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## The Histologic Changes Following Apicoectomy in the Root Filled Tooth of Dogs

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THE HISTOLOGIC CHANGES FOLLOWING APICOECTOMY  
IN THE ROOT FILLED TOOTH  
OF DOGS

by

SARIT K. GHOSE

A thesis submitted to the Faculty of the Graduate  
School of Loyola University in partial  
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## LIFE

Sarit K. Ghose was born in Kanpore, India, on January 25, 1936.

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He worked as House Staff in Oral Surgery Department of Calcutta Dental College and Hospital, in 1959 to 1960.

In July 1960, he began a two years Graduate Program leading to a Master of Science degree in Oral Biology at Loyola University School of Dentistry, Chicago, Illinois.

He has completed two years of residency in Oral Surgery at Cook County Hospital, Chicago, Illinois, in 1961 to 1963.

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## INTRODUCTION

Root resection, or apicoectomy is the surgical removal of a portion of the root apex of a tooth, exposing in cross-section the cementum, the dentin, and the root canal, with its content of the remaining stump to contact with the connective tissue of the bone. The outcome of this operation depends on the reaction of the connective tissue of bone to contact with these exposed surfaces.

Though apicoectomy has been practiced for a long time, there is no general agreement on the final outcome. There exists no uniform histological evidence regarding the type of tissue covering the resected root and filling the bony defect.

This experimental study is intended to:

1. Investigate in detail the nature of dental tissue covering the resected root surface and
2. The nature of the tissue filling the defect of the bone.

## REVIEW OF LITERATURE

## I. CONCERNING THE EXPERIMENTAL APICOECTOMY ON LABORATORY ANIMALS:

Bauer, in 1922, studied root resection of root filled teeth on cats. In his experiment six maxillary canine teeth were resected at their apices, and the cats were sacrificed after a period of one to six months. Bauer reported newly formed cementum covering the trans-sected root stump on both sides which in its turn had grown over by the periodontal membrane. Some osteoid-like tissue was found over the stump of the resected root.

Euler, in 1923, performed apicoectomy of root filled teeth on the canines of dogs, sacrificing the dogs from six to twelve weeks. He found no instance of complete healing and observed various degrees of inflammation and abscess formation.

Bauer, in 1925, again studied root resection using root filled maxillary canine of three dogs and two cats, sacrificing them from one to six months. He found regeneration of bone and periodontal ligament around the newly laid

cementum on the root stump. However, some specimens showed apical abcess formation.

Schachtel, in 1929, amputated the roots of ten maxillary and mandibular root filled canines in three dogs, sacrificing them from one to six months. Seven specimens showed regeneration of bone, periodontal membrane and cementum and three specimens showed infection and abcess formation.

Hill, in 1932, studied forty-eight teeth in dogs using the second and third maxillary and mandibular incisors. Two techniques were used: The pulp was removed, and with the root canals unfilled, the coronal ends were sealed. After pulp removal, sterilized paper points infected with pure culture of streptococci were inserted into the root canal. All of the teeth developed granulomas. He noted resorption of the root surface and said this condition is common on the roots of dog's teeth with granulomas.

Ojha, in 1961, performed apicoectomy on vital lateral incisors of ten healthy dogs. The animals were sacrificed at intervals from twenty-four hours to six months. Examination revealed that on the resected surface of the root resorption of cementum and dentin took place which was then followed by laying down of a very thin layer of new cementum



on the old cementum and a part of the dentin. The periodontal membrane returned to normal after the initial inflammatory changes. In the surgical defect a periapical cyst lined with stratified squamous epithelium was found in one six months specimen.

Bisch, in 1963, performed apicoectomy on teeth with vital pulps in dogs. The second lateral incisor bilaterally was used as the operative site. The animals were sacrificed at intervals of three weeks to six months. His findings were: the resected surface of the root was repaired by being covered with cementum. In the surgical defect, a residual periapical granuloma was found.

## II. CONCERNING APICOECTOMY OF HUMAN:

The studies done on human material are from teeth that had been treated and later extracted for some reason. Most of the reports described only the histological appearances of the dental tissue covering the resected root and not the surrounding supportive structures. No study has been reported in the literature on the histological appearance of an area of apicoectomy from human autopsy material.

Blayney and Wach, in 1924, reported a root resection on a mandibular left central incisor in a girl nineteen years

of age. Four years later the tooth was extracted because of a small radiolucent area immediately surrounding the root end was enlarging. On histologic examination, a deposit of calcified material was found on certain areas of the exposed dentin surface, and there was an extension of fibrous connective tissue from the periodontal membrane covering most of the surface.

Kronfeld, in 1928, reported of a mandibular second premolar that had been resected the previous year because of chronic apical periodontitis. He found a deposition of secondary cementum on the cross-section of the old cementum of the area resected.

Cook, in 1929, reported of a case where a granuloma on the resected end of the root was found when the tooth was extracted ten years after apicoectomy.

Coolidge, in 1930, cited a maxillary left first bicuspid tooth which was extracted fourteen years after resection. On histologic examination he found that new cementum had been laid down, covering the entire surface of the resected dentin. He also observed cementoblasts and a layer of uncalcified cementum. Across the exposed gutta-percha there was a dense band of fibrous connective tissue without any sign of in-

flammatory reaction. Bundles of normal appearing fibrous connective tissue extends from the surface of the new cementum to the alveolar bone.

Aisenberg, in 1931, reported a resected tooth that was extracted four years later because the crown had been fractured. Histologic examination, showed round cell infiltration and yet new cementum was being deposited in this area. Newly formed periodontal fibers were found embedded in this new cementum.

Parrot and Wellings, in 1934, presented the case of a twenty-five year old girl where the upper right lateral incisor had a root resection and was extracted seven years later. They demonstrated apposition of new cementum over the old cementum and dentin. The newly apposed cementum in its turn was covered by the periodontal ligament fibers. Also, a granuloma was present.

Hoenig, in 1935, reported six cases of resected human teeth. These teeth had root canal fillings of gutta-percha and iodoform cement. He found the deposition of new cementum at the cut root apex to occur simultaneously with the formation of granuloma and infection.

Moen, in 1940, demonstrated regeneration of cementum

over the entire resected surface except the canal foramen which was covered with connective tissue. The new cementum was then covered by the periodontal ligament.

Herbert, in 1941, reported the histologic findings of four root resected teeth. In two cases, there was regeneration of cementum on the resected surface. In the other two teeth, the resected surfaces remained free of new cementum.

Herbert, in 1943, again described a case of a thirty year old patient whose upper left central incisor was resected. He found newly apposed cementum covering the old cementum and dentin over the resected surface. The granulation tissue overlying the canal showed round cell infiltration.

The results of apicoectomy of human teeth as reported by various investigators, have included infection, abscess formation, cystic development and the normal repair of the root and bony wound.

## METHODS AND MATERIALS

Eight adult healthy dogs were used in this experiment. They were intraperitoneally anesthetized with an injection of 5% Nembutal, 1 c.c. per  $3\frac{1}{2}$  pounds of body weight.

Under aseptic conditions, an intraoral incision was made from the mid-line of the mandible up to the distal border of first premolar 2-3mm. below the crest of the gingiva with a number ten blade. The mucoperiosteal flap was raised. The thin labial plate of bone was cut with a fissure burr to make a window. The borders of the window was extended towards the coronal portion of the tooth with a rongeur for the exposure of the apex. The apical 3-4mm. of the root was resected and enucleated from the bony cavity.

With a small round burr access was made to the pulp canal through the lingual side above the cingulum. The pulp contents was taken out with a fine burr broach. The root canal was enlarged by different sizes of reamers and files to accommodate the smallest size (number 00) of gutta-percha points. The pulp canal was washed with normal saline. Sterile gutta-percha points were plugged into the

root canals and the part towards the apical end was sealed with a hot burnisher. The lingual opening of the tooth was closed with dental cement. The mucoperiosteal flap was replaced and sutured with size 0000 black silk. Post-operatively, the dogs were given 600,000 units of Bicillin, intramuscularly.

The dogs were maintained on Purina Dog Food, Lolly-Pup, Horse meat and water. They were kept under regular, periodic observation. They were sacrificed at the intervals of twenty-four hours, seventy-two hours, one week, two weeks, one month, two months, three months and six months. The block of jaw containing the tooth was sawed out and the specimen was fixed in 10% Formalin and submitted to the laboratory for the preparation of serial microscopic sections. The sections were cut at 12 microns and stained with hematoxylin and eosin and examined under microscope.

A BRIEF TABULAR SUMMARY OF THE EXPERIMENTAL DOG SURGERY

No.	Specimen	Wt. in Lbs. and Sex	Date of Surgery	Postop.	Date of Sacrifice
1	24 hrs.	19, F	11/15/61	Unevent.	11/16/61
2	72 hrs.	19, F	11/13/61	"	11/16/61
3	1 wk.	23, M	10/31/61	"	11/05/61
4	2 wks.	27, M	10/31/61	"	11/12/61
5	1 mo.	21, M	11/08/61	"	12/09/61
6	2 mos.	24, F	10/27/61	"	12/27/61
7	3 mos.	35, F	10/20/61	"	1/20/62
8	6 mos.	29, F	10/27/61	"	4/29/62

## FINDINGS

24 Hours

The surgically cut surface at the apical area of the tooth shows a fairly straight cut with the exposure of dental tubules. The lumen of the dental pulp is filled with dark staining gutta-percha filling material.

The cementum is normal and attached to the dentin.

The periodontal ligament is detached from the cementum at the very end of the cut root apex. It is infiltrated by polymorphonuclear leucocytes and shows slight edema. Figure I.

The alveolar bone is mainly of the cortical type. The defect is mostly in the cortical bone and extending into the cancellous bone. The marrow space is filled with few fragments (filings) of bone and many polymorphonuclear leucocytes.

Figure II.

The surgical defect is filled with a blood clot which appears adherent to the bony and dental walls. Polymorphonuclear leucocytes are found diffusely distributed within the clot. Figures IV, and V.



### 72 Hours

The cut surface of the root apex is irregular and thus exposed dentinal tubules to the periapical blood clot. Figure VII.

The lumen of the pulp canal contains grey-black staining gutta-percha filling material.

The cementum appears normally apposed to the dentin. Also, normal collagenous fiber bundles are embedded in the cementum.

The periodontal ligament at the very cut end of the root shows hyaline degeneration which are infiltrated with few polymorphonuclear leucocytes.

The alveolar bone tissue is sharply cut and the surgical defect is filled with a blood clot. Figure VI. The fibrin containing enmeshed hemolyzing red blood cells extend into the adjacent marrow spaces of the bone. Here also, polymorphonuclear leucocytes infiltration is seen. The labial plate of cortical bone is covered with a dense fibrous connective tissue flap. The inner surface of the flap shows collagenous fiber degeneration, edema and polymorphonuclear leucocyte infiltration. Figure VIII.

Around the surgical defect there are seen osteoclasts in Howship lacunae on bone trabeculae immediately deep to

the cut surface of bone. Mitotic figures of loose connective tissue cells are seen in the adjacent marrow. Also, young stellate cells and new blood vessels arising from the loose connective tissue in the marrow space are moving into the fibrin at the periphery of the blood clot.

### 2 Weeks

The specimen shows an irregularly cut root apex. This cut surface is one boundary of the apical surgical defect containing an organizing blood clot. The pulp chamber is filled with grey-black granular gutta-percha filling material.

The cementum is normal.

The periodontal ligament shows little infiltration of polymorphonuclear leucocytes and more of fibrosis as compared to twenty-four and seventy-two hours. The acute inflammatory process seems to be resolving at this time.

The surface of the alveolar bone is lined with osteoblasts apposing bone matrix. Much of the bone marrow contains differentiated fibroblasts and loose collagenous fibers.

The surgical defect is filled with fibrous tissue arising in the organized clot. Also forming are new coarse fibrillar bone trabeculae. The collagenous fibers are

loosely arranged containing fusiform fibroblasts and young capillaries. Little osteoclastic activity is seen on the surface of bone trabeculae of old bone. Figure IX.

### 1 Month

The surgically cut surface of the tooth apex is irregular. The pulp chamber is filled with dark grey-black granular gutta-percha filling material. Osteoblastic activity with new bone formation is seen on the cut root surface. This is in immediate continuity with new bone formation in the periapical healing defect.

The periodontal ligament shows increased collagenous fibrous tissue with new bone formation which also shows apposition of bone on the cementum surface creating ankylosis.

The osteoblastic and osteoclastic activities are seen in the old alveolar bone. However, there appears to be a net gain in new bone tissue in the marrow spaces and upon the cut surface of alveolar bone.

The surgical defect contains many undifferentiated connective tissue cells of which some are transforming into fibroblasts. Plasma cells and polymorphonuclear leucocytes are scattered all over the field. Figure X.

### 2 Months

The out surface of the root is irregular and shows osteoblastic activity with new bone formation and ankylosis to alveolar bone. Figure XI.

The cementum is increased in quantity and normally apposed to the dentin.

The periodontal ligament shows dense fibrous tissue and new bone formation. Very little inflammation and a small band of connective tissue is seen between the newly formed bone and root apex. There is resolution of the fibrous connective tissue adjacent to the alveolar bone tissues by new bone formation in the surgical defect.

The mucoperiostium shows evidence of acute inflammatory process surrounded by fibrous connective tissue. Few plasma cells and very little granulation tissue are seen within the actual fibrous connective tissue.

### 3 Months

The resected tooth apex shows a fairly smooth and straight surface. New bone formation is seen in the resected tooth area and other areas of the tooth show resorption by osteoclasts.

The cementum is increased in quantity and normally apposed

to the dentin.

The periodontal ligament fibers are swollen with interstitial edema and infiltrated with polymorphonuclear leucocytes.

The osteoblastic activity is seen with the deposition of new bone on the trabeculae of alveolar bone. The marrow spaces are moderately infiltrated with polymorphonuclear leucocytes. Figure XIII.

The surgical defect is filled with dense fibrous tissue of moderate vascularity which is infiltrated with plasma cells and polymorphonuclear leucocytes. At the apex of the root, there is a small granuloma composed of: polymorphonuclear leucocytes; lymphocytes; plasma cells and fibroblasts, and is surrounded by a fibrous connective tissue capsule. Figure XII.

#### 6 Months

The specimen shows the resected root surface covered with new bone.

The cementum is increased in thickness and is normally apposed to the dentin.

The periodontal ligament shows dense collagenous fiber

bundles embedded in the alveolar bone and the cementum. There is no evidence of any inflammatory cells.

The pulp canal apex is filled with capillaries and loose young connective tissue cells.

Increased osteoblastic activity is seen in the alveolar bone with new bone formation. The marrow spaces are filled with new blood vessels. Also, there is evidence of inflammation.

The surgical defect is filled with mature fibrous tissue and new bone. Figure XIV.

## DISCUSSION

Clinical examination of the specimen at the time of sacrifice revealed no significant changes in the oral tissues. The only evidence of the apicoectomy procedure was the presence of a scar at the site of incision. Bisch, in 1963, reported the same findings.

When the experimental apicoectomy is performed on a root filled tooth of a dog, immediate protective response occurred in the mucosa, periodontal ligament, dentin, cementum, alveolar bone and bone marrow. According to Boyd, in 1956, no matter how aseptic an operation may be it must inevitably be accompanied by some degree of inflammation.

The sequence of events will be discussed accordingly; the injury, proliferation, differentiation, repair and resolution.

The trauma made by the experimental surgery causes division of small blood vessels namely arterioles, capillaries and venules, with occurrence of corresponding hemorrhage in the operative field. In a few minutes the blood loses its fluidity and clots, which under the microscope consists

of a mesh of very delicate fibrils, among which were entangled, as in a net, the red and white cells and many fragmented platelets of the blood. The fibrils are composed of and formed by the conversion of the fibrinogen of the plasma from a soluble (hydrosol) into an insoluble form (hydrogel) and thus forms a clot (Bert and Taylor, 1961).

The surgical defect is filled with various elements of an inflammatory exudate, chiefly fibrin bound together with interlacing strands to form a firm clot. This clot functions by glueing the adjacent structures together, which seals the wound from any infection until the arrival of the polymorpho-nuclear leucocytes and macrophages, and thus sets the stage for repair. This stage occurs within twenty-four hours. The unorganized blood clot, in the surgically produced periapical alveolectomy is adherent to the alveolar bone, cementum and dentin. It thusly fills the surgical defect and seals all the surfaces.

After the first day or two hemolysis of the red blood cells occurs with the liberation of haemosiderin and haemotoidin pigments. The number of mononuclear cells is increased by the local transformation of primitive mesenchymal cells of the connective tissue into histiocytes which phagocytizes such



pigments.

There is proliferation of reserve connective tissue cells which is totipotent. The osteoclast in Howship lacunae and osteoblast of bone trabeculae as we see in the seventy-two hours specimen are derived by the proliferation of the reserve connective tissue cells.

Once the mucoperiosteal flap is closed with sutures, the narrow space between the apposed tissue becomes filled with blood which coagulates to form a firm fibrin clot that fills and seals the operative defect. Some neutrophils, lymphocytes and monocytes are present at the margin of the incision.

The young fibroblasts from the fixed cells and the sprouting endothelial cells from the adjacent capillaries form a solid bud of growing endothelial cells. These growing endothelial cells follow the course of the migrating fibroblasts along the fibrin meshwork, advancing at about the same rate as fibroblasts. In three to four days the blood clot in the wound becomes populated by the highly vascularized, actively growing, connective tissue, a process referred to as organization of the blood clot.

The oral epithelial cells proliferates from the apposed edges of the flap to fill the mucosal incision while the

underlying fibroblastic proliferation is filling up the surgical defect and thus the continuity of the oral epithelium is established.

Concurrently with the other injured tissue the cementum, is laid down in one month over the resected end after apicoectomy. This was also seen by Bauer, Euler, Hill, Ojha, Bisch and others.

The injury to the periodontal ligament and the periostium of the alveolar bone causes proliferation and differentiation of connective tissue cells to form osteoclasts. After surgical removal of the tooth apex, the surface of the remaining tissues shows differentiation of osteoclasts which resorbs the dentin, the regulation of the extent of resorption is not known. According to Sicher, in 1955, aging of bone tissues contributes to the formation of osteoclasts and bone resorption.

The small, scalloped resorptive defects on the dentin is covered by newly apposed cementum, Sicher, in 1955, points out that the functional age of the tooth is only that of the last layer of cementum.

In the present investigation, the new cementum is both cellular and acellular and covers the resorptive defect

of the dentin as a thin layer. This is mainly a normal physiological reaction of repair. This experiment confirms the findings of Coolidge, Aisenberg, Ojha and Bisch, that a thin new layer of cementum is laid down on the old cementum and part of the dentin following their initial resorption.

In the area of the surgically created bony defect, organization of the blood clot takes place and forms granulation tissue between the bone and the resected root end. The differentiation from granulation tissue of both osteoblasts and fibroblasts are responsible for the new bone formation and new fibers of the periodontal ligament. New collagenous fibers are embedded in the new cementum as it is apposed to the resected root, forming the periapical alveolar group of fibers.

On the other hand, in the surgical defect chronic inflammatory reaction continues to operate more or less continuously for long period. In these chronic inflammatory reactions the degree of local injury is seldom sufficient to restrain the reparative reactions of the fixed mesenchymal cells. Hence the phenomena of inflammation and repair may go on hand in hand.

The face of granulation tissue is in contact with the

resected root and with cancellous bone which remains indefinitely the element of granulation tissue, e.g. capillaries, and indifferent connective tissue cells. However, macrophase, plasma cells and polymorphonuclear leucocytes infiltrate the area, that is why in three months specimen we see a residual periapical granuloma.

In six months time the apical tissue resolves by the increased osteoblastic activity in the alveolar bone with new bone formation and by maturation of fibrous connective tissue.

## SUMMARY AND CONCLUSION

This investigation was undertaken to study the nature of dental tissue covering the resected root surface and the nature of tissue filling the defect of the bone. Eight dogs were used as experimental animals. The lower canine and lower second lateral incisor were used as the operative site. The animals were sacrificed at the intervals of twenty-four hours to six months. The section of the jaw containing the tooth was fixed in 10% Formalin, embedded in paraffin and stained with hematoxylin and eosin. The resected root and the area of bony defect were examined in detail.

Conclusion

1. The lumen of the dental pulp is filled with dark staining gutta-percha filling material.
2. The cementum is normal and attached to the dentin. The repair of the resected surface is by deposition of new cementum.
3. The periodontal ligament shows increased collagenous fibrous tissue with new bone formation which also shows apposition of bone on the cementum surface

creating ankylosis.

4. Increased osteoblastic activity is seen in the alveolar bone with new bone formation.
5. The marrow spaces are filled with new blood vessels, also there is evidence of inflammation.
6. The surgical defect is filled with mature fibrous tissue and new bone.

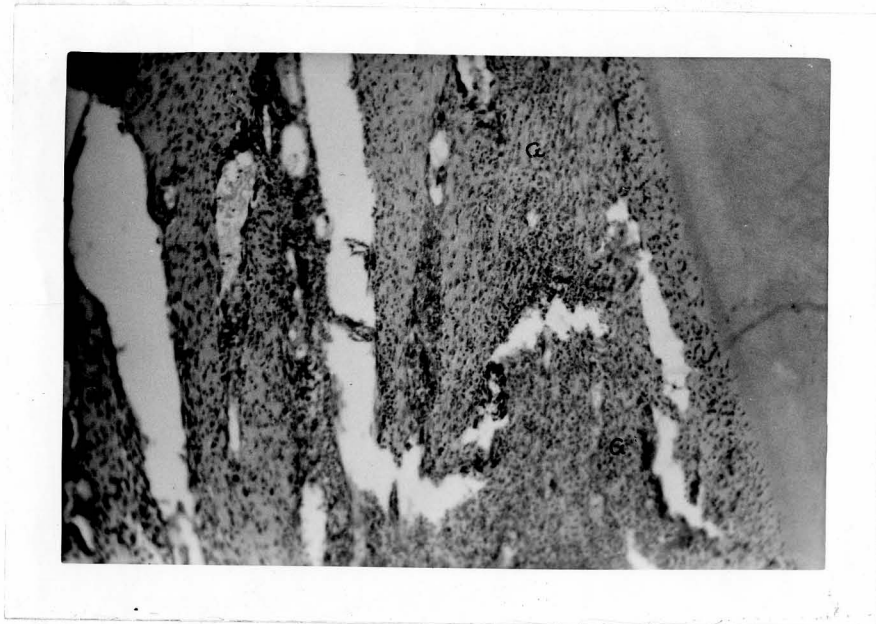


FIGURE I

Photomicrograph of twenty-four hours specimen shows infiltration of polymorphonuclear leucocytes in the periodontal ligament, a, (10X10).

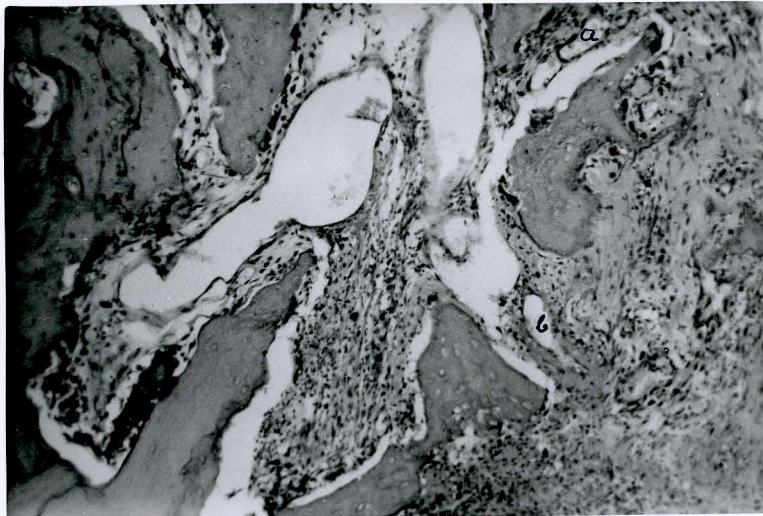


FIGURE II

Photomicrograph of twenty-four hours specimen shows dilated capillaries, a, b, the edematous marrow spaces are infiltrated with polymorphonuclear leucocytes. Note the osteoclastic resorption of the bony trabeculae. The lacunae of the bone were empty and necrotic. (10 X 10).



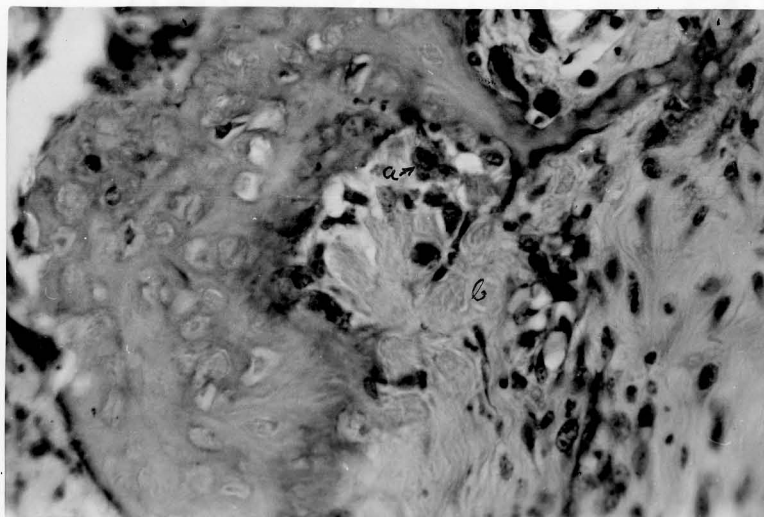


FIGURE III

Photomicrograph of twenty-four hours specimen shows mitotic figures, a, in the periodontal ligament, b, near the resected root surface. (10 X 40).

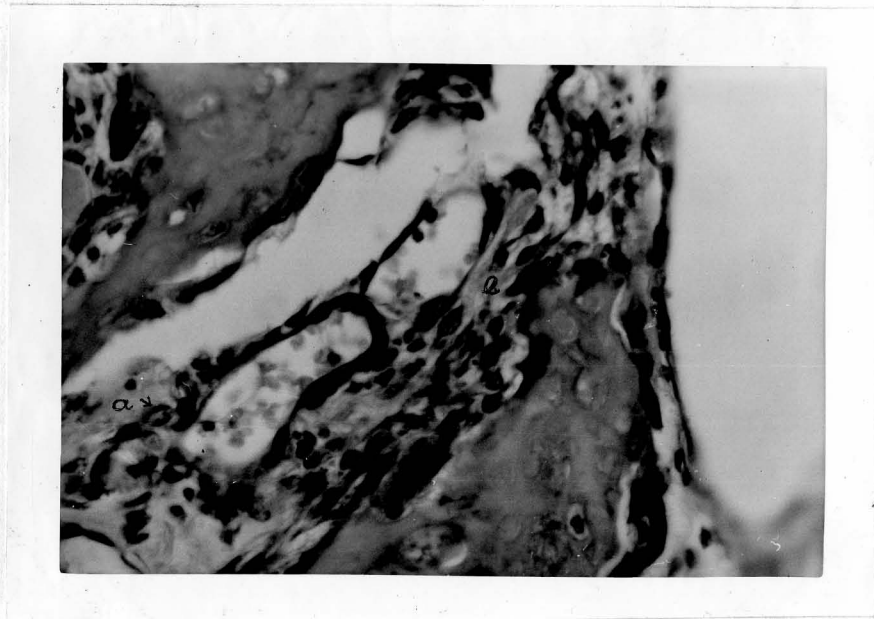


FIGURE IV

Photomicrograph of twenty-four hours specimen shows perivascular mitotic figures, a, and the loose connective tissue in the adjacent marrow spaces, b, note osteoclastic resorption, c. (10 X 40).

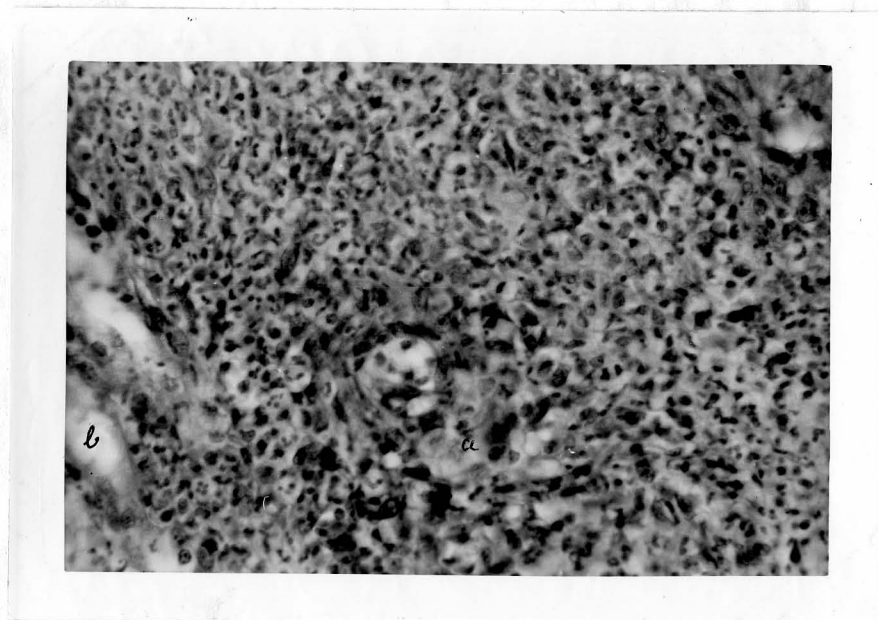


FIGURE V

Photomicrograph of twenty-four hours specimen shows fibropurulent clot, a, and surviving capillaries in the surgical defect, b. (10 X 40).

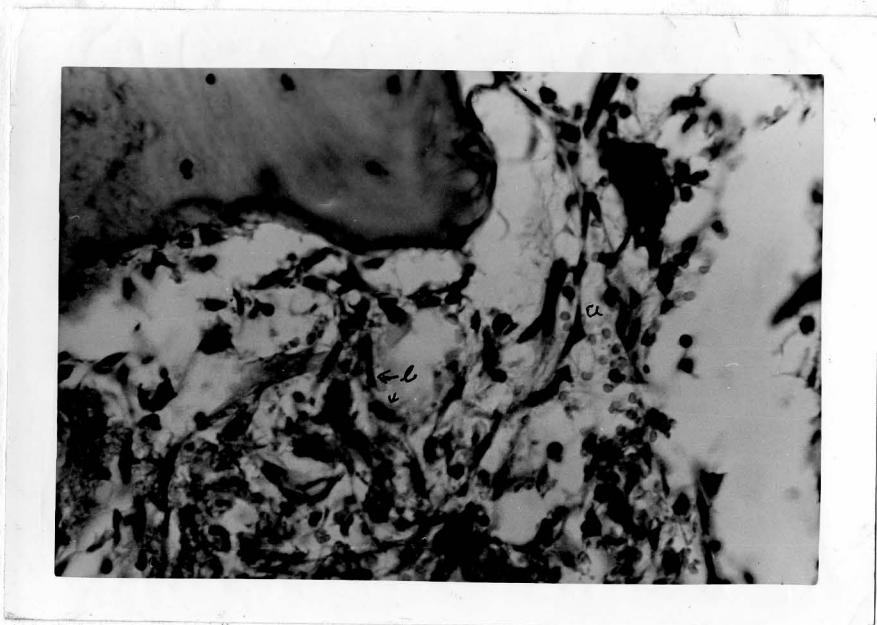


FIGURE VI

Photomicrograph of seventy-two hours specimen shows organized blood clot which extends into the adjacent marrow spaces. Note the young capillaries, a, and loose connective tissue cells, b, around the clot border. (10 X 40).



FIGURE VII

Photomicrograph of seventy-two hours specimen shows periodontal ligament, a, at the resected root surface, b, with infiltration of polymorphonuclear leucocytes. (10 X 10).

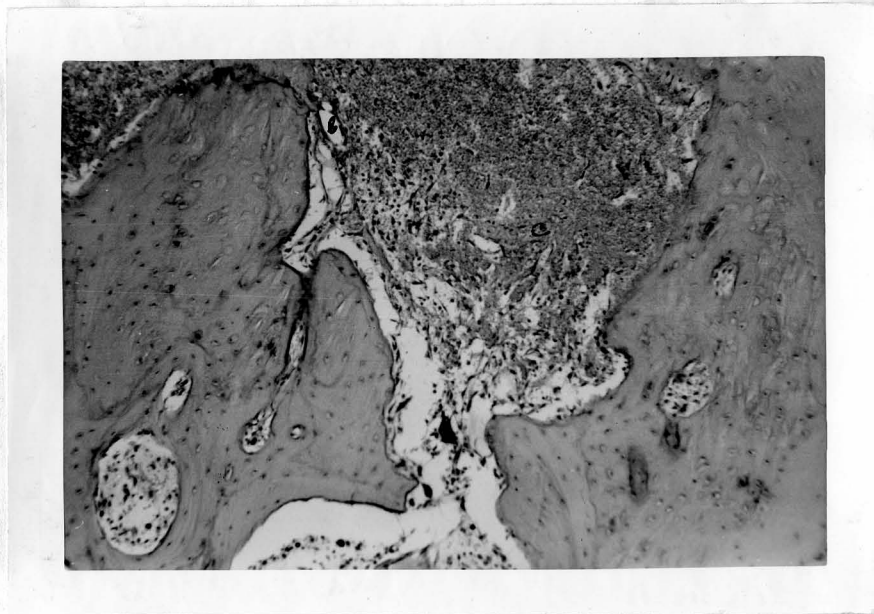
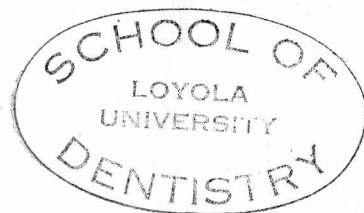


FIGURE VIII

Photomicrograph of seventy-two hours specimen shows stellate cells, a, new blood capillaries, b, and fibrin clot, c, occupying the surgical defect. (10 X10).



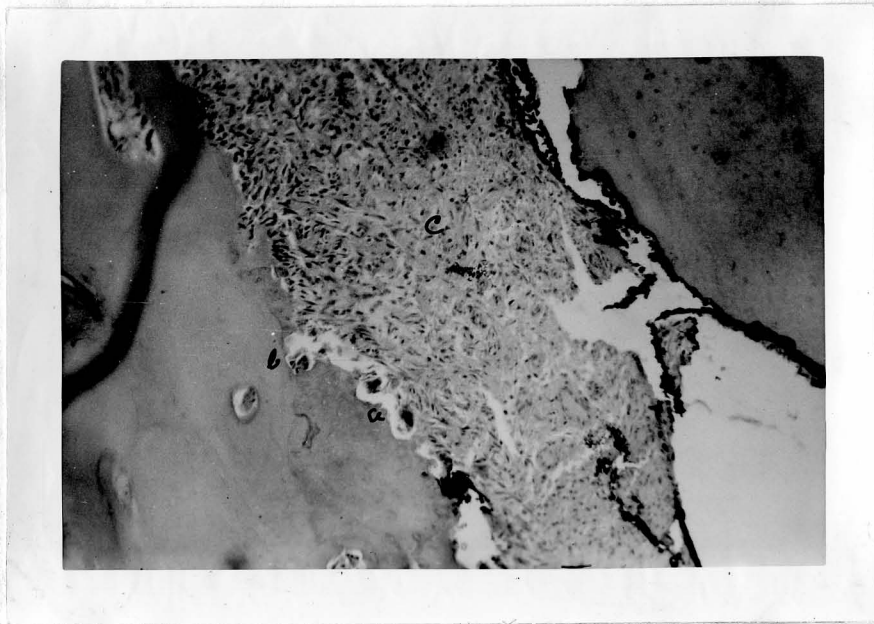


FIGURE IX

Photomicrograph of two weeks specimen shows resorption of the old bone, a, by osteoclasts, b, note fibrosis of the new connective tissue at the apical periodontium, c. (10 X 40).

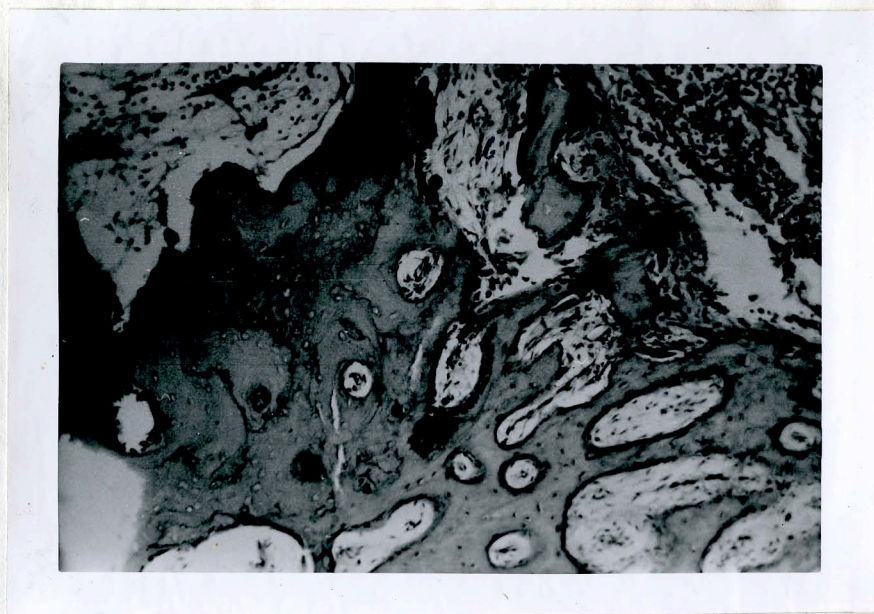


FIGURE X

Photomicrograph of one month specimen shows apposition of new bone on old alveolar bone as indicated by the reversal lines. Osteoclastic and osteoblastic activity are seen in the alveolar bone, a. The adjacent surgical defect is filled with fibrous connective tissue, b, infiltrated with polymorphonuclear leucocytes. (10 X 10).



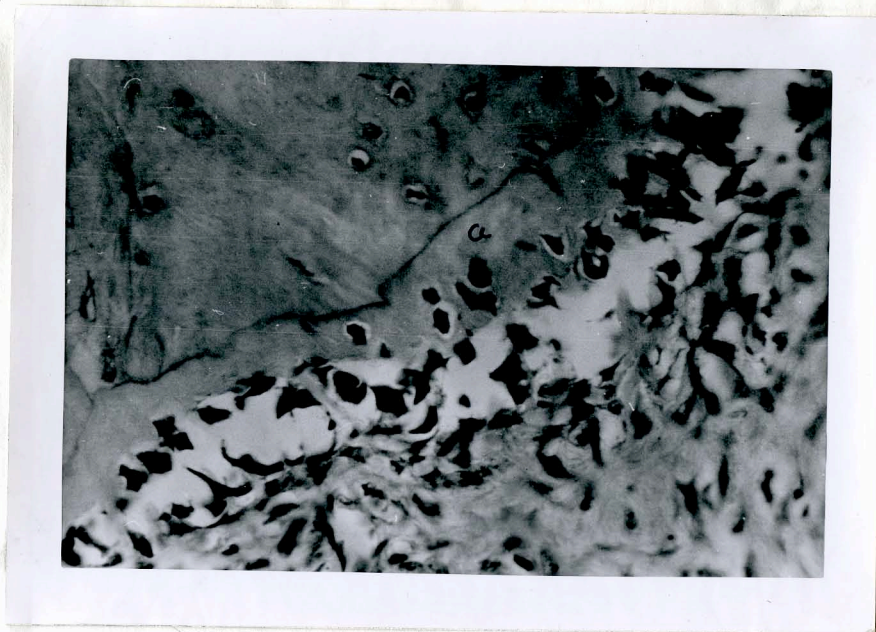


FIGURE XI

Photomicrograph of two months specimen shows apposition of new cementum on the resected root surface. Note the new bone formation in the old surgical defect, a,. (10 X 40).



FIGURE XII

Photomicrograph of three months specimen shows resorption of the resected root surface, a. Note the gutta-percha, b, at the root apex and granulation tissue, c. (10 X 40).

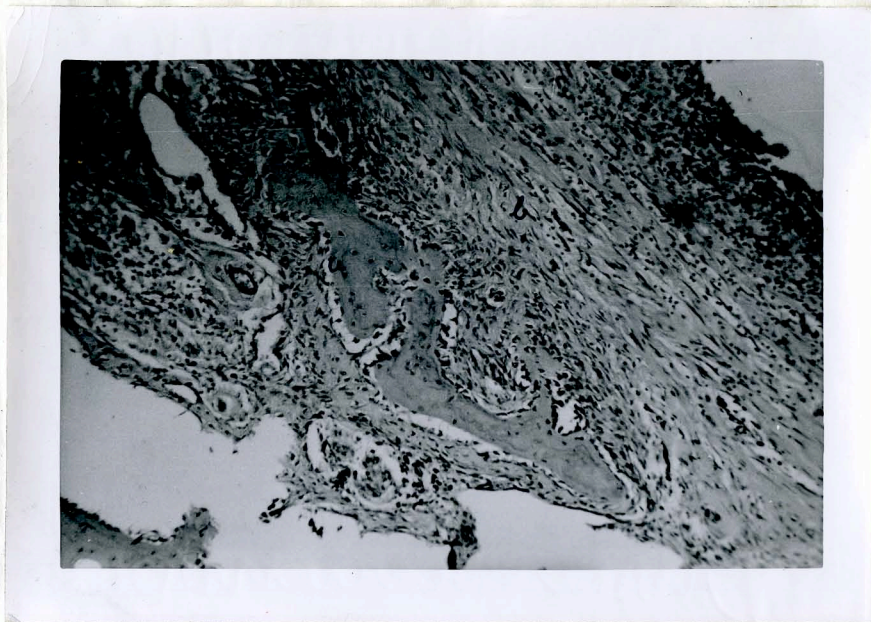


FIGURE XIII

Photomicrograph of three months specimen shows new bone formation, a. The resected area is occupied by fibrous connective tissue, b, and granulation tissue, c. (10 X 10).



FIGURE XIV

Photomicrograph of six months specimen shows new cementum apposition at the cut root surface, a. Note the young trabecular bone in a moderately vascular field of fibrous connective tissue. Artefact present at the resected surface, b. (10 X 10).

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APPROVAL SHEET

The thesis submitted by Dr. Sarit K. Ghose has been read and approved by three members of the Department of Oral Biology.

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

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

Date

4/30/64

Nicholas Charles Hoff

Signature of Adviser