of inflammation and necrosis on the surface of the transplantation area. (H & E, 44x, enlarged three times)

Figure 19A. Higher power view of cut dentinal wall, superficial inflammation and necrosis in transplantation area. The "giant cells" probably represent a coagulum of degenerating cells. (H & E, 300x, enlarged three times)

Figure 19B. Higher power view of superficial inflammation and regenerating fibrous connective tissue in transplantation area. (H & E, 300x, enlarged three times)


19
A
19B
f
19


19A


19B

Plate 7

Three Week Host Teeth

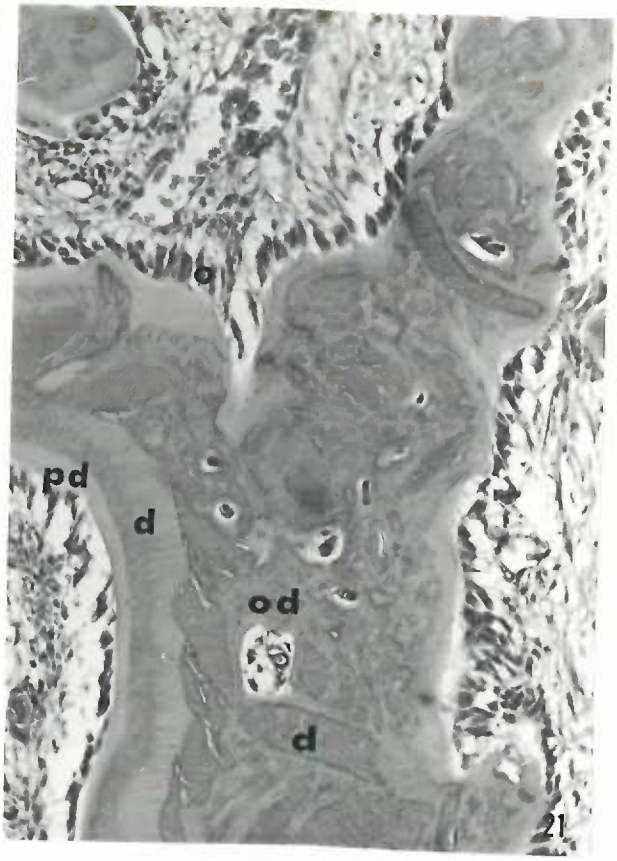
Figure 20. Moderately inflamed connective tissue in superficial portion of transplantation area. The lateral dentinal wall exhibits resorption with associated multinucleated giant cells. (H & E, 150x, enlarged three times)

Figure 21. Deep portion of transplantation area containing fragments of dislodged tubular dentin (d) encompassed in a mass of osteodentin (od) which in turn is overlaid by tubular dentin (d). Well-differentiated odontoblasts (o) are lined up next to the predentin (pd). (H & E, 150x, enlarged three times)

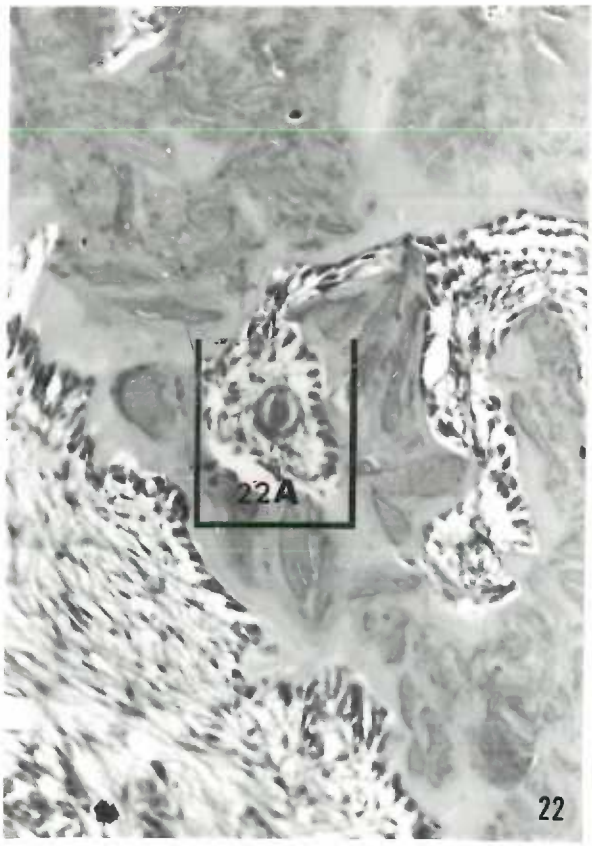
Figure 22. Giant cell in region of pulpal dentin masses. (H & E, 150x, enlarged three times)

Figure 22A. Higher power view of giant cell surrounding fragment of necrotic tubular dentin. (H & E, 785x, enlarged three times)

20



21



22

22A

Plate 8

Three Week Host Teeth

Figure 23. Deep portion of transplantation area exhibiting numerous young fibroblasts, collagen fibrils, a few chronic inflammatory cells and osteodentin. (H & E, 300x, enlarged three times)

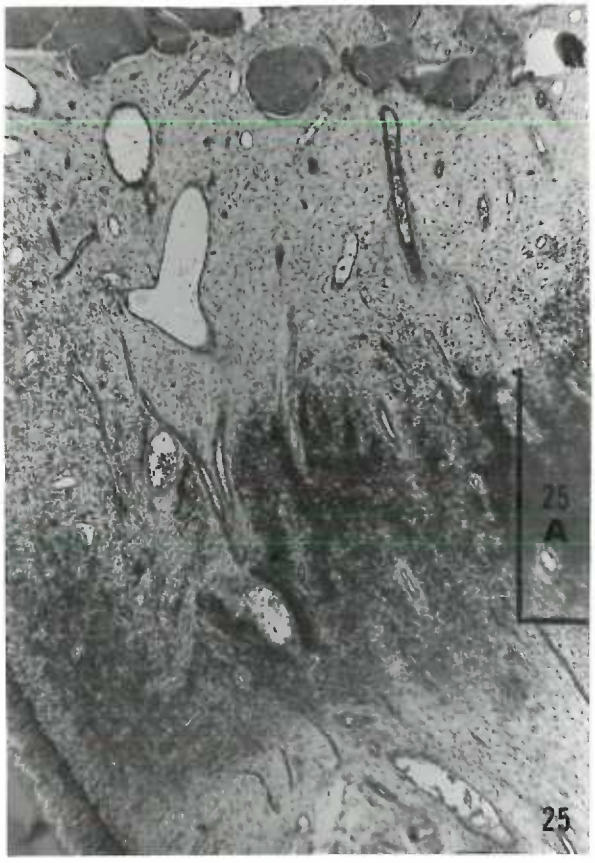
Figure 24. The wall of the operative area is lined by osteodentin above the notch or groove (arrow) that marks the radicular margin of the transplantation area. Below the notch a calciotraumatic line (cl) separates the postoperatively formed tubular dentin from the preoperative dentin. (H & E, 150x, enlarged three times)

Figure 25. Well-delineated band of hemorrhage below the pulpal dentinal masses extending between the lateral walls of the root canal. (H & E, 44x, enlarged three times)

Figure 25A. Higher power view of the hemorrhagic band containing hemosiderin pigment. (H & E, 150x, enlarged three times)


23


cl


25
A
25


25A

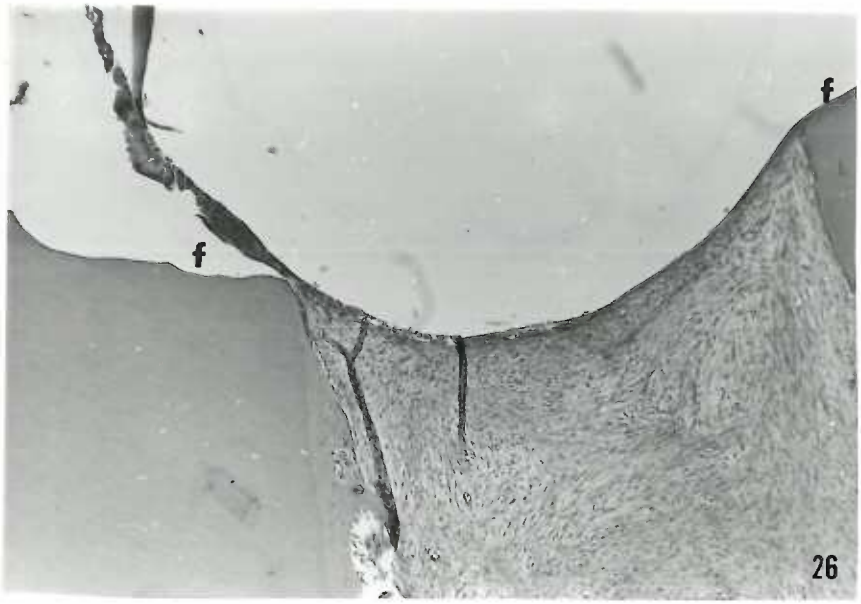
Plate 9

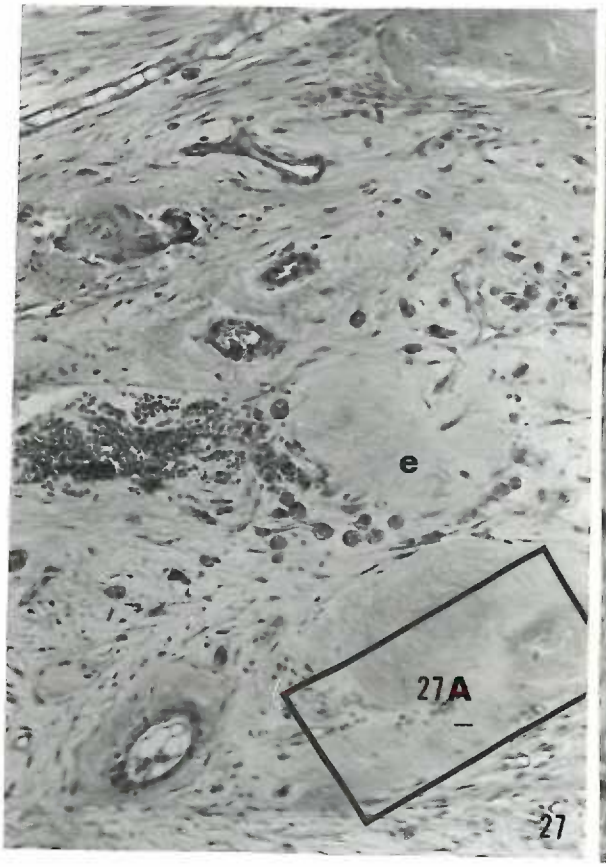
Five Week Host Teeth

Figure 26. Floor of cavity preparation (f) and superficial connective tissue in transplantation region. The inflammatory reaction is less severe than in the three week time interval. (H & E, 44x, enlarged three times)

Figure 27. Eosinophilic fibrillar or "cotton candy" structures (e) in transplantation area. These fibrous masses are often associated with and, in fact, may represent the sclerosing degenerating blood vessels which commonly occur in the transplanted tissues. (H & E, 150x, enlarged three times)

Figure 27A. Higher power view of the fibrillar eosinophilic structures. Several of these masses contain a central lumen-like space. (H & E, 300x, enlarged three times)


f
f
26


e
27A
27


27A

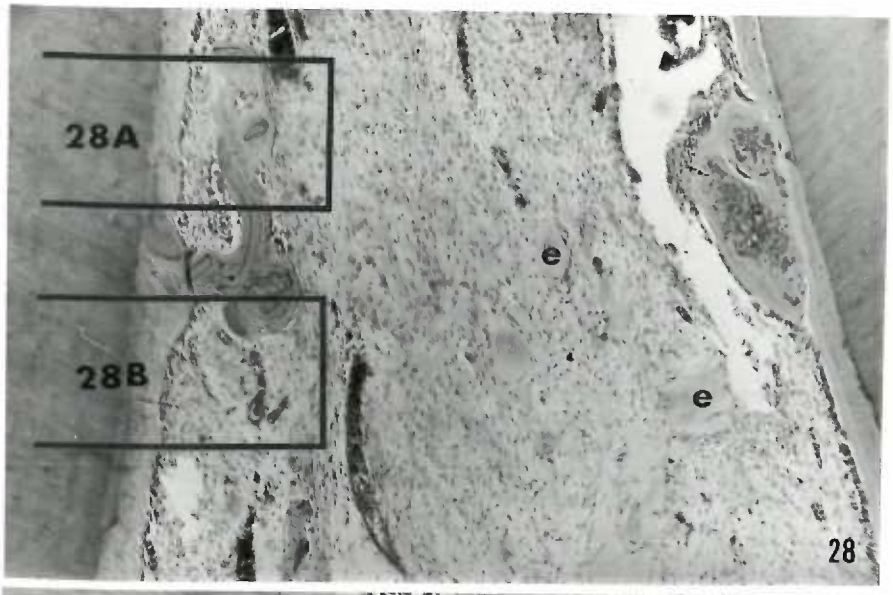
Plate 10

Five Week Host Teeth

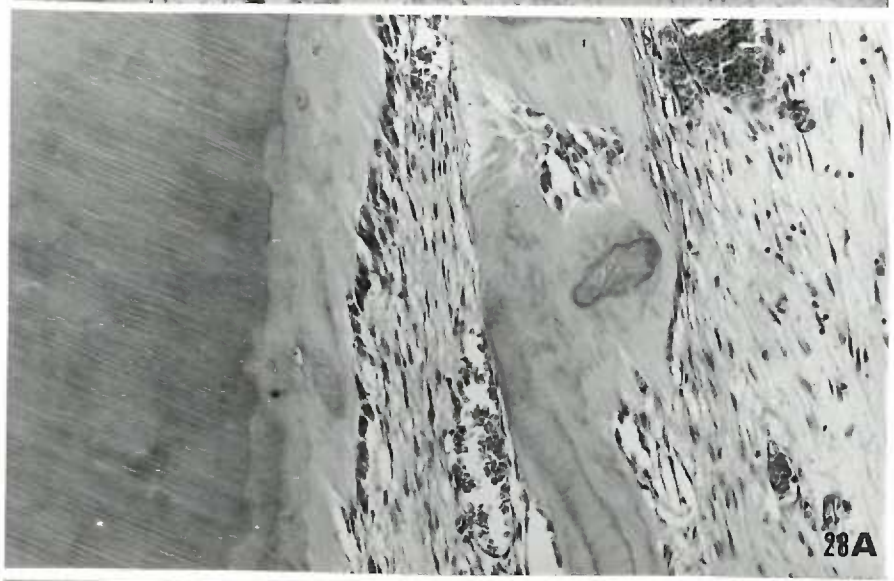
Figure 28. Low power view of transplantation area. The mildly inflamed fibrous connective tissue contains several eosinophilic fibrillar structures (e). (H & E, 44x, enlarged three times)

Figure 28A. Higher power view of the dentinal wall in the transplantation area. The osteodentin laid down on the cut surface of the wall has been overlaid with irregular tubular dentin. (H & E, 150x, enlarged three times)

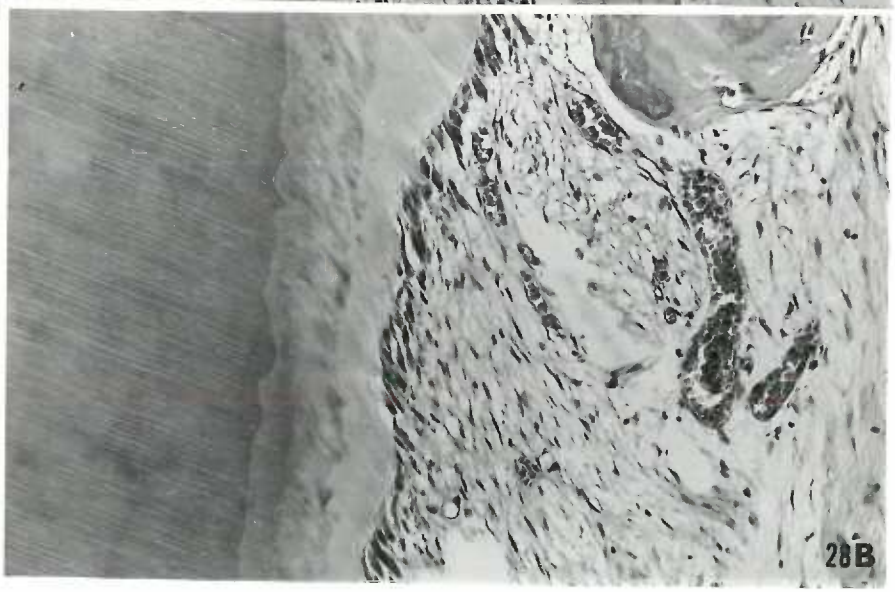
Figure 28B. Higher power view of the region below the dentinal fragments. The preoperatively formed tubular dentin is covered with postoperatively formed irregular tubular dentin. (H & E, 150x, enlarged three times)



28



28A



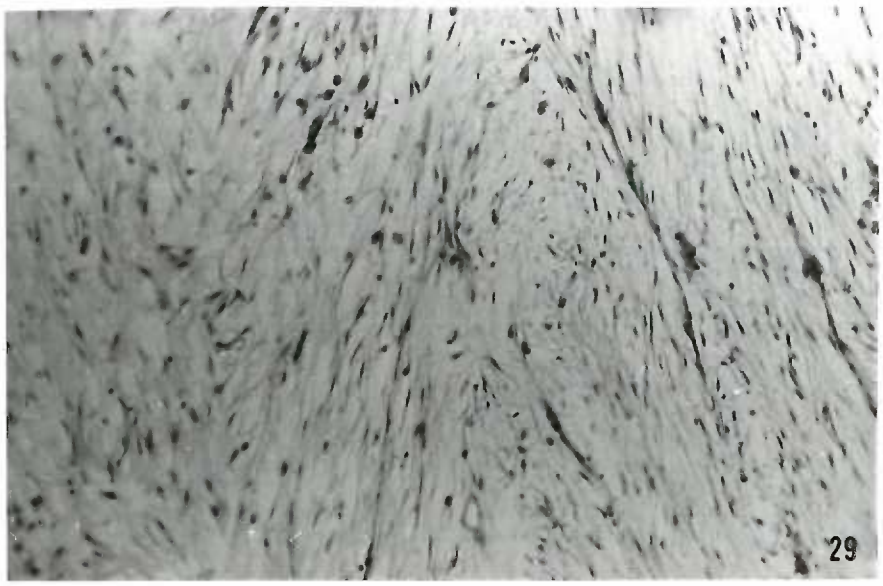
28B

Plate 11

Five Week Host Teeth

Figure 29. Youthful connective tissue in transplantation area. Except for a few inflammatory cells the tissue resembles normal pulp. (H & E, 150x, enlarged three times)

Figure 30. Radicular pulp below dentinal fragments containing some hemorrhage and engorged blood vessels but no distinct hemorrhagic band as was present in some three-week host teeth. (H & E, 44x, enlarged three times)


29

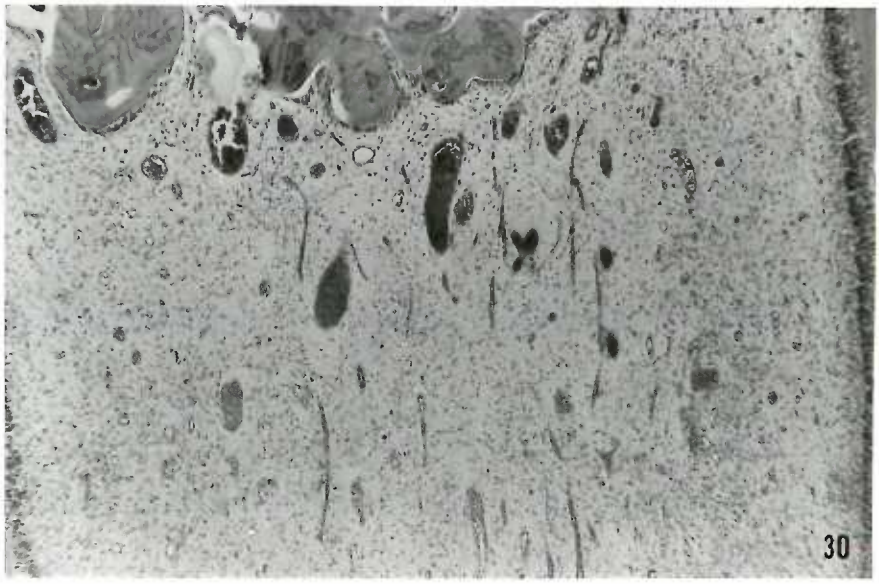

30

Plate 12

Nine Week Host Teeth

Figure 31. Low power view of the cavity preparation and surface of the transplantation area. Note the supporting ledge (sl). (H & E, 27x, enlarged three times)

Figure 31A. Higher power view of the superficial inflammatory exudate and the incisal portion of the transplantation area. The connective tissue contains numerous clear spaces and engorged blood vessels. (H & E, 44x, enlarged three times)


sl
sl
31

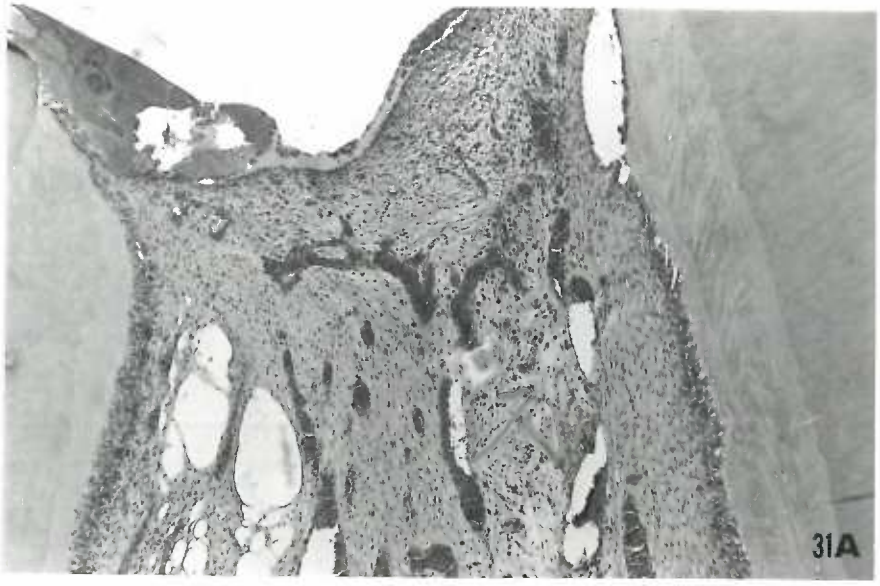

31A

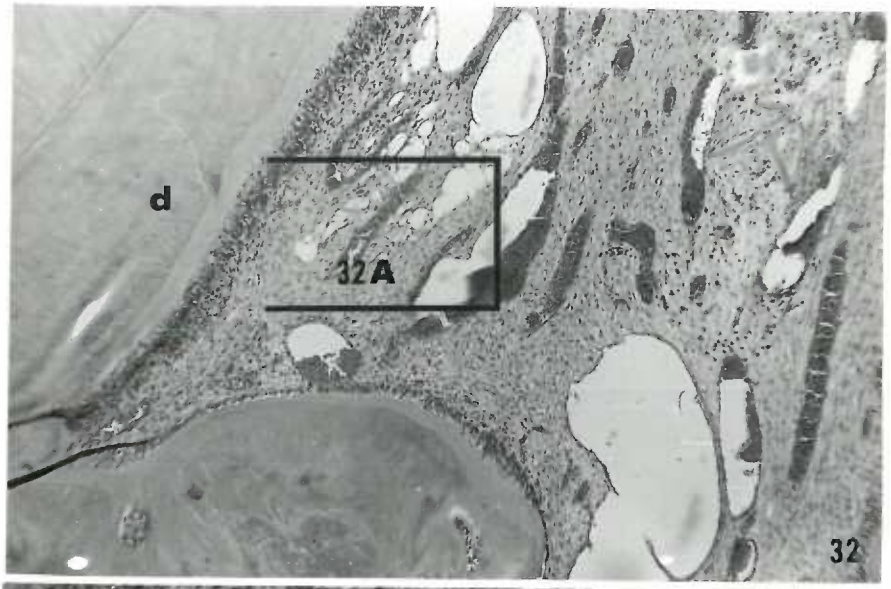
Plate 13

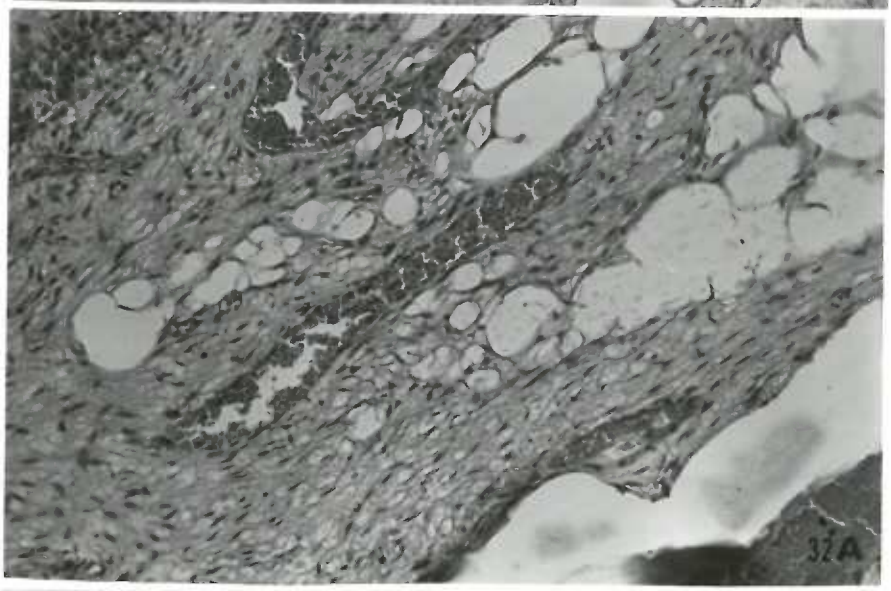
Nine Week Host Teeth

Figure 32. Dentinal wall and attached dentinal mass covered with a thick layer of postoperatively formed tubular dentin (d). The connective tissue contains numerous clear spaces of varying sizes and shapes. (H & E, 44x, enlarged three times)

Figure 32A. Higher power view of the boxed area in Figure 32 showing the connective tissue containing the clear spaces. Some of the spaces appear to be large venous channels devoid of blood cells and others to represent areas previously occupied by masses of dentin; however, in many instances it was impossible to establish any direct relationship. This type of clear space formation could lead to what is generally called reticular degeneration. (H & E, 150x, enlarged three times)

Figure 33. Foreign body type giant cells surrounding an amorphous rod-like structure in the transplantation area. The foreign structure may possibly be a fragment of one of the paper points used in taking the bacterial cultures. (H & E, 300x, enlarged three times)


d
32A
32


32A

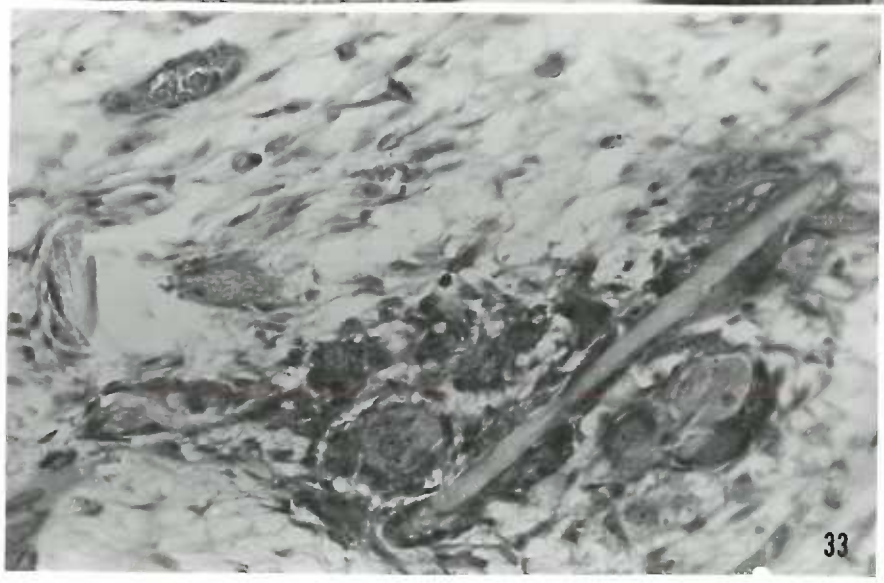

33

Plate 14

Three Week Sham-operated Teeth

Figure 34. Superficial band of necrosis and large subjacent accumulation of inflammatory cells and serum. The large clear space at the bottom of the field was probably filled with inflammatory exudate which was lost during processing. (H & E, 44x, enlarged three times)

Figure 34A. Higher power view of the inflammatory exudate consisting of neutrophils, a few mononuclear cells and serum. (H & E, 300x, enlarged three times)

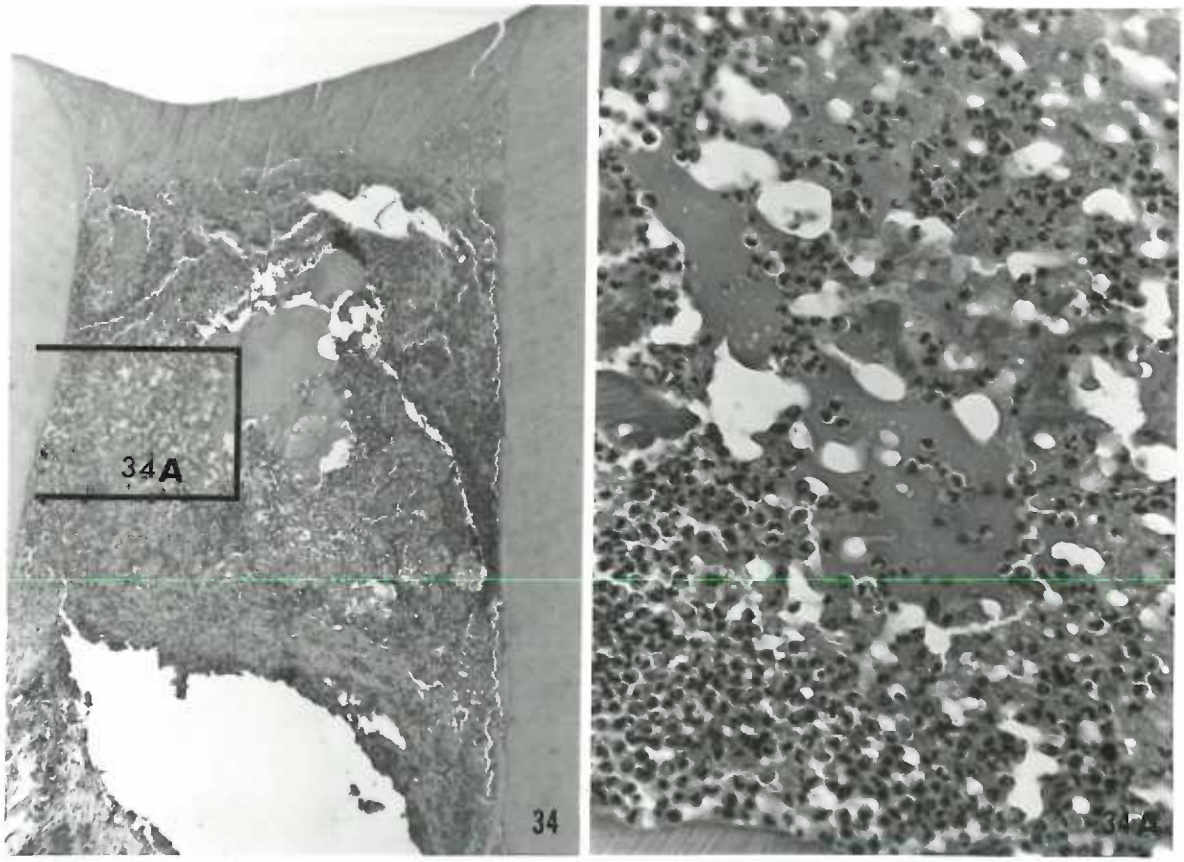
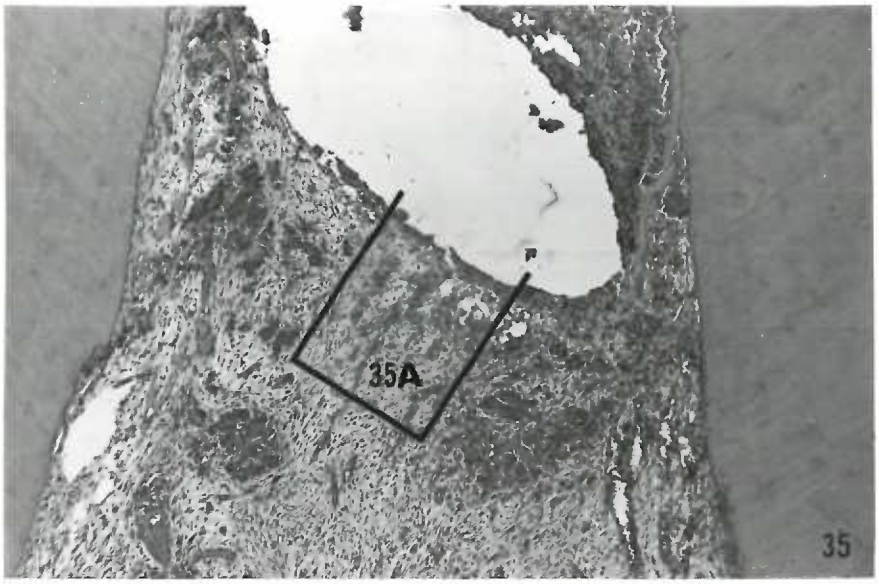

34A
34
34A

Plate 15

Three Week Sham-operated Teeth

Figure 35. Clear space seen at the bottom of Figure 34 formerly occupied by inflammatory exudate, and chronically inflamed connective tissue in deep portion of the extirpation area. The connective tissue contains fragments of dislodged tubular dentin and the surrounding cut dentinal walls contain little or no postoperatively formed dentin. (H & E, 44x, enlarged three times)

Figure 35A. Higher power view of the chronically inflamed connective tissue below the clear space. (H & E, 150x, enlarged three times)


35A
35

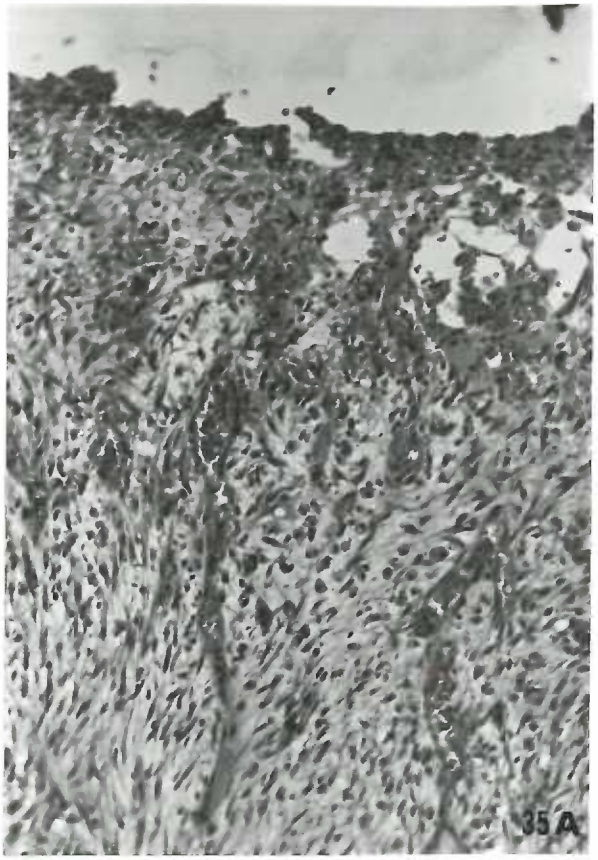

35A

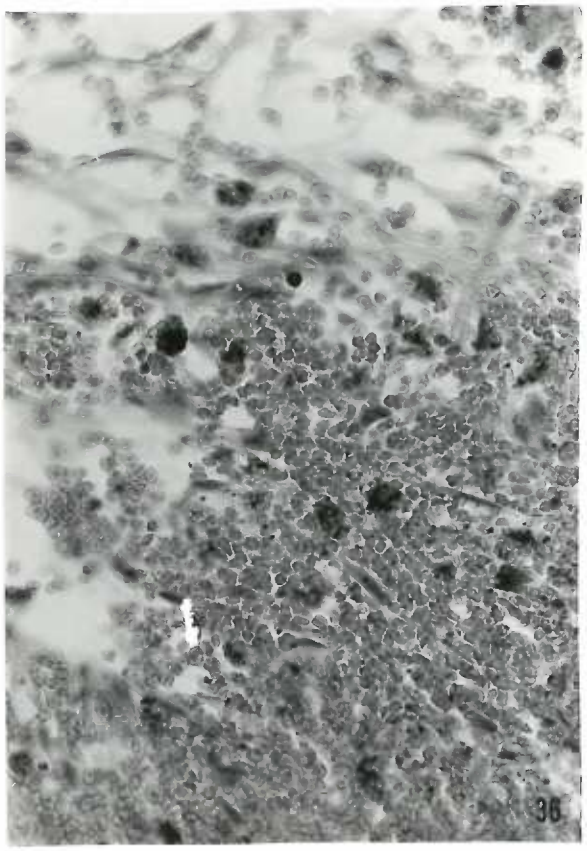
Plate 16

Five Week Sham-operated Teeth

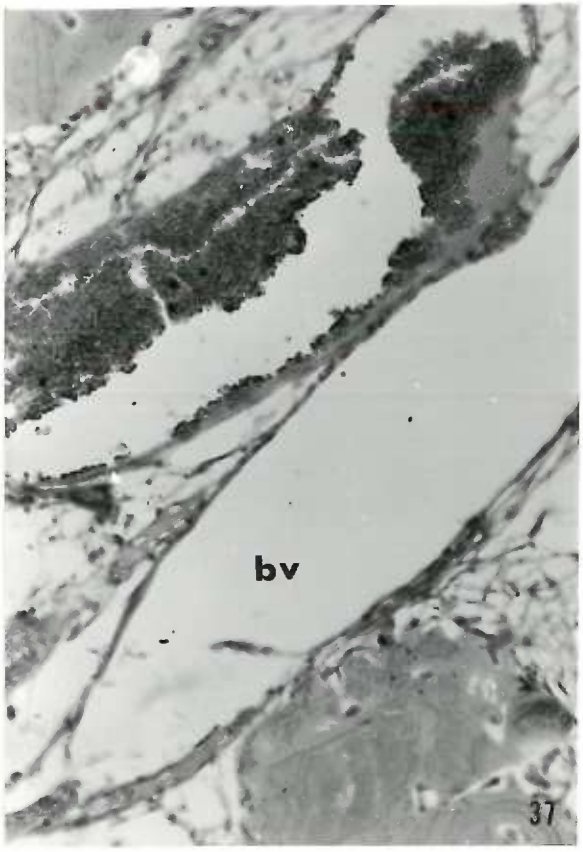
Figure 36. Hemorrhage, hemosiderin-laden macrophages and regenerating connective tissue in extirpation region. (H & E, 300x, enlarged three times)

Figure 37. Large dilated venous channels in extirpation area. Degenerating vessels (bv) are devoid of red cells, and coalescence of similar vessels of varying sizes can resemble "reticular degeneration". (H & E, 150x, enlarged three times)

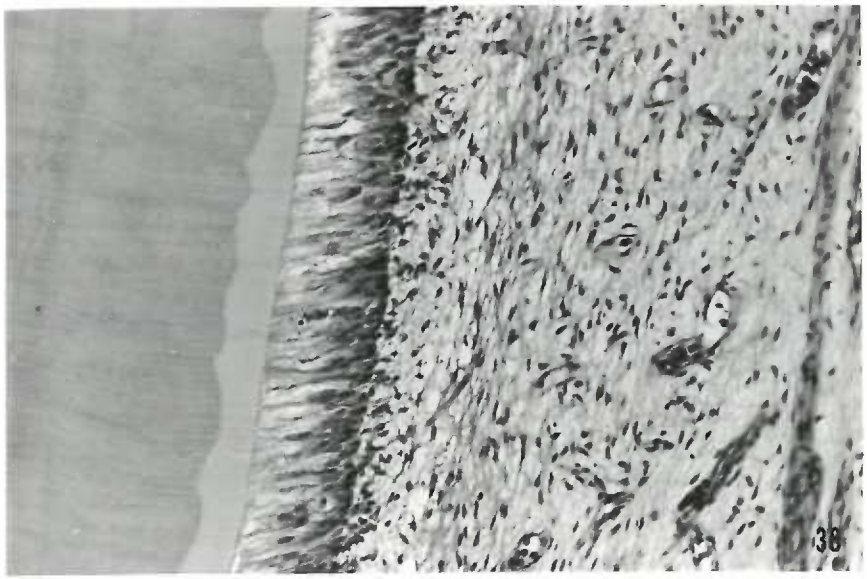
Figure 38. Relatively normal dentin and pulp tissue below extirpation area. (H & E, 150x, enlarged three times)



36



37



38

Plate 17

Five Week Sham-operated Teeth

Figure 39. Floor of cavity preparation (f), narrow superficial band of exudate and moderately inflamed regenerating connective tissue in extirpation area. Postoperatively formed dentin lines the cut dentinal walls in the middle and lower portion of the area. (H & E, 44x, enlarged three times)

Figure 40. Cut dentinal wall in the upper portion of the field is covered with osteodentin which gradually merges into irregular tubular dentin in the lower portion. The narrow cracks between the preoperatively and postoperatively formed dentin are a fairly consistent sectioning artifact. (H & E, 150x, enlarged three times)

39

40

Plate 18

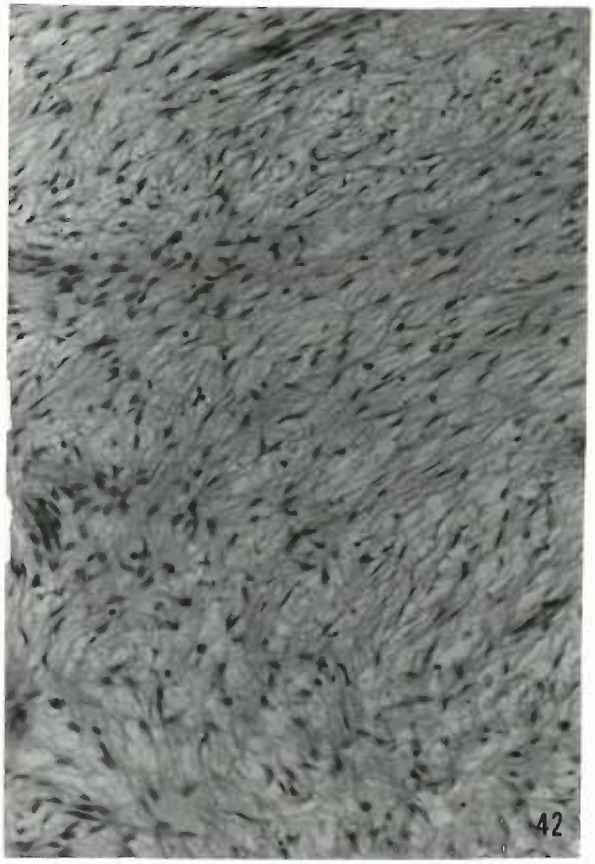
Nine Week Sham-operated Teeth

Figure 41. Regenerated fibrous connective tissue and thick layer of postoperatively formed dentin in extirpation area. This was the only sham-operated tooth to contain the eosinophilic fibrillar structures (e) routinely found in the host teeth. (H & E, 44x, enlarged three times)

Figure 42. Youthful connective tissue in superficial portion of the extirpation area containing a few chronic inflammatory cells. (H & E, 150x, enlarged three times)

Figure 43. Dentin, predentin, odontoblastic layer and youthful vascular connective tissue in deep portion of the extirpation area. Connective tissue in the area contains hemorrhage and a mild chronic inflammatory cell infiltrate. (H & E, 150x, enlarged three times)


e
41


42

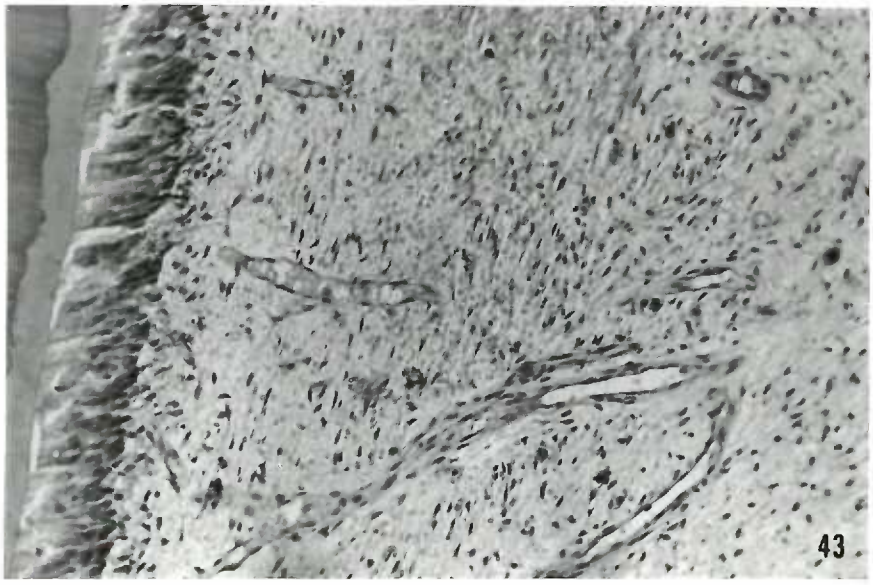
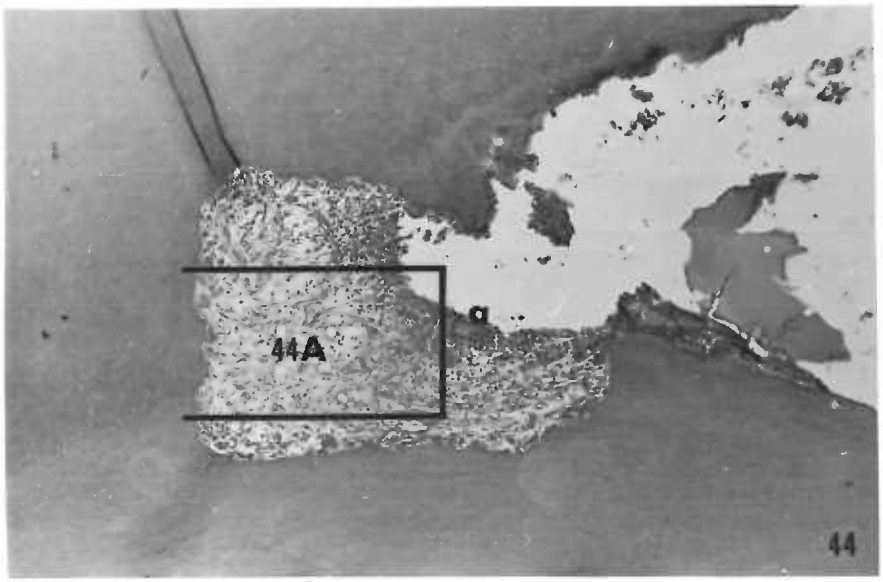

43

Plate 19

Three Week Donor Tooth
(With periapical lesion)

Figure 44. Amputation site of distal coronal pulp tissue (a), and empty mesial coronal chamber containing debris. The amputation site contains a dense band of chronic inflammatory cells which are diffusely distributed in the underlying pulp tissue. The dentinal wall exhibits areas of resorption with associated giant cells. (H & E, 44x, enlarged three times)

Figure 44A. Higher power view of chronically inflamed pulp tissue near amputation site, and dentin resorption with giant cells. (H & E, 150x, enlarged three times)


44A
44

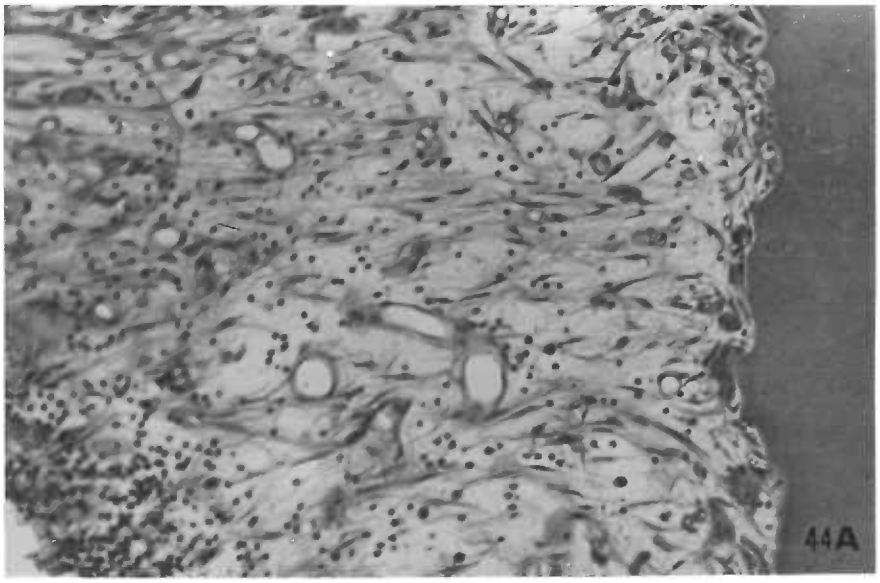

44A

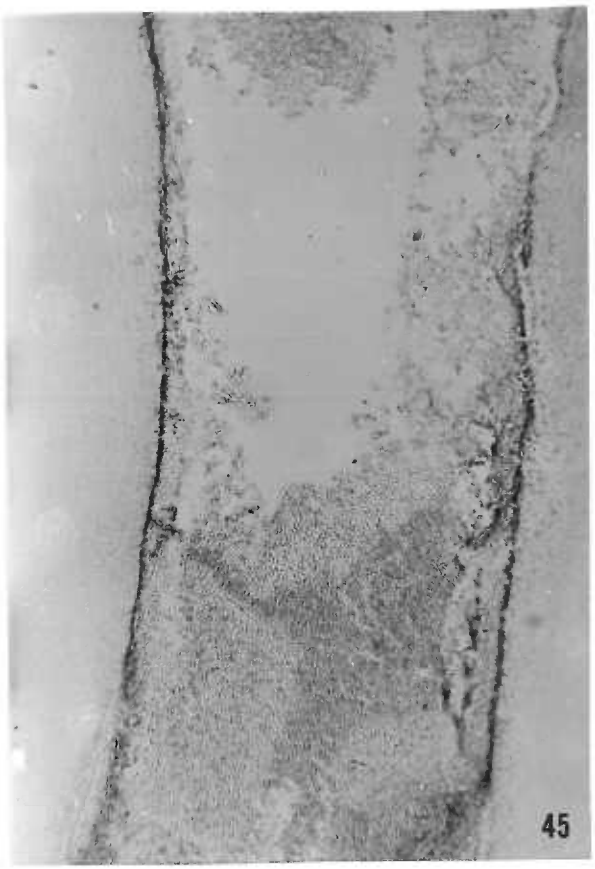
Plate 20

Three Week Donor Tooth
(With periapical lesion)

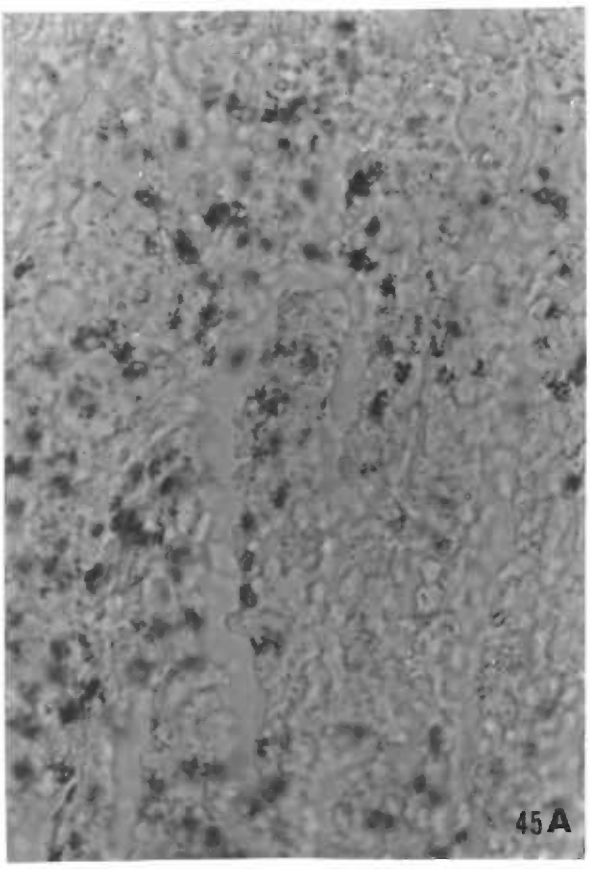
Figure 45. Middle third of mesial donor canal containing numerous Gram positive bacteria and inflammatory cells. The bacteria are concentrated along the dentinal walls. (Brown and Brenn tissue Gram stain, 44x, enlarged three times)

Figure 45A. Higher power view of inflammatory exudate deeper in pulp, with macrophages containing phagocytized bacteria. (B & B, 500x, enlarged three times)

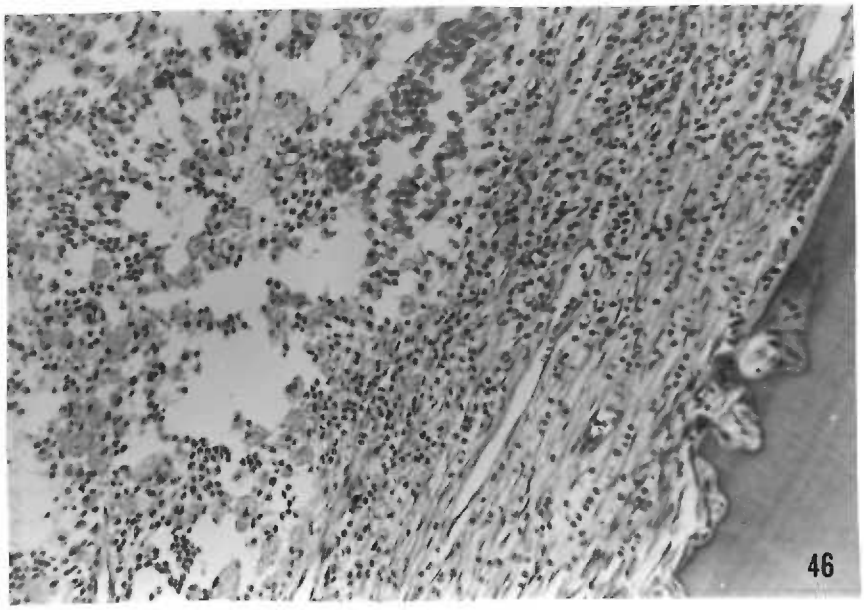
Figure 46. Chronically inflamed regenerating connective tissue and focal areas of resorption in mesial donor canal. The proliferating connective tissue filled the apical fourth of the canal, and may have arisen from the periapical tissues. (H & E, 150x, enlarged three times)



45



45A



46

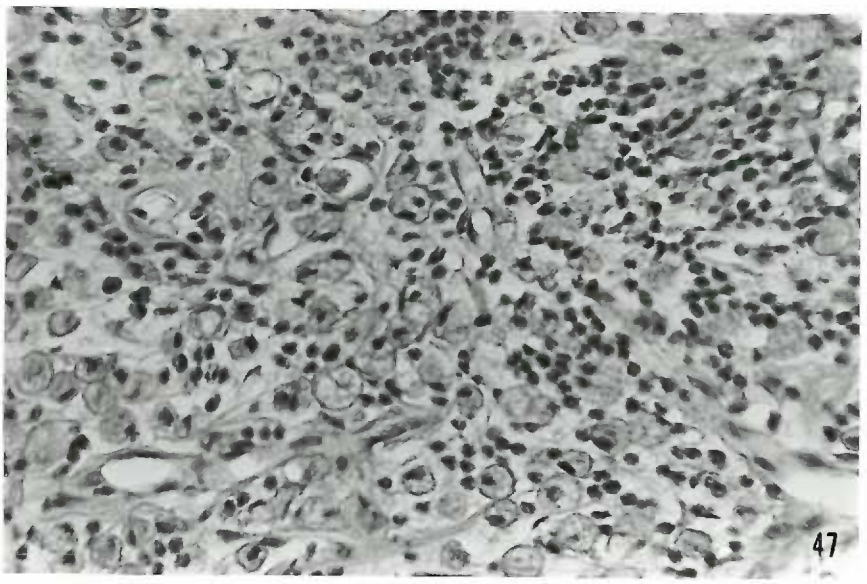
Plate 21

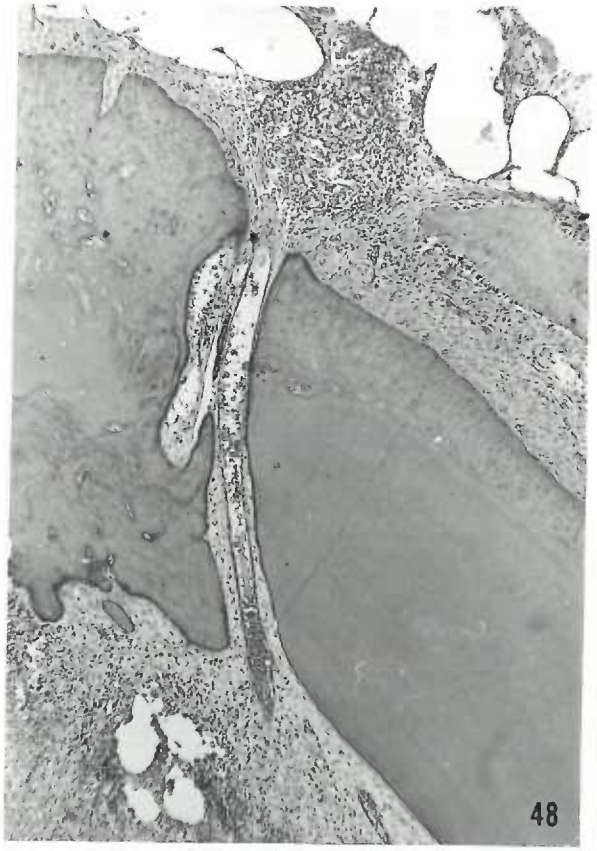
Three Week Donor Tooth
(With periapical lesion)

Figure 47. Higher power view of chronic inflammation in mesial donor canal with numerous foamy macrophages. (H & E, 300x, enlarged three times)

Figure 48. Apical foramen connecting donor canal with a small focal area of chronic periapical inflammation. (H & E, 44x, enlarged three times)

Figure 48A. Higher power view of the periapical lesion containing phagocytized bacteria. (B & B, 500x, enlarged three times)


47


48

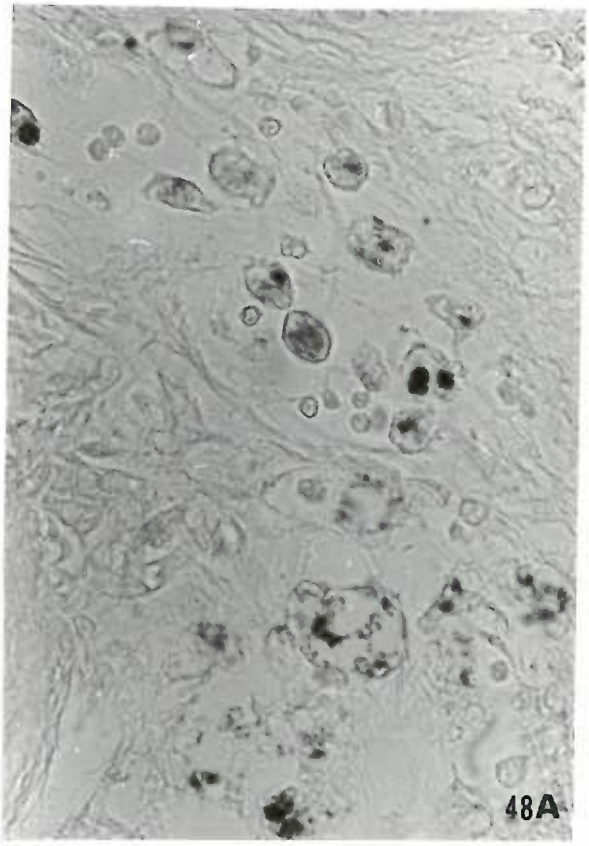

48A

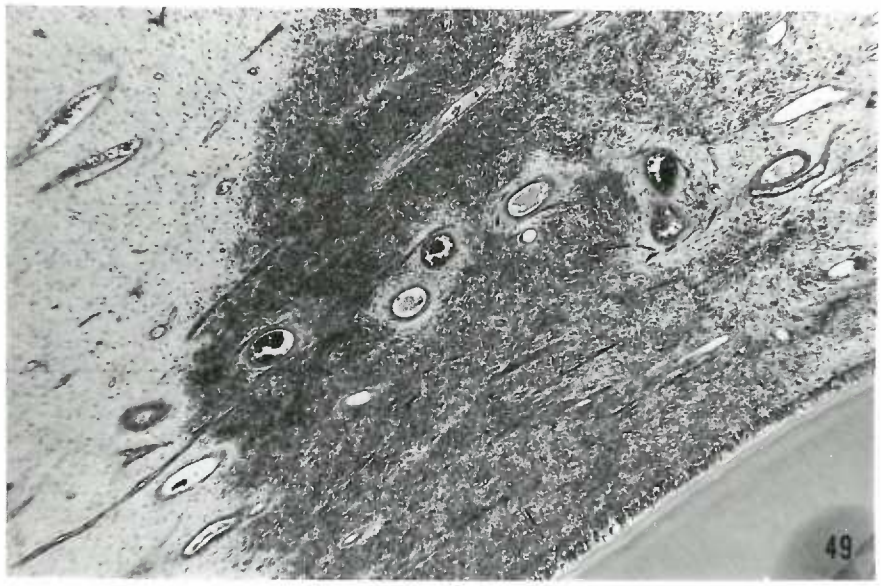
Plate 22

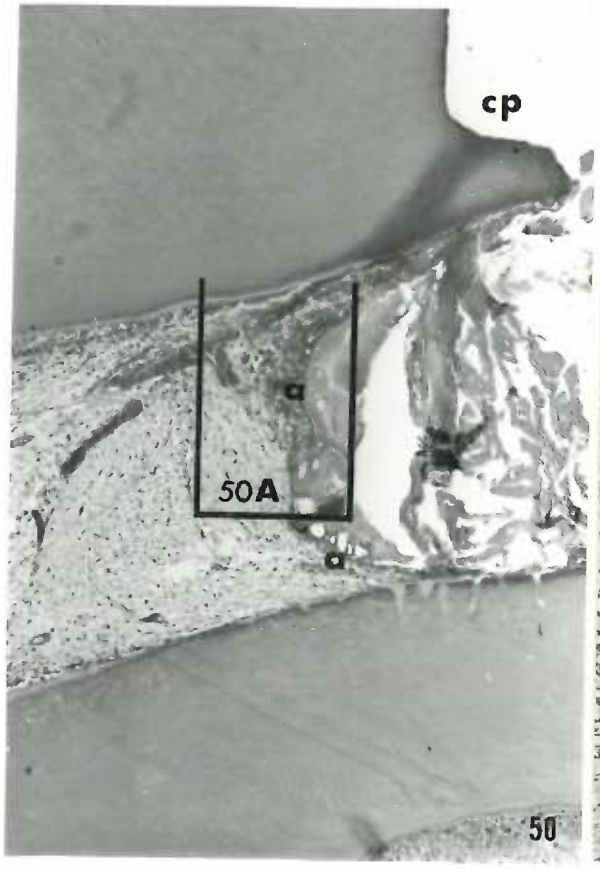
Three Week Donor Tooth
(Without periapical pathology)

Figure 49. Dense hemorrhagic band in distal coronal pulp tissue. The hemorrhage occurred three millimeters distal to the amputation site and contains both intercellular and phagocytized hemosiderin pigment. (H & E, 44x, enlarged three times)

Figure 50. Amputation site of distal coronal pulp tissue (a) and cavity preparation (cp). (H & E, 44x, enlarged three times)

Figure 50A. Higher power view of the distal amputation site which contains a well-delineated band of chronic inflammatory cells next to the homogeneous eosinophilic coagulum. (H & E, 150x, enlarged three times)


49


cp
a
50A
a
50

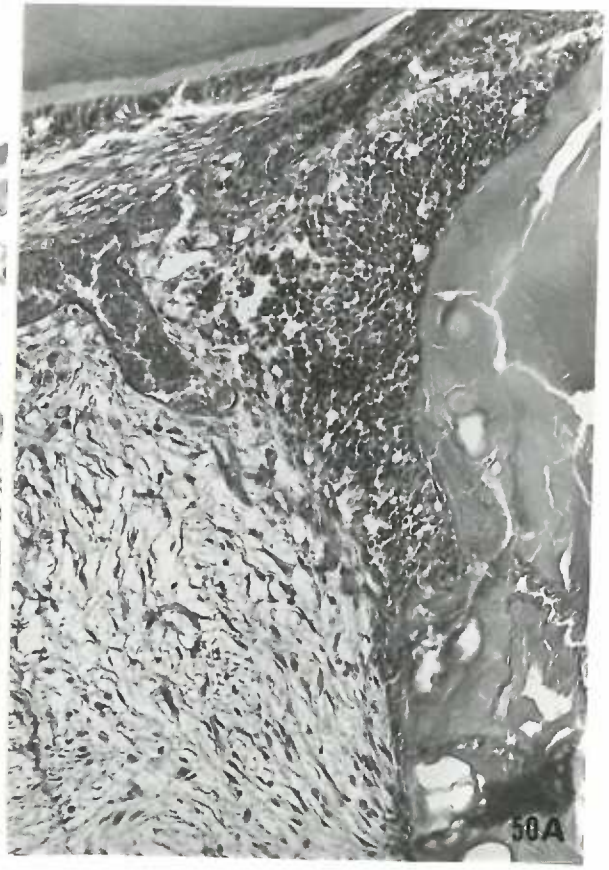

50A

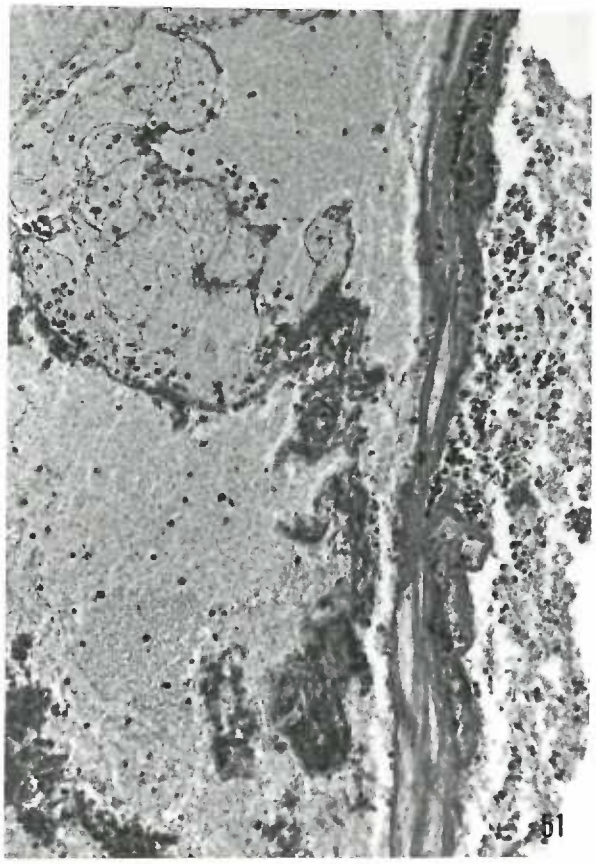
Plate 23

Three Week Donor Tooth
(Without periapical pathology)

Figure 51. Hemorrhage, inflammation and necrotic debris in the extirpated mesial coronal pulp chamber occlusal to the regenerating connective tissue. (H & E, 150x, enlarged three times)

Figure 52. Occlusal margin of the chronically inflamed regenerating connective tissue which fills the apical half of the mesial donor canal. (H & E, 150x, enlarged three times)

Figure 53. Chronically inflamed connective tissue in the apical region of the mesial canal from which the donor pulp was taken. (H & E, 44x, enlarged three times)


51


52

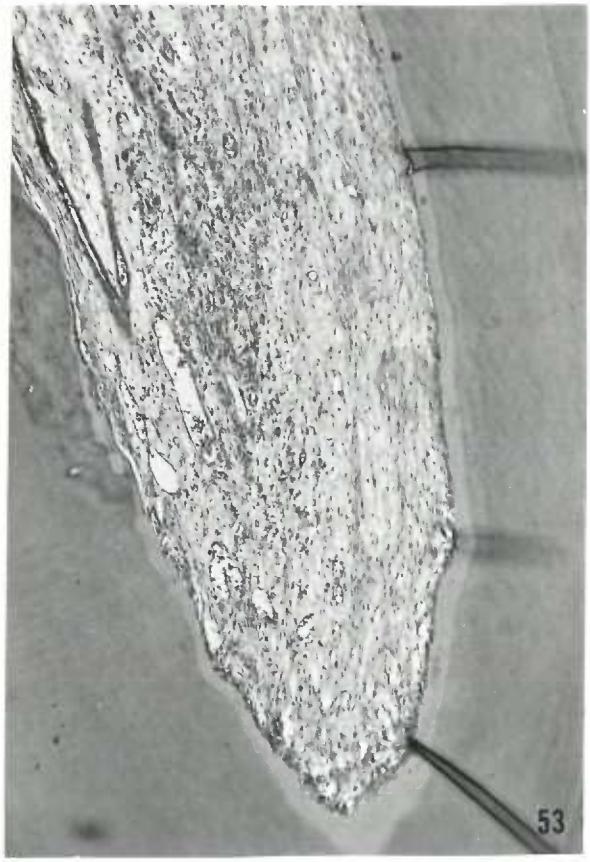

53

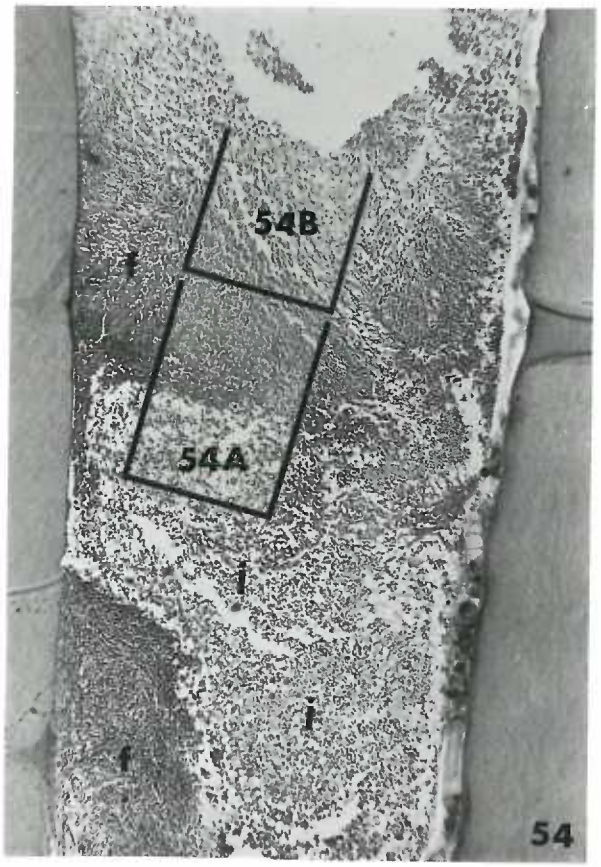
Plate 24

Five Week Donor Tooth
(With periapical lesion)

Figure 54. Inflammatory exudate (i) and avascular foci of young fibroblasts (f) occlusal to the regenerating connective tissue in the mesial donor canal. (H & E, 44x, enlarged three times)

Figure 54A. Higher power view of an avascular focus of young fibroblasts (f) growing in a tissue culture-like pattern adjoining inflammatory cells (i). The inflammatory cell exudate consists of neutrophils, macrophages, lymphocytes and plasma cells. (H & E, 150x, enlarged three times)

Figure 54B. Higher power view of another focus of fibroblasts growing in a tissue culture-like pattern. (H & E, 300x, enlarged three times)


54B
54A
i
i
i
54


i
54A

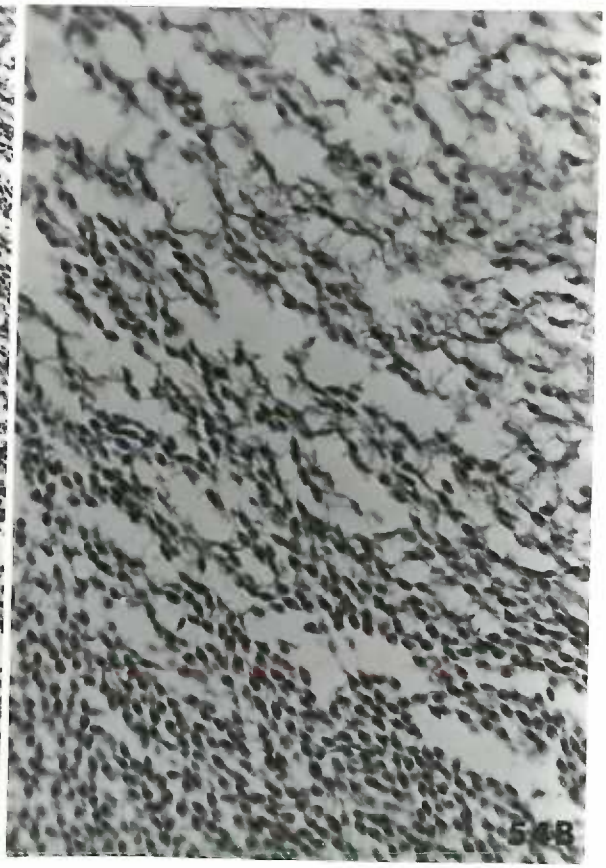

54B

Plate 25

Five Week Donor Tooth
(With periapical lesion)

Figure 55. Apical foramen connecting the mesial donor canal with the large area of periapical inflammation. (H & E, 44x, enlarged three times)

Figure 55A. Higher power view of the apical foramen showing the infiltrate of lymphocytes, neutrophiles, macrophages and plasma cells. (H & E, 150x, enlarged three times)


55A
55


55A

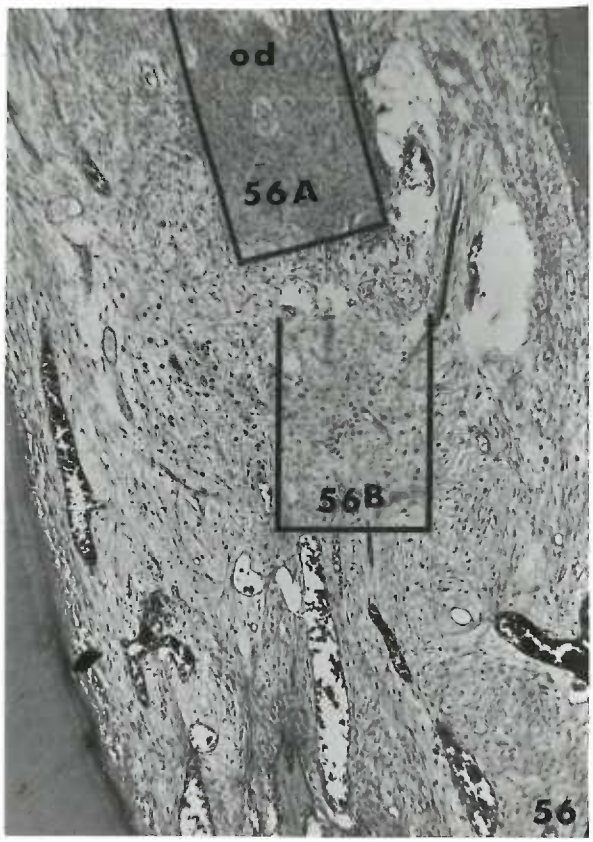
Plate 26

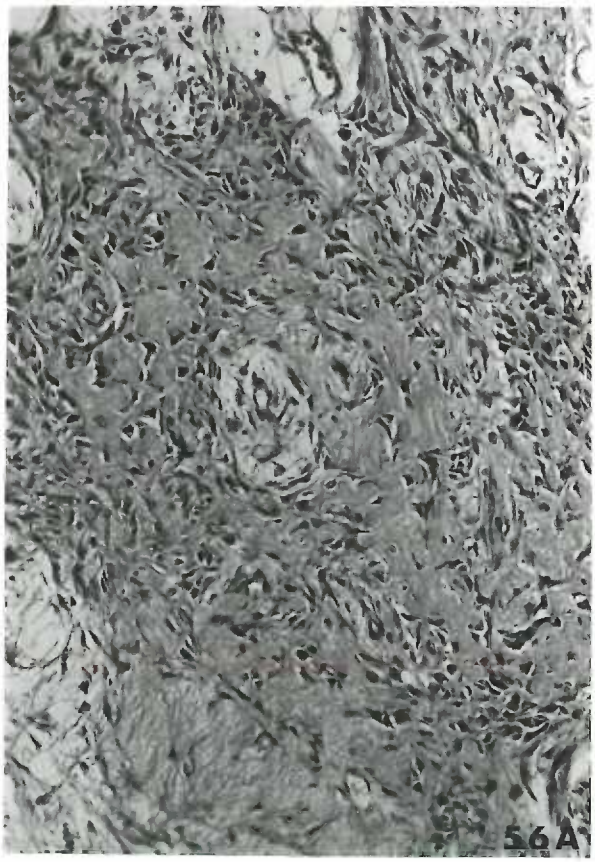
Five Week Donor Tooth
(Without periapical pathology)

Figure 56. Regenerated connective tissue in the middle third of the mesial donor canal containing an island of newly formed osteodentin or cementum (od), mild chronic inflammation and large vascular channels. (H & E, 44x, enlarged three times)

Figure 56A. Higher power view of the pulpal island of osteodentin or cementum. (H & E, 150x, enlarged three times)

Figure 56B. Higher power view of the regenerated connective tissue containing large pigment-laden macrophages. (H & E, 300x, enlarged three times)


od
56A
56B
56


56A

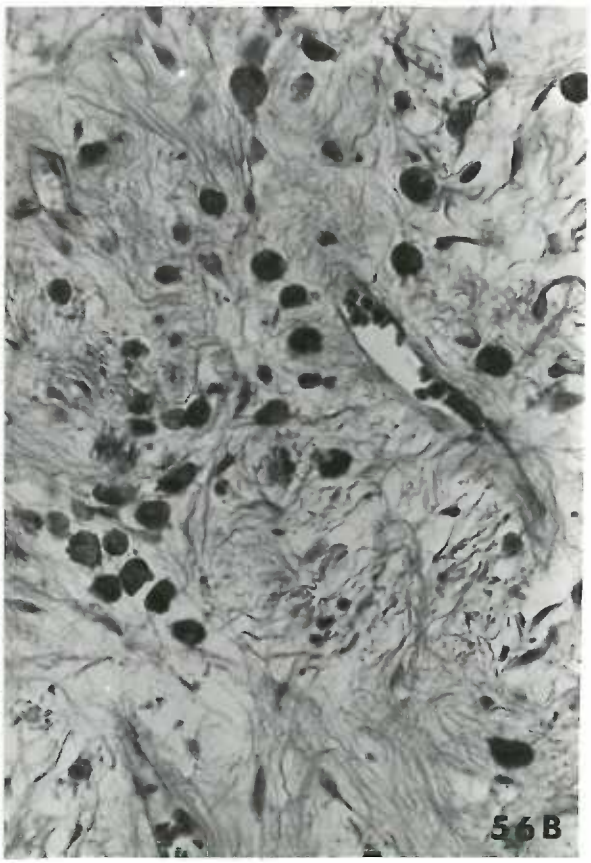

56B

Plate 27

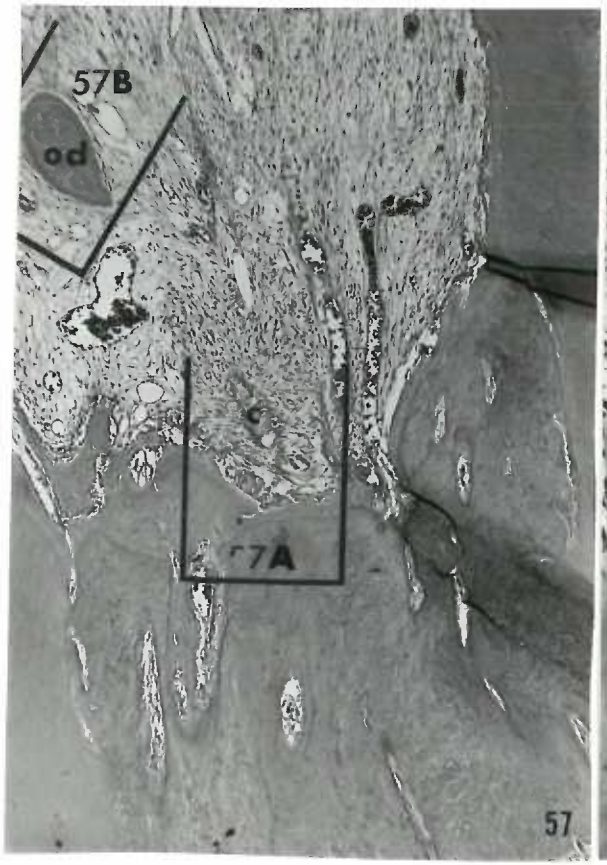
Five Week Donor Tooth
(Without periapical pathology)

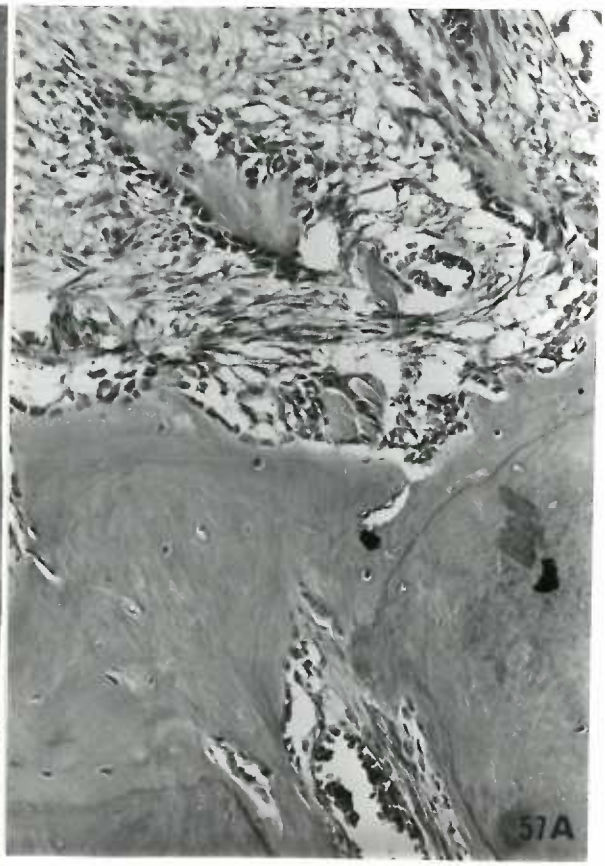
Figure 57. Connective tissue in apical portion of mesial donor canal containing finger-like projections and/or islands of cementum (c), and an island of osteodentin (od). (H & E, 44x, enlarged three times)

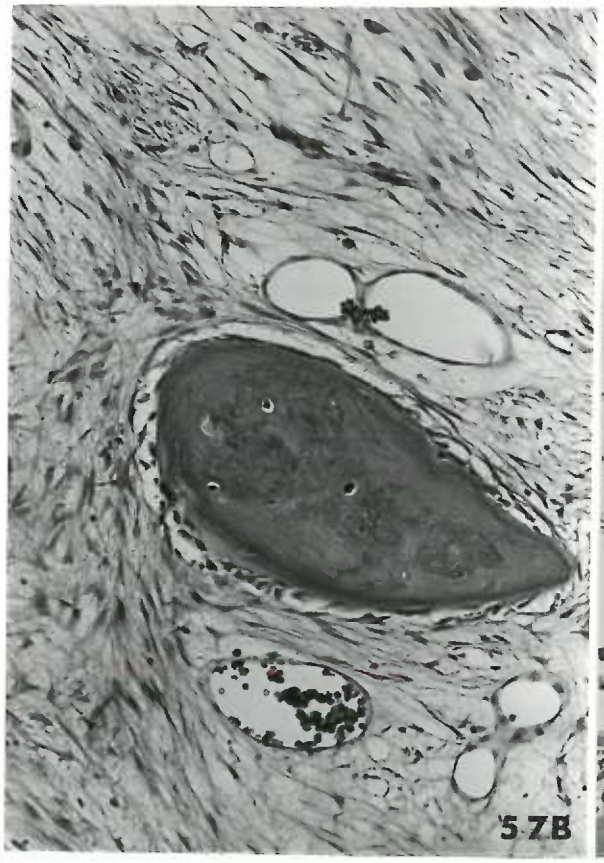
Figure 57A. Higher power view of an island of cementum. The island may actually be the end of a finger-like projection arising from the apical cementum. (H & E, 150x, enlarged three times)

Figure 57B. Higher power view of the osteodentin island. The center of the mass appears to contain several fragments of dentin which may have been chipped off the wall during removal of the donor pulp tissue. (H & E, 150x, enlarged three times)

Figure 58. Bone marrow space in periapical region containing a few inflammatory cells. (H & E, 150x, enlarged three times)


57B
od
57A
57


57A


57B

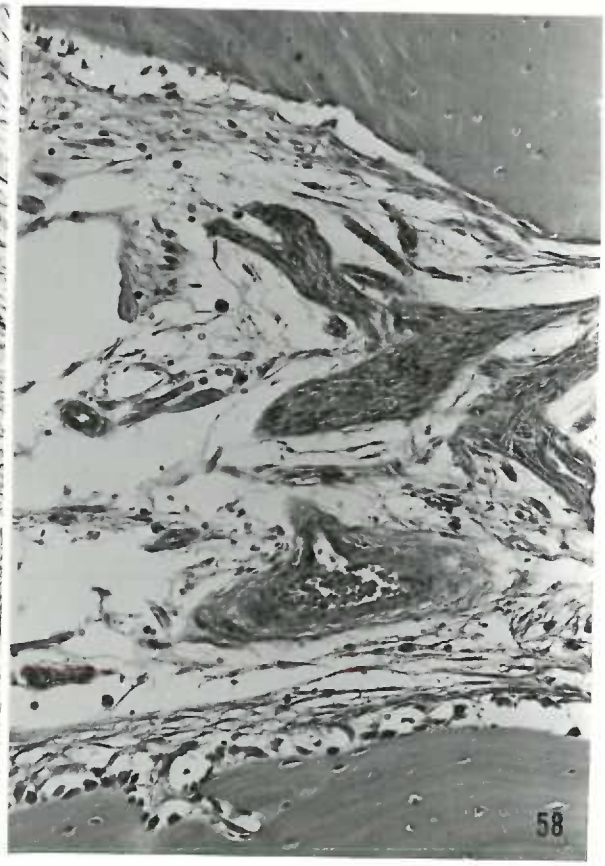

58

Plate 28

Nine Week Donor Tooth

Figure 59. Dentin and regenerated connective tissue in the mesial donor canal. The connective tissue fills three-quarters of the canal and contains a mild chronic inflammatory cell infiltrate and a focus of hemorrhage (h). Postoperatively formed osteodentin (od) covers the preoperatively formed tubular dentin (d). (H & E, 44x, enlarged three times)

Figure 60. Dentin and connective tissue in the apical area. The only postoperatively formed tubular dentin in the donor canal was in the apical area separated from the preoperatively formed dentin by a calciotraumatic line (cl). (H & E, 44x, enlarged three times)


o
d
d
59

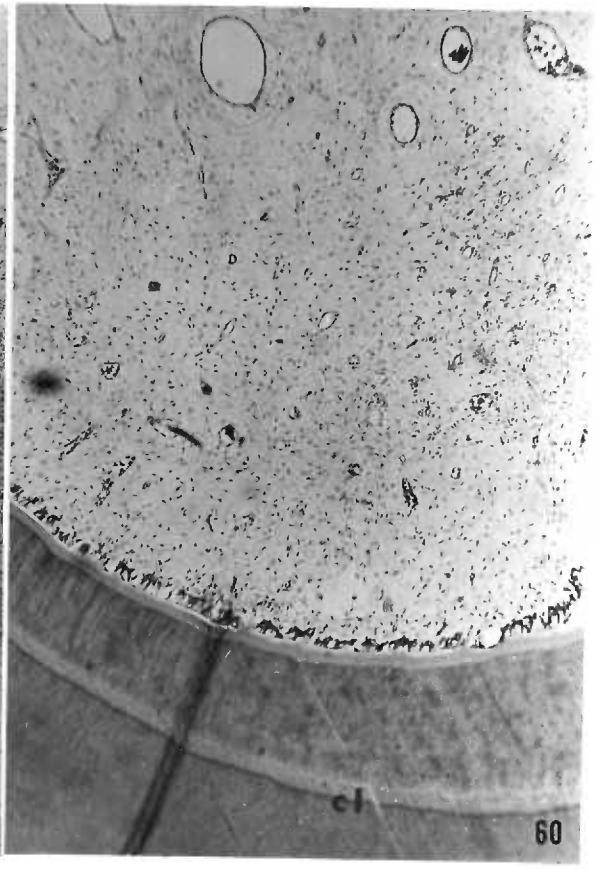
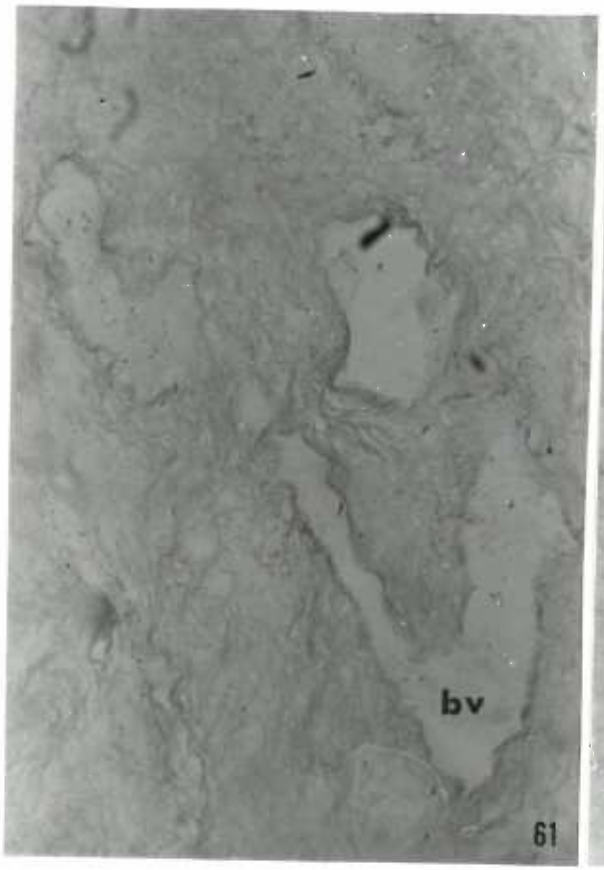

cl
60

Plate 29

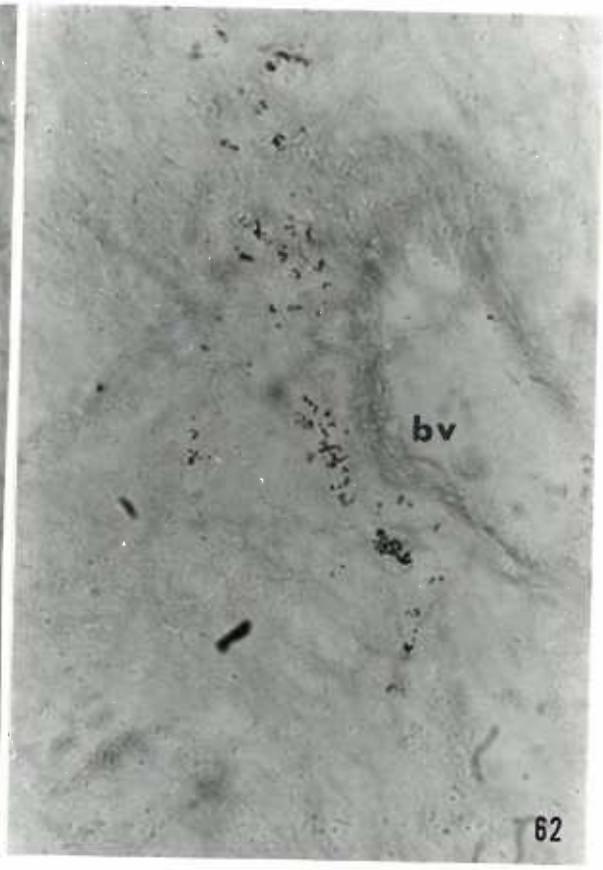
Nine Week Host Tooth
(Undecalcified)

Figure 61. Necrotic radicular host pulp tissue free of liquefaction degeneration and inflammatory cells. The area exhibits the ghost-like outlines of the necrotic vascular channels (bv). This was the only tooth to exhibit any postoperative sequelae. (Weigert's Gram stain, 150x, enlarged three times)

Figure 62. Necrotic pulp tissue in another area of the same tooth demonstrating the Gram positive bacteria and the ghost-like blood vessels (bv). This was the only tooth which gave a positive bacterial culture. (Weigert's Gram stain, 500x, enlarged three times)



61



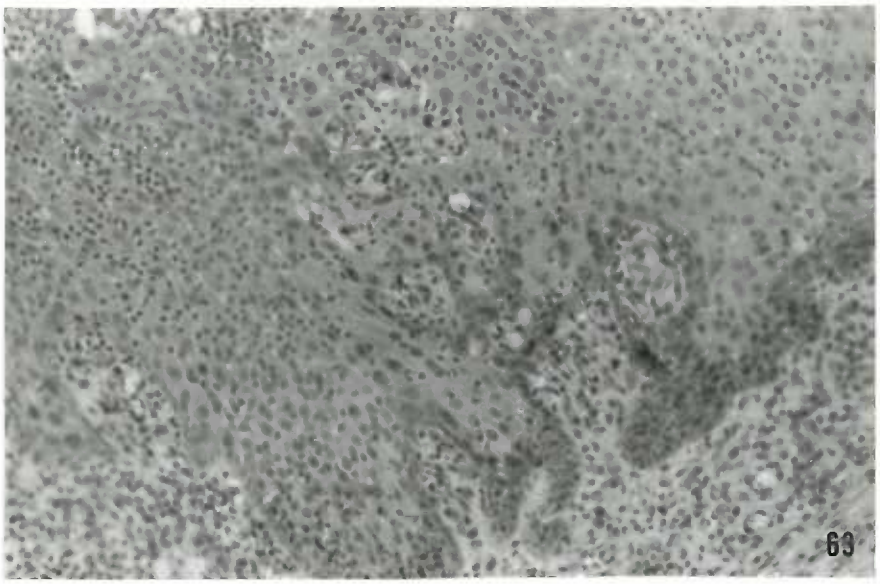
62

Plate 30

Nine Week Host Tooth
(Undecalcified)

Figure 63. Proliferating stratified squamous epithelium surrounding the cystic periapical lesion of the host tooth with the necrotic pulp. The epithelium contains numerous neutrophils and lymphocytes. (H & E, 150x, enlarged three times)

Figure 64. Extension of the periapical inflammation into the surrounding marrow space. The area exhibits chronic inflammation and bone resorption. (H & E, 300x, enlarged three times)


63

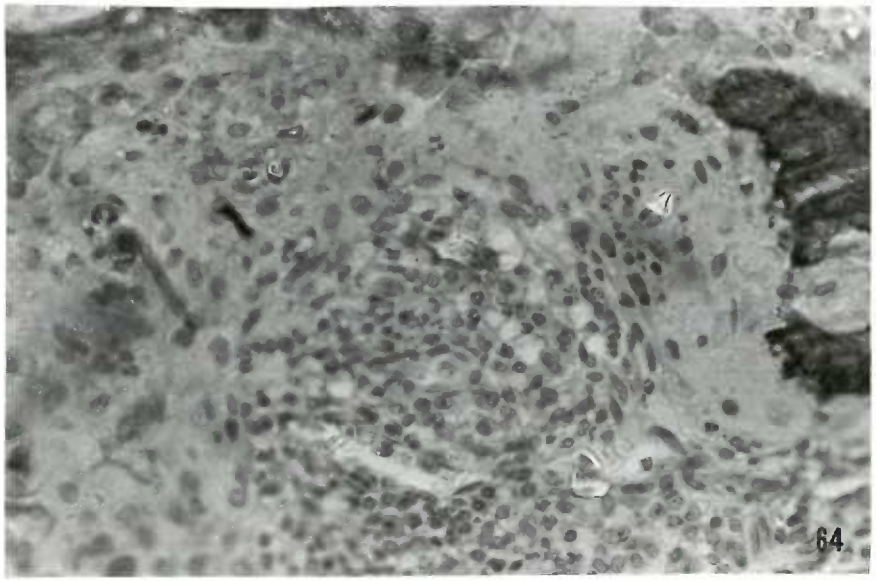
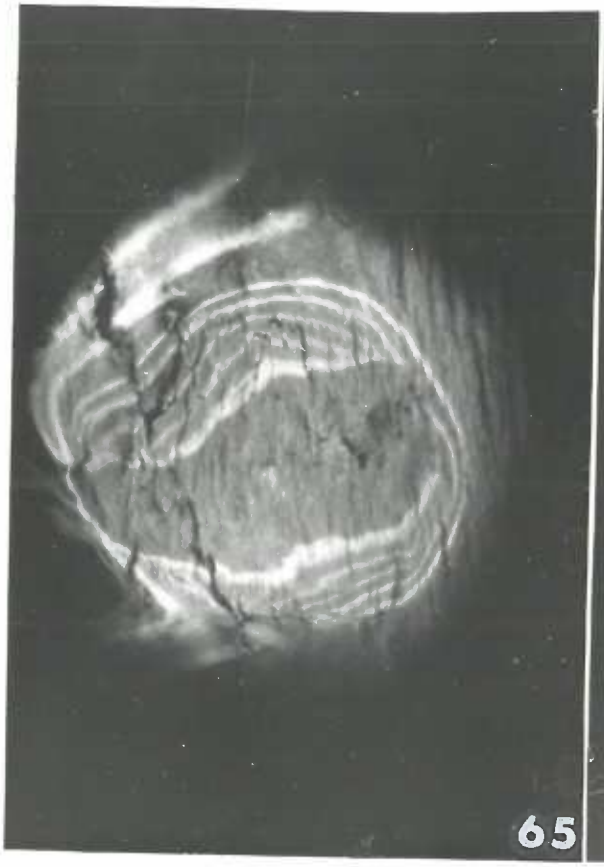

64

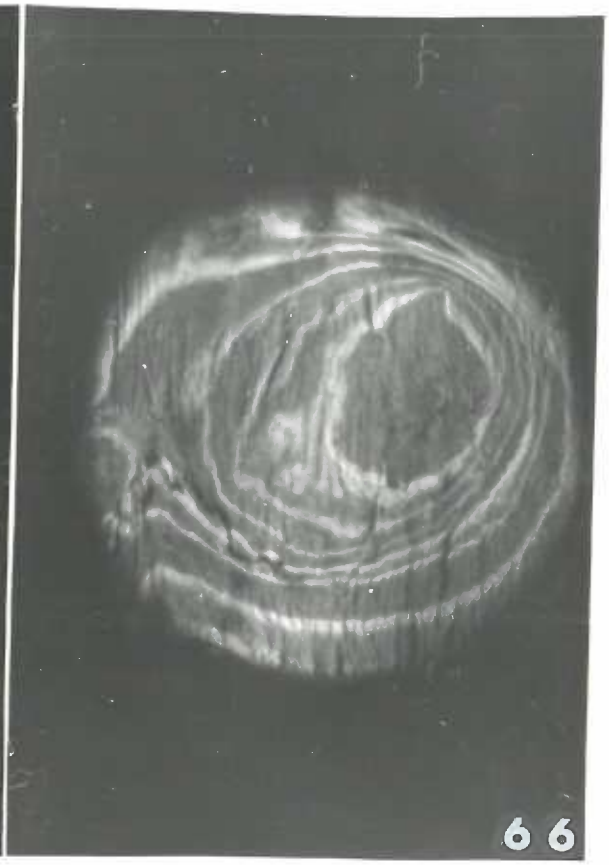
Plate 31

Nine Week Host Tooth
(Undecalcified)

Figure 65. Dentin mass in transplantation area illuminated with blue-violet light. The dentin mass was in the viable pulp tissue of the other host tooth in the animal whose tissues were sectioned undecalcified. The central core, consisting of a fragment of preoperatively formed tubular dentin, is free of fluorescence and is surrounded by postoperatively formed dentin containing seven concentric fluorescent rings. The fluorescent rings on the periphery of the mass are incomplete. (Unstained, 150x, enlarged three times)

Figure 66. Another dentin mass demonstrating the seven asymmetric fluorescent rings which represent the tetracycline hydrochloride incorporated into the postoperatively formed dentin. The first of the seven doses of the drug was administered at the end of the second postoperative week and the last dose two days before sacrifice. (Unstained, 150x, enlarged three times)


65


66