THE LOCAL EFFECTS OF CORTICO-STEROIDS AND ANTI-INFECTIVE AGENTS ON THE PROLIFERATION OF GRANULATION TISSUE INTO PULPECTOMIZED AND APICOECTOMIZED DOG'S TEETH
(A Histological Study)

by

Herbert C. Berquist, B. S., D. M. D.

A THESIS

Presented to the Department of Pharmacology
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
June 1960

AN ABSTRACT OF THE THESIS OF Herbert C. Berquist
(Name)

for the degree of _____ in _____
(Department)

to be taken May 19, 1960

Title: The Local Effects of Cortico-steroids and Anti-infective Agents on the Proliferation of Granulation Tissue into Pulpectomized and Apicoectomized Dog's Teeth.

Approved _____
(Thesis Advisor)

Little is known concerning the effects of local applications of corticosteroids or their anti-inflammatory activity on the periapical tissues of teeth. Suppression of inflammation during the healing process of epicoectomized and pulpectomized teeth should allow proliferation of granulation tissue into the root canal and help maintain the structural integrity of the tooth.

Experimental and control observation on thirty-three (33) epicoectomized and pulpectomized teeth of nine (9) dogs were made after local application to the periapical defect of fludrocortisone acetate, hydrocortisone and prednisolone sodium succinate and methyl prednisolone. The steroids were used alone or in conjunction with anti-infective drugs such as iodoform, iodochlorhydroxyquin, nitrofurazone and tetracycline hydrochloride. After 50-119 days in Series I and 35 to 56 days in Series II, histological examination of the sections of the teeth with their periapical tissues was made, noting specifically (1) type and degree of inflammation (2) presence or absence of epithelial proliferation, (b) fistulation, and (c) bacterial contamination in the root canal, and (3) proliferation of granulation tissue into the root canal.

The results indicate that the soluble cortico-steroids effectively suppress inflammatory response following trauma of periapical surgery and allow proliferation of granulation tissue into the pulpless root canal regardless of bacterial contamination. Iodoform, an anti-infective when used alone also showed some local tissue stimulation and a beneficial action on wound healing.

Perhaps a combination of iodoform and soluble cortico-steroid may prove more effective in stimulating proliferation of granulation tissue into pulpless root canal - a possibility to be tested in the future.

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INTRODUCTION

During the past few years there has been an increasing therapeutic usage of cortico-steroids in medicine as well as in dentistry. These compounds have been found useful in replacement therapy, in treatment of rheumatoid and collagen diseases and in various dermatological and allergic disorders. Much experimentation has been carried out in tissue culture to show the effects of these corticosteroids on specific tissue cells, with particular reference to healing, growth and development. Considerable work has also been done showing the effects of systemic administration of these compounds on the growth and development of various organized tissues. However, little is known concerning the effect of locally applied corticosteroids on the periapical tissue of teeth with particular reference to: 1) Reduction of the inflammatory response following traumatic endodontic surgery, 2) Proliferation of granulation tissue into a pulpless root canal, 3) Bacterial growth in the root canal, 4) Fistulous tract formation and epithelial proliferation following the surgical procedure and 5) Preservation of the integrity of the periodontal structures of the tooth. The purpose of this investigation is to study histologically the effectiveness of various selected corticosteroids and anti-infective agents, applied locally, in an attempt to

promote proliferation of granulation tissue into a pulpless root canal. The desired basic clinical benefits of having a pulpless tooth filled with granulation tissue compared to that of a root canal filling are: 1) Retaining of an essentially functioning non-vital tooth, 2) A lessened dehydration and brittleness of the remaining tooth structures due to the presence of a living tissue within the root canal, 3) Lessened discoloration of the crown and the root of the tooth subsequent to dehydration and penetration of pigments into the permeable dentin and 4) The elimination of the procedure of obdurating the root canal with filling materials. If the preceding benefits can be achieved successfully, an improved therapeutic procedure will be available for the treatment and care of the dental patient.

The therapeutic use of the cortico-steroids requires adequate knowledge of their normal and toxic biological actions at both the systemic and at the tissue cell level.

It is seen that abnormally heightened permeability of the capillaries in injured tissue can be lessened or abolished by adrenal cortico-steroids[33,55]. These agents seem to possess the power of counteracting the formation of exudates on inflamed tissues by restoring the usual restraining power of capillary endothelium on the extravasation of plasma proteins. This deterrent effect on the formation of exudate and on the migration of polymorphonuclear leucocytes, followed by the depression of reparative activity of the fixed mesenchymal cells, seems to be responsible for the therapeutic

effectivness of adrenal cortico-steroids in controlling the inflammatory phenomena.

THE ROLE OF INFLAMMATION IN THE PROTECTION OF TISSUE

The basic inflammatory reaction plays an important role in the defense of the body against injurious agents. Three main components of this reaction are 1) Vascular changes, 2) Formation of exudate and 3) the characteristic behavior and movements of polymorphonuclear leucocytes. As a consequence of cellular injury the following chain of events is initiated. With cellular damage, histamine, or a histamine-like substance, together with local nervous mechanisms, produces dilatation of capillaries and arterioles in the affected region. The resultant increased mean capillary pressure, as well as the increased permeability of the endothelium of the vessels permit an escape of plasma proteins into the extravascular spaces with an accompanying excessive loss of fluid from the capillaries. This loss of fluid leads to an increased blood viscosity within the vessels and to subsequent slowing or stasis of blood flow. The increased concentration of cells per unit volume of blood and the slow velocity of flow favors the accumulation of polymorphonuclear leucocytes along the vessel walls. Tissue breakdown products in the injured area exert a chemotactic influence on the polymorphonuclear leucocytes and promote their migration into the affected region[22]. If an infectious process exists in the area, either primarily or secondarily, the exudate from the

inflammation offers protection in the following ways: 1) By diluting the toxins of bacteria and thus lessening their damage to tissue cells, 2) By throwing a cordon of fibrin around the affected area and 3) By coating the invading bacteria with a film of protein which promotes phagocytosis by making them less offensive to the polymorphonuclear leucocytes which engulf them. Inflammation may therefore be defined as the process by means of which cells and exudate accumulate in irritated tissues and usually tend to protect them.

The hormones of the adrenal cortex may be divided into three general groups according to their target functions, 1) Those which influence carbohydrate and protein metabolism, 2) Those which have important actions on the salt and water balance of the body and 3) Those which affect gonadal activity. The glucocorticoids, such as cortisone, hydrocortisone and their synthetically produced derivatives are most active in counteracting inflammation. Following is a brief review of the literature pertinent to the influence of cortico-steroids on inflammation, repair and tissue growth, and a review of the various anti-infective agents used in this investigation.

REVIEW OF THE LITERATURE

The response of fibroblasts in vitro to cortisone has been investigated by Burlinger and Dougherty[3]. They observed that fibroblasts, treated with hydrocortisone (cortisol), round up and in this state resist destruction in areas of inflammation. Radioactive hydrocortisone tends to localize "mainly either at the surface of fibroblasts or within them". It has been suggested by these investigators that this hormone-induced change in fibroblasts enhances their resistance to cellular damage and thus interupts a chain reaction of cellular destruction which ordinarily potentiates inflammation. In addition the fibroblasts are inhibited in their growth by this same compound. The inhibition of growth of fibroblast cultures by hydrocortisone in concentrations of 10, 20 and 50 micrograms per milliliter was noted by Holden and Adams[23]. To determine whether this effect on the cells by the steroids was reversible, these workers transferred the treated fibroblast cultures to media free of the test agent. Complete recovery of the culture with growth equal to that of the control ensued. Ten to twenty micrograms of hydrocortisone per milliter produced about 50 per cent fewer cells than controls. Fifty micrograms of hydrocortisone per milliliter was twice as inhibitory as ten to twenty micrograms. Inhibition by cortisone was less than that by hydrocortisone. Burlinger and Dougherty[3] also

observed this difference in potency between cortisone and hydrocortisone. They speculated that fibroblasts are capable of transforming the chemical structure of cortisone into hydrocortisone. It may be that cortisone and hydrocortisone are interconvertible and possible that hydrocortisone alone is anti-inflammatory and that cortisone must by converted to hydrocortisone for this hormone to be antiphlogistic. This may explain the difference in potency of these two compounds.

Sobel et al[46], observed that cortisone decreases the content of the hexosamine-containing mucopolysaccharides in the connective tissues of the skin and femurs of the rat. It has been assumed that the changes, observed in skin and femurs following cortisone administration, occurred in connective tissues in general. The ratio of hexosamine, arising from ground substance, to the collagen, was used as an index of fibrillar density. Since fibrillar density is related to the relative amount of collagen present, the results indicate that in the presence of cortisone this ratio was markedly depressed, however, the ratio was soon restored to normal following the discontinuence of the steroid. The time lag of recovery was proportional to the depression of this ratio. It was also noted that recovery in the hexosamine/collagen ratio occurred after the removal of cortisone, but when depression was greatest, recovery lagged the most.

Schayer et al[41], noticed an inhibition by cortisone of the binding of new histamine in rat tissues. Their results present direct evidence that pre-treatment with cortisone produces a large decrease in

the rate of binding of new histamine in the rat abdominal skin.

Moon and Tershakovec[35] used the Trypan-blue reaction for measuring permeability changes following the injection of protein digestion products with and without systemic administration of cortisone. They showed that migration of polymorphonuclear leucocytes from blood vessels was inhibited after cortisone administration. They also reported[36] that diapedesis, capillary hemorrhage and edema were quantitatively much less in cortisone-treated animals than in controls after burning with hot water and dry ice.

Howes et al[24], investigated the healing process following the use of cortisone. They found that eight days after wounding the ears of rabbits, the blood vessels in the skin defects remained uncovered by granulation tissue when 6-10 mgm/kg of cortisone was administered intramuscularly three days prior to the wounding and subsequently continued. At the time of operation no difficulty with hemorrhage was encountered with these animals, and subsequently, a small layer of fibrin formed in the wound. Grossly these wounds remained free of exudate and after eight days, were remarkedly unchanged from the original appearance. However, some epithelialization took place from a center island and from the edges of the wound, although not to the same extent as in the controls. Microscopically the tissues at the base of the wound did not increase in thickness. They contained dilated large blood vessels surrounded by an increased number of round cells and fibroblasts with small nuclei. No new blood vessels or new reticulin fibrils were present.

Mucopolysaccharides were diminshed and exudation on the surface was somewhat less than over the control wounds. Smaller doses of cortisone, 2 to 3.6 milligrams per kilogram, delayed the onset of the appearance of granulations for a shorter period. When granulations did appear, blood vessels, fibroblasts and fibrils seemed to appear almost simultaneously, although in numbers the new blood vessels were in excess. Cancellation of cortisone administration resulted in the reappearance of granulations within four to five days. Their findings on the measurements of tensile strength of sutured wound in rats revealed that the bursting strength of the healing wounds on the sixth postoperative day was markedly less in cortisone-treated animals than in the controls.

The healing of wounds in adrenalectomized rats and those adrenalectomized and treated with cortisone (2.5-5 mgm. daily) was studied by Pernokas et al[38]. Polyvinyl alcohol pledgets were implanted subcutaneously and sutured over. Four, eight, twelve and twenty days later the pledgets were removed and tissue samples were taken from the sponges and analyzed for hydroxyproline from which the total collagen content was computed. The authors concluded that adrenalectomy did not significantly affect production of collagen or granulation tissue. Cortisone decreased collagen production significantly at dosages which did not appear to affect total granulation tissue production. Their findings of granulation tissue production, following cortisone administration, differed from that of Howes et al[24]. Howes and his group found marked depression of granulation tissue with cortisone

administration following the traumatic wounding of rabbits ears; but this type of injury differs from that produced by the implantation of alcohol sponges done by Pernokas et al [38]. The continuous irritation produced by the alcohol sponge would appear to produce more total granulation tissue.

The effect of systemic administration of cortisone upon the peridontium of white mice was investigated by Glickman et al[20]. Ten animals, four to six weeks old, were given 0.5 mgm. of cortisone intramuscularly at twenty-four hour intervals, while ten controls were given normal saline solution in a similar manner. The animals were maintained for 43 days before being sacrificed. Microscopic examination of the histological sections of the maxilla and mandible of the cortisone-treated animals revealed osteoporosis, reduction in height of alvoelar bone and edema of the periodontal membrane with a reduction in the number of fibroblasts and collagen fibers. The osteoporosis was characterized by reduction in the number of osteoblasts and amount of newly formed osteoid matrix. These changes were unrelated to gingival inflammation which occurred in isolated areas associated with local irritation. Changes in the alvoelar bone and periodontal membrane were similar to those in bone and periosteum in other areas of the body. As a part of their study on rats, Applebaum and Selig[2] found that cortisone administration produced a loss of supporting bone in the jaws. However, they found a reduction in height of alvoelar bone in adrenalectomized animals.

The antiphlogistic activity of cortisone and other cortico-steroids has been studied quantitatively by the granuloma pouch technique of Selye[44]. The principle of the granuloma pouch technique is that by injecting a given amount of air into the loose subcutaneous tissue of a rat, a symetrical or spherical space, of any desired size can be created. This acts as a mold for the subsequent formation of a granulomatous membrane, into which the inner surface of this cavity can be readily transformed, by injection of an irritant into the air space. The thickness and structure of the granuloma pouch as well as the quality and constitution of the fluid which gradually replaces the air, can be largely determined at will by the selection of appropiate irritants. The introduction of small measured amounts of cortico-steroids directly into the wall of the pouch, prevents or suppresses the development of these inflammatory structures. By measuring the size and weight of the excised granuloma pouch a quantitative measurement can be made, between the experimentals and controls, of the quantity of granulomatous tissue produced, resulting from the severity of the inflammatory response due to the irritant. Other reported methods[51] using comparative quantitative measurements of anti-inflammatory activity between cortisone and other cortico-steroids, are based on: 1) The eosinophilic response in adrenalectomized rats, 2) The liver glycogen deposition in adrenalectomized rats and 3) The amount of involution of the thymus gland in immature rats.

Tolksdorf et al[51], assayed the anti-inflammatory and adreno-

cortical activity of two new cortico-steroids, prednisone and prednisolone. These two compounds are modifications of the chemical structure of cortisone. This structural modification increased the adrenocortical activity to three to four times that of hydrocortisone. Lyster et al[31], used the granuloma pouch technique in the assay of the anti-inflammatory activity of methylprednisolone, a new derivative of cortisone. This compound demonstrated six times greater anti-inflammatory activity, and ten times greater glycogen deposition than hydrocortisone. Another new cortisone derivative, 9-alpha-fluoro-hydrocortisone, has been used for controlling the electrolyte balance in patients with Addison's disease[47]. Satisfactory salt retention is maintained with dosages of 0.1 to 0.3 mgm. daily, however, marked diuresis of potassium occurs. These undesireable side effects limit its usefulness mainly to topical therapy.

Fleming[19] observed the effect of cortico-steroids on the growth of tooth germs of guinea pigs. Tooth germs of 20 day old guinea pig embryos were transplanted to the anterior chamber of the eye of an adult animal. These preparations were divided into two groups, one recieving 2.5 mgm. of crystalline cortisone acetate intramuscularly daily, and the untreated control group. Cortisone therapy was continued for eight days. Transplants in the experimental group appeared to vascularize within 24 hours, whereas four days were needed for vascularization in the control group. Once vascularization had occurred in the control group, growth was rapid and exceeded that of

the experimental group. Lymphocytic infiltration was significantly depressed in the experimental group, but resumed on the discontinuence of the test agent. The control group having surived the intial lymphocytic attack began to grow readily. Fewer transplants survived in the cortisone-treated animals compared with the controls. Resorption of many transplants in the treated animals occurred when the steroid injections were discontinued. Overall growth was superior in the controls. A significant absence of osteodentin was noted in the cortisone-treated animals. It would be interesting to note the growth results of tranplants of the experimental group if the steroid administration had been decreased gradually over a period of several days, or more, rather than to be abruptly discontinued.

Clinical use of the cortico-steroids in endodontics was investigated by Wolfsohn[54]. He flushed a suspension of hydrocortisone into the apical region of teeth undergoing root canal therapy, for the alleviation of apical periodontitis. The inflammatory changes resulting from trauma to the periodontal membrane by mechanical manipulation, or irritation by the electrolyte used in the ionization sterilization process, were diminished when this steroid was employed. Sterility of the root canal was found to be essential if hydrocortisone were to be used, for exacerbation of the disease process can occur in infected pulps and periapical tissues. Blitzer[6] used the same compound combined with anti-bacterial preparations and hyaluronidase placed in the root canal for the treatment of inflamed teeth under-going root canal

therapy. He observed that these inflamed teeth became much more comfortable and ready to fill in a shorter period of time provided that the root canal was rendered sterile before using this treatment. Stewart[48], and Stewart and Chilton[49], observed the reduction of postoperative sequelae following endodontic surgery with the systemic use of corticosteroids alone or in combination with antihistamines. Penicillin was used for supportive antibiotic therapy. Patients receiving medication had less postoperative swelling, pain, discoloration and a good healing of the surgical wound. Following the cessation of cortico-steroid therapy, some patients exhibited a slight swelling and occasionally discoloration, as if these common postoperative sequelae had been held in abeyance by the drug.

Ross and White[40], in a double blind study, observed a significant reduction of postoperative edema, pain and trismus following oral surgical procedures with the use of systemically administered hydrocortisone.

Rapoport and Abramson[39] applied a topical suspension of hydrocortisone acetate to exposed pulps in their pulp capping and pulpotomy procedures. A high degree of sucess, using positive vitality tests of the teeth as criteria, was achieved in both sterile (80%) and non-sterile (93%) preparations.

Using damaged and infected pulps of molar teeth of rats as test material, Kiryati[26] compared tissue reactions resulting from treatment with hydrocortisone alone, hydrocortisone in combination with various

antibiotics and antibiotics alone, either singly or in mixture. Hydrocortisone alone yielded 22 per cent complete healing, hydrocortisone plus neomycin and bacitracin, or oxytetracycline and chloramphenicol, 63 per cent healing, neomycin-bacitracin combination 18 per cent healing and oxytetracycline 35 per cent healing. In comparison the controls showed little or no healing. He found no evidence that hydrocortisone interfered with pulpal repair.

The effectiveness of nitrofurazone as an antibacterial agent in the treatment of surface infections has been reported by Shipley and Dodd[45]. With the use of nitrofurazone 0.2 per cent in a water soluble base (Furacin Soluble Dressing), they observed a rapid decrease in odor and drainage from the wounds with no clinical evidence of retradation of granulation tissue formation or epithelialization of the healing wound. A very high suscepibility to the gram-positive and gram-negative organisms which are common in surface infections, was observed with the use of this compound. Weiner and Fixler[53] also noted the effectiveness of nitrofurazone in the topical treatment of bacterial dermatitides. Satisfactory results were observed in 83.5 per cent of 212 patients treated. Jeffords and Higerty[25] observed the healing of donor sites from which a split thickness graft was taken with a dermatome and dressed, followed with the use of nitrofurazone soluble dressing (Furacin Soluble Dressing) and petrolatum (Vasoline), impregnated in a fine mesh gauze. Half of the wound was dressed with the nitrofurazone-impregnated gauze and the other half with petrolatum-impreganted gauze. Their results

showed that epithelialization following the use of nitrofurazone soluble dressing was superior to that of petrolatum, and with no evidence of sensitization of the patient by nitrofurazone. In all instances it was noted that more rapid healing occurred when secondary infection was prevented.

Tetracycline hydrochloride has been well established as an effective broad spectrum antibiotic[15]. In addition to its inhibition of bacterial cell growth, Bevelander et al[5], observed the inhibition of skeletal formation in chick embryos following the administration of this substance into the yolk sac of fertile chicken eggs on the eighth day of incubation. Reduction in bone growth occurred with a decrease in the amont of calcified trabeculae. Tetracycline or its derivative was found to be incorporated and retained by the bones over long periods of time as indicated by the presence of a fluorophore.

Iodoform has been used over many years in dentistry for stimulating growth of tissue and "drying up abcess cavities"[1]. Its use in endodontics for sterilization of the root canal has given way to more effective sterilizing agents in the more recent years.

Laws[28] has reported a technique of filling root canals using an antiseptic mixture containing iodoform, menthol, para-monochlorphenol and camphor, commercially known as Kri-1, in combination with gutta percha. He utilized the sectional filling method of removing and coating the apical one-third of the gutta perchascone with this mixture and inserting it into place, followed by cementing the remainder of

the cone into place with root canal cement. Castognola and Orlay[7] have also reported filling root canals, after sterilization, with this mixture alone. Accidental overfilling of the canal with this mixture, in their opinion, produces no ill effect, and may be desirable if a gangrenous pulp had been present before obduration of the root canal, as excess paste is resorbed by the tissues in a few days. Their radiographic and histological findings indicated that disappearance of the radiopaque antiseptic mixture from the canal was a sign of success, this being interpreted as the replacement of this mixture by granulation tissue moving up into the canal. However, to date, no one seems to have confirmed histologically that this process occurs. The appearance of osteclasts and the formation of new bone is the histological proof, according to Castognola and Orlay, that the area is free from infection and toxins. They prefer to use these particular anti-infective agents, as their objections to the use of antibiotics in root canal and pulpal therapy are: 1) Sensitization of the patient to the antibiotic, 2) Antibiotics act by preventing the mitotic reproduction of cells. This operates on unicellular organisms as well as on the cells of the human body and may be the reason why treatment with antibiotics, according to the authors, is bound to fail in vital pulpal therapy and 3) Strains of bacteria may develope resistance to the antibiotics. The findings of Bevelander et al[5], showing the depression of skeletal growth in chick embryos by an antibiotic, tetracycline, does partially substantiate the depression of cell growth,

as stated by Castognola and Orlay.

Iodochlorhydroxyquin (Vioform) is another water-insoluble organic iodine compound, but unlike iodoform, has a hydroquinone structure. It was introduced in 1931, and has been used extensively in the treatment of amebiasis. Topically it is used as an antiseptic dusting powder and in suppository form to control trichomonas vaginalis[16]. It is also available in combination with hydrocortisone in a water-washable base, as a cream and lotion and as an ointment in a petrolatum base.

METHODS AND MATERIALS

This investigation made two series of observations; the first employing five dogs, and the second four. The many operative procedures and drugs used in the first series were designed to develope a standard surgical and drug-selection approach for the second series. In the first series a control (sham) operative procedure was performed on each dog (except the 4th) in order to compare the reactions of medicated and non-medicated tissues. A complete histological evaluation of the tissue responses to the various drugs used in Series I was made before Series II was started. Dogs numbered from one through five are included in Series I, and those numbered from six through ten, constitute Series II. This numerical listing of the experimental dogs will be referred to in the succeeding portions of this paper.

Surgical Procedures

The dogs were anesthetized with 2½ per cent thiopental sodium, administered intermittently through a continous intravenous drip of 5 per cent dextrose in water. Atropine sulfate, 0.4 mgm. intravenously, was used preoperatively to control salivary secretions. An adequate airway was maintained by means of a cuffed endotracheal tube which also directed

the respiratory gases away from the operative field and allowed packing the throat with gauze for cleanliness. The maxillary incisor teeth were numbered one through six consecutively, from right to left, for ease in recording. After cleansing the teeth and adjacent tissues with tincture of Metaphen(R), the pulp chamber of each experimental tooth was opened on the lingual aspect of the crown with a No. 2 or No. 4 steel round bur. After establishing an adequate opening into the pulp chamber, the pulp was removed with barbed broaches and enlarged and smoothed with reamers and files. The root canal was then cleansed with sterile saline solution. Hemostasis was accomplished by pressure with sterile cotton points and cotton pellets. Utilizing the standard apicoectomy approach, an elliptical flap was raised to expose the bone. A fissure bur was then used to expose and excise the apex of the root and create a small pocket for the reception of the medication. Reamers from within the canal assured visually that the apicoectomy opening communicated with the root canal. The root canal and apicoectomy opening were irrigated with sterile normal saline solution to remove all loose particles of dentin, bone and other debris. Hemostasis in the apicoectomy defect was obtained with the use of pellets of cotton saturated with epinepherine solution, 1:1000. The root canal was again dried with cotton points and, in Series I, a layer of paraffin wax was sealed in the coronal portion of the tooth followed by the placement of a silver amalgam filling. In series II, a fast setting zinc-oxide and eugenol cement was used in place of the wax be-

neath the silver amalgam filling. In Series I (Fig. 1) the experimental drugs were placed in the apicoectomy defect and the soft tissue flap reapproximated with interupted 00000 chromic gut sutures. The placement of drugs in Series II (Fig. 2) differed from Series I in that the experimental drugs were placed in both the apicoectomy defect and in the coronal portion of the root canal beneath the zinc-oxide and eugenol filling and before that was inserted. The extent of the periapical defect was determined by roentgenograms taken at the conclusion of the surgical procedure. The endotracheal tube was removed when the dog responded to swallowing reflexes, indicating that he could maintain his own airway, and returned to the cage. The dogs were housed in clean, individual enclosures in an adequately ventilated room with a runway in which they could excercise daily. During the interval of time from placement of drug to sacrifice, the dogs were fed a standard diet of commercial canned horse meat and hard dog biscuits daily, with water ad-libitum. The experimental procedures on the teeth of dogs numbered two and five, were done on two different occasions, at 15 and 29 days interval respectively. The surgical preparation of the teeth in all other dogs was done in a single operation.

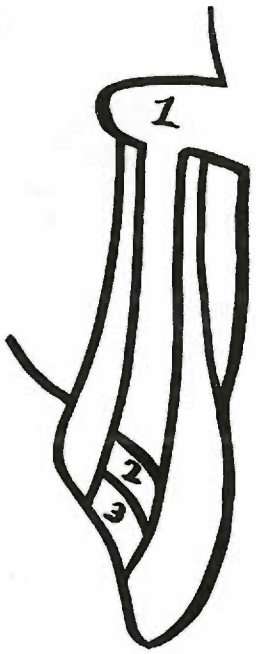


Fig. 1. Sketch of the tooth and periapical region in Series I. Apicoectomy defect (1) in which drug or drugs were placed. Paraffin wax (2) in the coronal portion of the root canal beneath the silver amalgam filling (3).

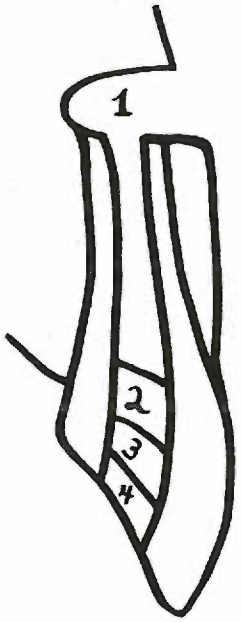


Fig. 2. Sketch of the tooth and periapical region in Series II. The apicoectomy defect (1) and coronal portion of the root canal (2) in which the drug was placed. Zinc-oxide and eugenol cement (3) in the coronal portion of the root canal beneath the silver amalgam filling (4).

DRUGS USED

The drugs used on the individual teeth are:

SERIES I

1. Fludrocortisone acetate ophthalmic ointment 0.2% (Florinef(R)) 25 mgm.

2. Fludrocortisone acetate ophthalmic ointment 0.2% (Florinef(R)) 15 mgm. with nitrofurazone 0.2% in a water soluble ointment base (Furacin Soluble Dressing(R)) 15 mgm.

3. Nitrofurazone 0.2% in a water soluble ointment base (Furacin Soluble Dressing(R)) 25 mgm. with iodoform (N.F.) 50 mgm.

4. Nitrofurazone 0.2% in a water soluble ointment base (Furacin Soluble Dressing(R)) 25 mgm. with iodochlorhydroxyquin (Vioform(R)) 50 mgm.

5. Prednisolone sodium succinate (Meticortelone(R) Soluble) 15 mgm. in lanolin (U.S.P.) 25 mgm.

6. Lanolin (U.S.P.) 25 mgm.

7. Iodoform (N.F.) 50 mgm.

8. Tetracycline hydrochloride (Panmcin Hydrochloride(R) I.V.) 50 mgm.

9. Hydrocortisone sodium succinate (Solucortef(R)) 25 mgm.

SERIES II

1. Prednisolone sodium succinate (Meticortelone(R) Soluble) 25 mgm.

2. Methylprednisolone (Medrol$^{(R)}$) 25 mgm.

3. Iodoform (N.F.) 100 mgm.

HISTOLOGICAL PREPARATION

After placement of the drugs, the dogs were sacrificed at intervals of time ranging in Series I from 50 days to 119 days, and in Series II from 35 days to 56 days. At the time of sacrifice another set of periapical roentgenograms were taken of the maxilla which served by comparison with the earlier ones for positive identification of the animal. The maxilla was removed from the animal and block sections of the teeth and associated structures were separated with the use of a fine coping saw and immersed in a 10 per cent formalin solution for three days. Following the formalin fixation, the blocks were decalcified in formic acid solution over time intervals varying from two to four weeks. The decalcified sections, after being embedded in paraffin, were cut longitudinally with a microtome into serial sections. Three to five of the most representative sections cut through the root canal and periapical regions were affixed to slides, stained with hematoxylin-eosin and mounted under a cover slip with balsam of peru. Special note of the following periapical features were made in the microscopic examination.

1. Type and degree of inflammation.
2. Presence or absence of epithelial proliferation.
3. Presence or absence of a fistulous tract to the oral cavity.

4. Presence or absence of dentinal resorption.

5. Amount of granulation tissue within the root canal.

6. Presence or absence of bacteria within the root canal.

RESULTS

The results of the microscopic examination of the sections of teeth are recorded in the following protocol with photomicrographs (magnification 30x) and descriptions. All photographs, except fig. 3, are oriented so that the apical opening of the root canal appears at the upper border of the picture.

The degree of inflammation, both acute and chronic, is classified as: 1) Severe, 2) Moderate, 3) Mild and 4) None. Severe acute inflammation is designated as one in which large numbers of granulocytes, both viable and degenerative, are present covering a large area around the apex of the root. Moderate acute inflammation is designated as one in which granulocytes are present and are mostly viable in character and confined to a small area. Minimal acute inflammation is designated as one in which only a sprinkling of granulocytes is present in the inflamed area. Severe chronic inflamation is desigated as one where large numbers of lymphocytes are present covering a large area. Moderate chronic inflammation is desiganted as one in which lymphocytes are present but confined to a small area. Minimal chronic inflammation is designated as one where only a sprinkling of lymphocytes is evident.

SERIES NO. I

Controls

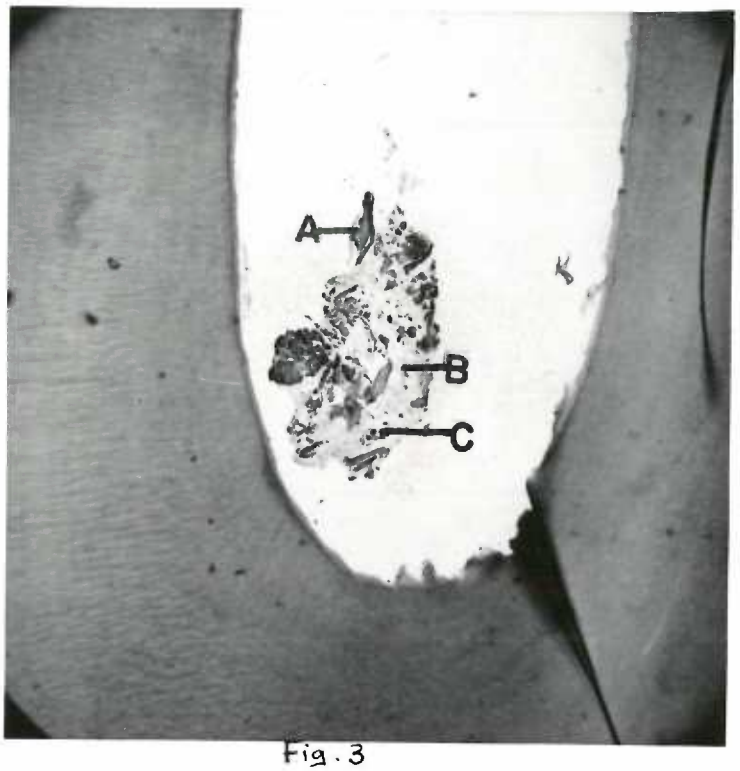


Fig. 3

Dog No. 1. Tooth No. 6. 50 days. The apex of the root has been removed in the sectioning process. The root canal is essentially empty except for a few particles of debris consisting of dentinal fragments (A), bacterial colonies (B), degenerating cells and a sprinkling of cells with a large dark staining nucleus (C) which may be lymphocytes or yeast cells. No dentinal resorption is present inside the root canal.

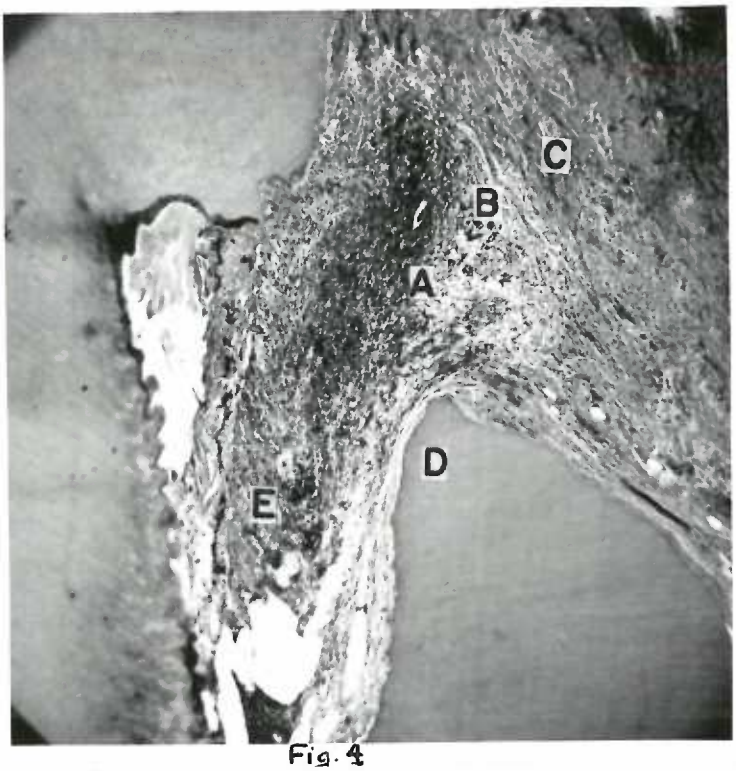


Fig. 4

Dog No. 2. Tooth No. 5. 91 days. A mixed moderate acute (A) and a severe chronic (B) inflammation is present at the apex of the root. Dense connective tissue (C) surrounds the inflammatory area which is infiltrated with lymphocytes, plasma cells and macrophages. Dentinal resorption (D) is present at the apex of the root and within the canal. No epithelial cells or fistulous tracts are present. Granulation tissue densely infiltrated with granulocytes, lymphocytes and macrophages has moved high into the root canal (E) preceded by a layer of viable and degenerating granulocytes and colonies of bacteria. Some dentinal fragments are present within the root canal.

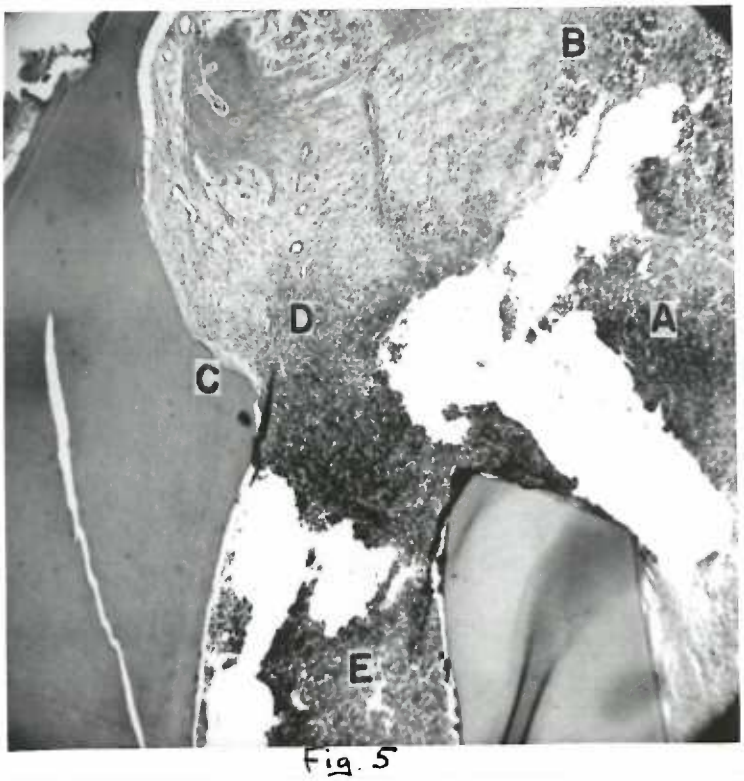


Fig. 5

Dog No. 3. Tooth No. 1. 119 days. A massive area of severe acute inflammation (A) with necrosis is present at the apex of the root and extends to the oral mucosa. An area of severe acute chronic inflammation (B) surrounds this acutely inflammed area. Dentinal resorption (C) is present at the apex. A large mass of granulocytes in a matrix of granulation tissue (D) is present at the apex of the root. Within the root canal are large masses of viable and degenerating granulocytes (E) preceded by colonies of bacteria and dentinal fragments with an absence of granulation tissue.

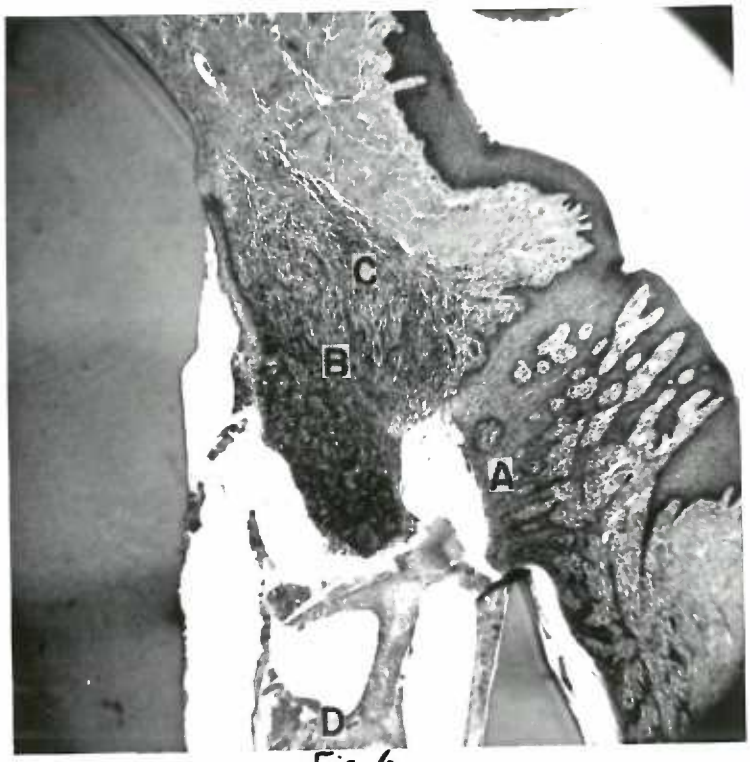


Fig. 6

Dog No. 5. Tooth No. 4. 62 days. The apex of the root is covered with squamous epithelium which is continuous with the surface epithelium (A). Outermost from this epithelial layer is a zone of mixed mild acute (B) and severe chronic (C) inflammation with the cellular population consisting of mainly lymphocytes with a sprinkling of granulocytes, plasma cells and macrophages. The root canal is empty except for degenerating granulocytes, colonies of bacteria and dentinal fragments (D). Dentinal resorption is absent.

Fludrocortisone Acetate Ophthalmic Ointment

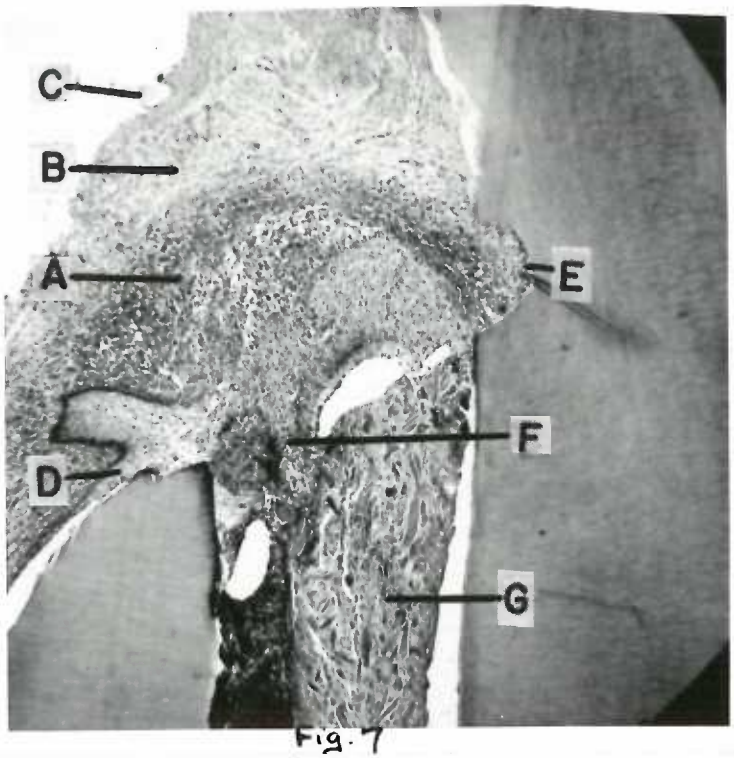


Fig. 7

Dog No. 1. Tooth No. 4. 50 days. A large, severe, acute suppurative inflammatory area with abscess formation (A) surrounded by a small zone of moderate chronic inflammation (B) is present at the apex of the root. Numerous lymphocytes, plasma cells and macrophages are present in the zone of chronic inflammation. A sprinkling of foreign bodies (C) which appear to be food particles are observed outermost from this chronic inflammatory area. Epithelial proliferation (D) which appears to be continuous with the surface epithelium is present at the apical opening of the root canal. Dentinal resorption (E) is present in the opening of the root canal, preceded by a dense mass of granulocytes which contain colonies of bacteria. Fragments (G) of dentin are also present in the root canal.

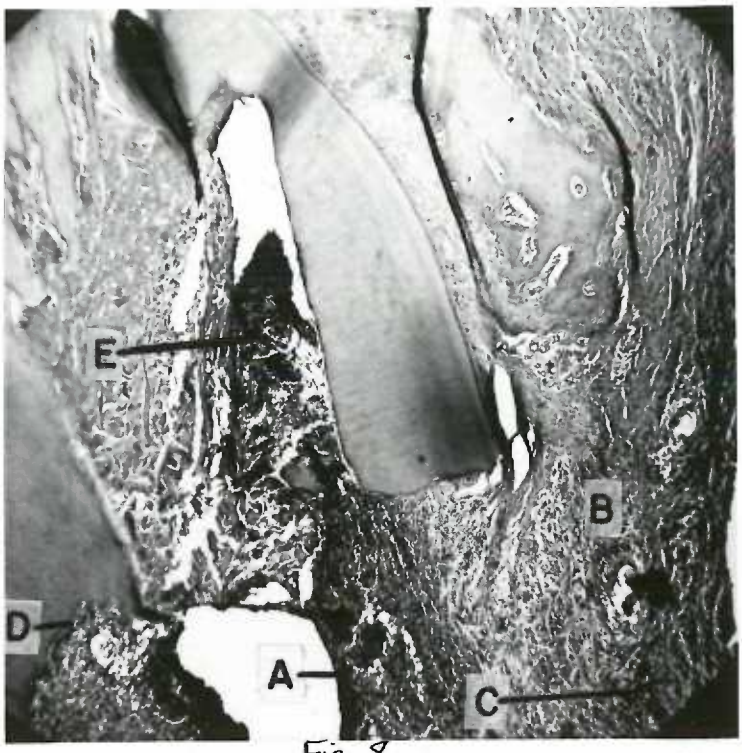
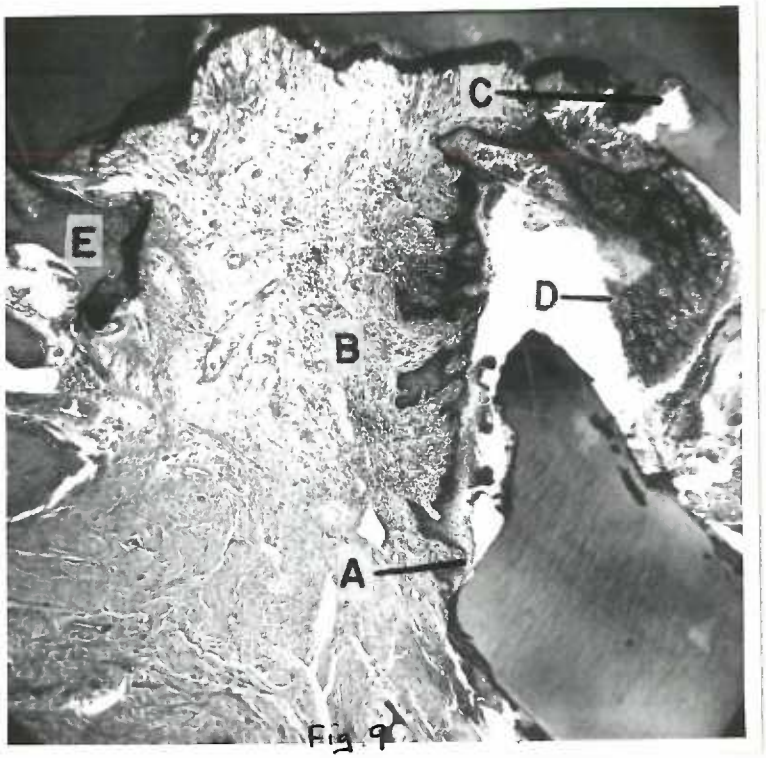


Fig. 8

Dog No. 1. Tooth No. 5. 50 days. The root has been perforated near the apex. A large area of severe acute inflammation (A) with abscess formation is present at the apex of the root. A zone of severe chronic inflammation (B) infiltrated with lymphocytes, plasma cells and macrophages surrounds the area of acute inflammation. Partial encapsulation of this area with surface epithelium (C) has occured. An epithelialized fistulous tract (C) to the oral cavity is present. Active dentinal resorption (D) is occurring within the root canal. Dentinal fragments, viable and degenerating granulocytes, macrophages and colonies of bacteria are present in the root canal (E) with absence of granulation tissue.



Dog No. 3. Tooth No. 2. 119 days. The root has been perforated near the apex. Proliferating epithelium (A) arising from the gingival crevice has moved into the lateral defect of the root canal and over the apical opening. Mild acute inflammation is characterized by a sprinkling of granulocytes in this epithelial layer. Outermost from this layer is a small localized area of moderate chronic inflammation (B) which is infiltrated with lymphocytes, plasma cells and macrophages. Active dentinal resorption (C) is occurring within the root canal. Viable and degenerating granulocytes, colonies of bacteria and fragments of dentin (D) are present within the root canal with absence of granulation tissue.

Fludrocortisone Acetate Ophthalmic Oint. with Furacin Soluble Dress.

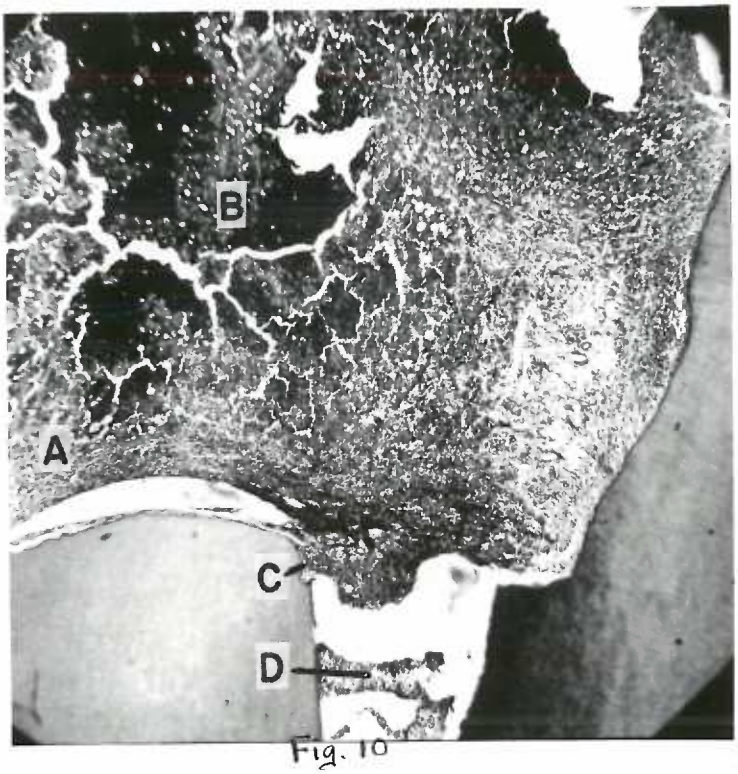


Fig. 10

Dog No. 2. Tooth No. 1. 76 days. The root has been perforated laterally near the apex. A dense band of connective tissue (A) infiltrated with lymphocytes, plasma cells, macrophages and granulocytes covers this lateral perforation. Inside this band, a large area of severe acute inflammation (B) is present surrounded by a zone of mild chronic inflammation. Inward from this inflammatory area is quite vascular granulation tissue, densely infiltrated with lymphocytes, plasma cells, macrophages and granulocytes. Dentinal resorption is present. A small amount of granulation tissue (C) has moved upward into the root canal preceded by viable and degenerating granulocytes, dentinal fragments and

colonies of bacteria (D). No epithelial proliferation or fistula to the oral cavity is present.

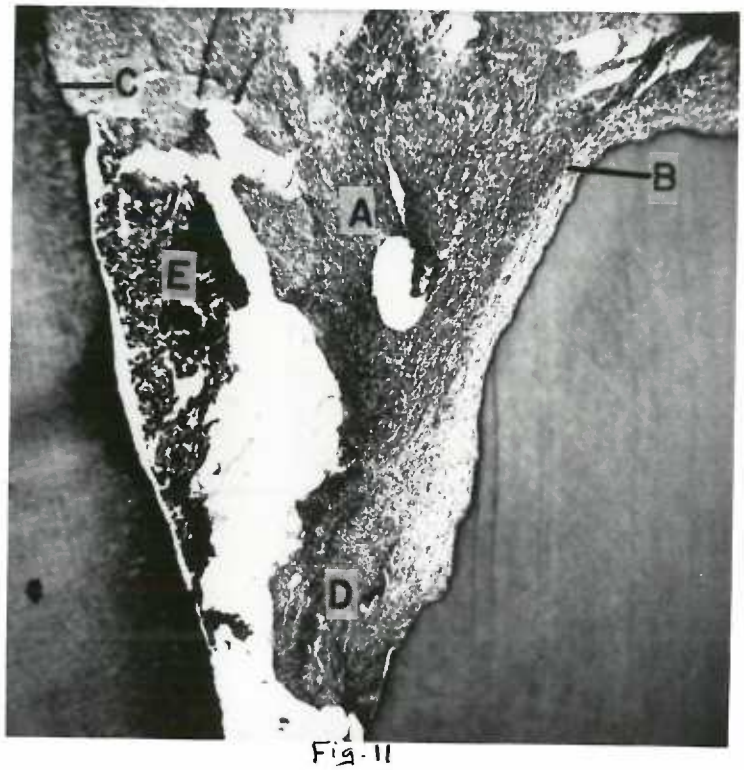


Fig-11

Dog No. 2. Tooth No. 6. 76 days. A large mass of severe acute and a small area of mild chronic inflammation (A) is present at the apex of the root. This inflammatory mass is densely infiltrated with granulocytes, macrophages, lymphocytes and plasma cells. Connective tissue (B) having direct connection to the periodontal membrane is moving into the root canal along one dentinal wall. The periodontal membrane appears to be proliferating in the area of the apicoectomy defect. Few inflammatory cells are present outward from the apex of the root. No epithelial proliferation or fistulous tract is present. Dentinal

resorption (C) is occuring within the root canal. Granulation tissue (D) containing many macrophages, lymphocytes and granulocytes is present within the root canal. Dentinal fragments (E) are present in the root canal.

Fig. 12

Dog No. 4. Tooth No. 1. 105 days. The root has been perforated laterally near the apex. A layer of proliferating squamous epithelium (A) covering the apex of the root appears to act as a barrier in preventing granulation tissue from entering the root canal. Mild acute inflammatory cells have infiltrated this epithelial layer. Adjacent to this layer are dilatated blood vessels (B) surrounded by a small region of moderate chronic inflammation with cells consisting of lymphocytes, plasma cells, macrophages and granulocytes. Bone (C) has nearly filled in the apicoectomy

defect. Dentinal resorption is absent and no fistulous tract is demonstrable. The root canal contains particles of dentinal fragments, degenerative and viable granulocytes, macrophages and colonies of bacteria. (D) Fluid is present in the root canal in which particles of hemosiderin appear. (E)

Furacin Soluble Dressing With Iodoform

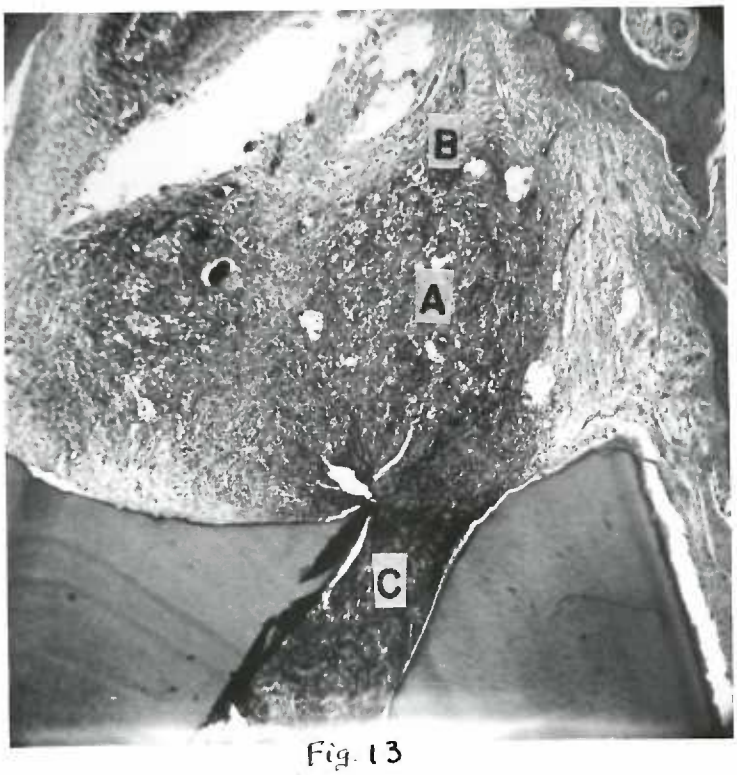


Fig. 13

Dog No. 4. Tooth No. 6. 105 days. A small area of moderately acute inflammation (A) which appears to be encapsulated with connective tissue is present at the apex of the root. On the outskirts of this inflammatory is a zone of mild chronic inflammation (B) consisting of lymphocytes and a sprinkling of plasma cells and macrophages. Dentinal resorption is absent. No fistulous tracts

or epithelial cells are demonstrable. An area of very vascular granulation tissue (C) containing acute inflammatory cells, has moved upward into the root canal. Dentinal fragments and viable and degenerating granulocytes are also present in the root canal.

Furacin Soluble Dressing With Vioform

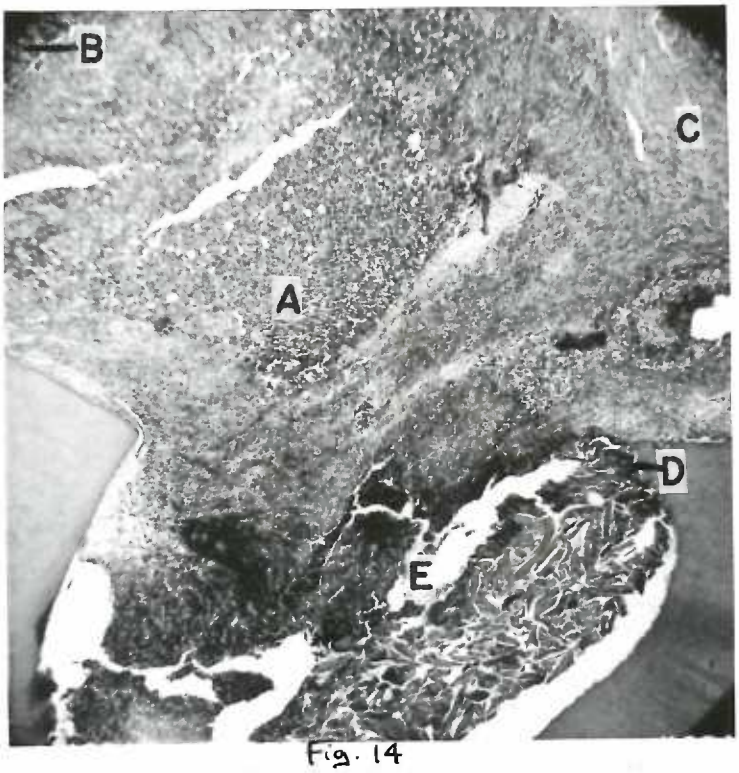


Fig. 14

Dog No. 4. Tooth No. 4. 105 days. A massive area of severe acute inflammation (A) with suppuration extends from the opening of the root canal nearly to the oral mucosa. Dense connective tissue (B) has partially walled off this acutely inflamed area. An area of moderate chronic inflammation (C) is present on the periphery of this connective tissue. Numerous macrophages are present in the area of chronic inflammation. A localized osteomyelitis is

present in the adjacent bone. No epithelial proliferation is present and a fistulous tract is not demonstrable. Active dentinal resorption (D) is present at the apical opening of the root canal. The root canal is filled with viable and degenerating granulocytes, and dentinal fragments. A minimal amount of granulation tissue has entered the apical opening of the root canal.

Prednisolone Sodium Succinate in Lanolin

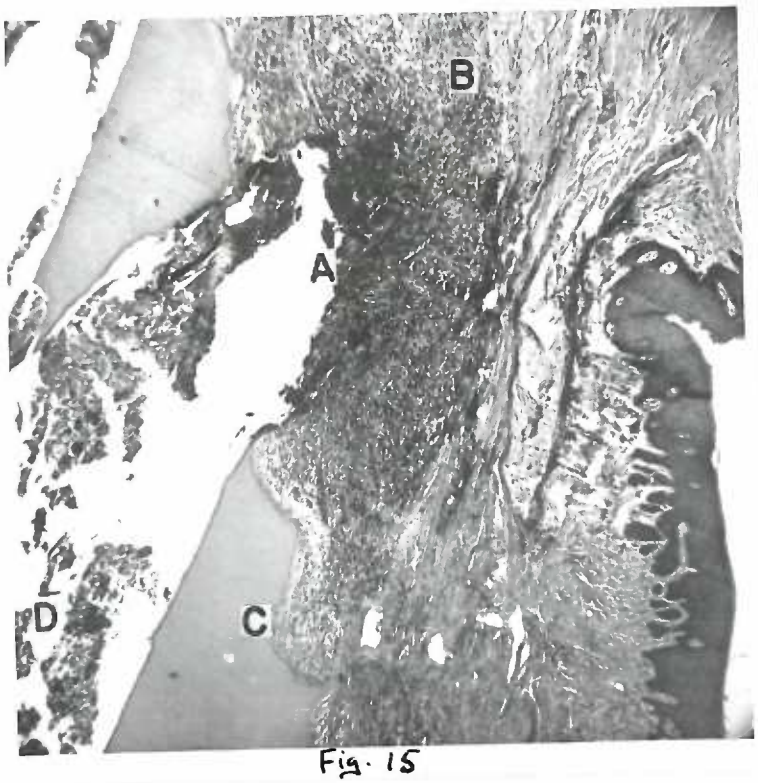


Fig. 15

Dog No. 5. Tooth No. 6. 91 days. A layer of stratified squamous epithelium appears to cover a portion of the apex of the root. Outermost from this layer is a zone of severe acute inflammation (A) extending almost to the oral mucosa. An area of moderate

chronic inflammation (B) containing hyperplastic endothelium, lymphocytes, macrophages and plasma cells, surrounds the acutely inflammed area. Active dentinal resorption (C) is present. The root canal is filled with colonies of bacteria, viable and degenerating granulocytes and dentinal fragments with an absence of granulation tissue (D). The presence of a fistulous tract is questionable.

Lanolin

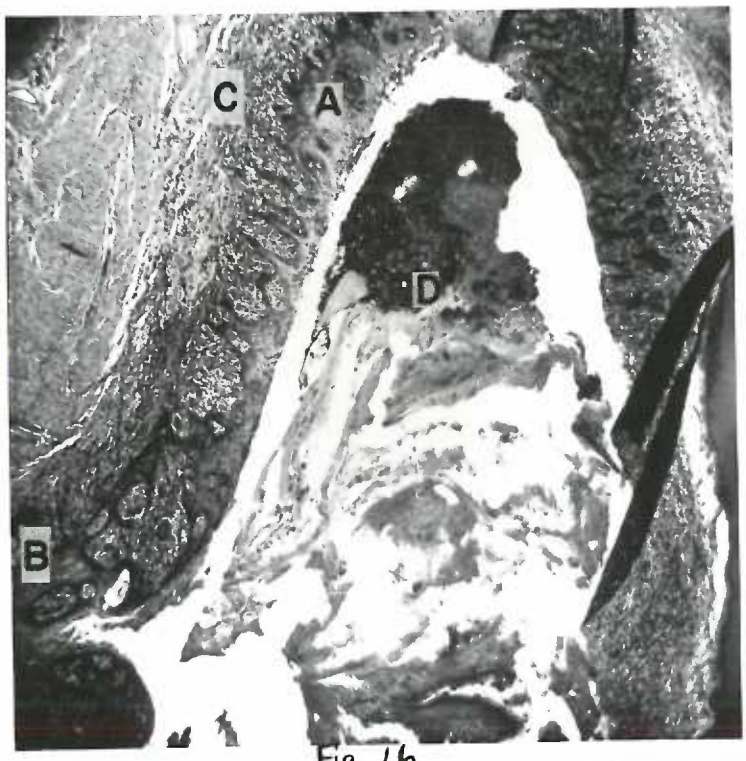


Fig. 16

Dog No. 5. Tooth No. 2. 62 days. The apex of the root canal is enclosed by a layer of squamous epithelium (A), infiltrated with lymphocytes and plasma cells, which appears to be continuous with

the epithelium of the oral mucosa (B). Outermost from this epithelial layer is a zone of moderately acute inflammation (C) surrounded by an area of moderate chronic inflammation. Dentinal resorption is absent. The root canal contains coagulated blood, colonies of bacteria and fragments of dentin.

Iodoform Powder

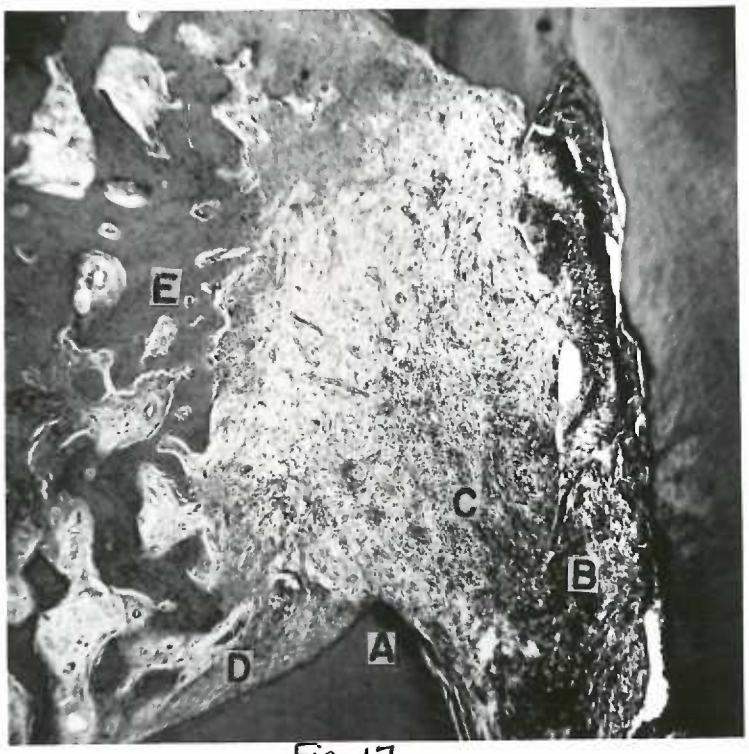


Fig. 17

Dog No. 2. Tooth No. 2. 91 days. The root has been perforated laterally near the apex. Absence of epithelial and inflammatory cells is noted outermost from the apex of the root. Dentinal resorption (A) with repair by an osteodentin type material is present within the root canal. A small area of mild acute inflammation (B) is present within the root canal containing colonies

of bacteria. Vascular granulation tissue (C) containing macrophages and plasma cells has spread into the root canal extensively. No fistulous tract or epithelial proliferation is demonstrable. The periodontal membrane has moved partly into the root canal (D). High in the root canal is an area of osteod tissue lining the dentin. Good bony regeneration is present in the apicoectomy defect.

Tetracycline Hydrochloride

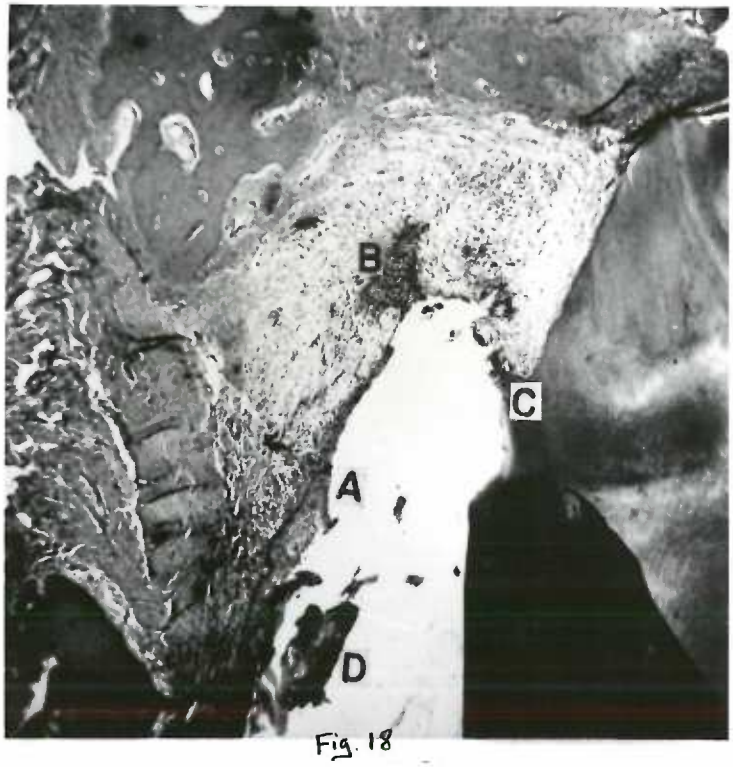


Fig. 18

Dog No. 5. Tooth No. 3. 91 days. Squamous epithelium continuous with that of the oral cavity (A) has proliferated into the periapical region nearly enclosing the root canal opening. Outermost from this epithelial layer is a small area of moderate

acute and minimal chronic inflammation (B) containing large numbers of multinucleated macrophages, plasma cells, granulocytes and lymphocytes. Dentinal resorption is present (C). The root contains degenerative granulocytes and fragments of dentin with an absence of granulation tissue (D).

Hydrocortisone Sodium Succinate

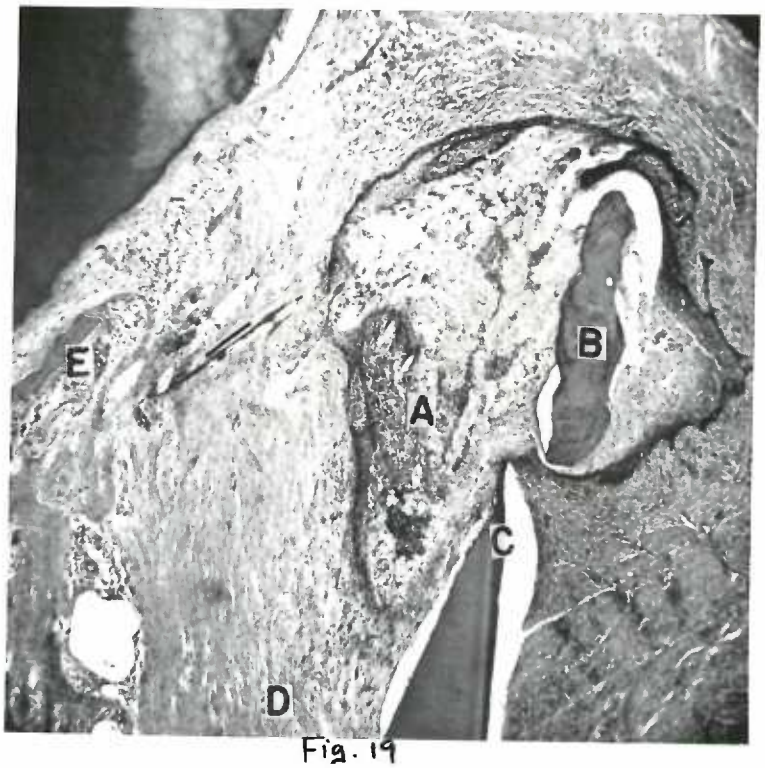


Fig. 19

Dog No. 5. Tooth No. 5. 62 days. A small area of mild acute inflammation is present at the apex of the root which appears to be enclosed by an epithelial sac (A). A spicule of newly formed bone (B) appears in the sac. A fistulous tract is not demonstrable. Minimal resorption of dentin in the root canal has occurred (C). Vascular granulation tissue infiltrated with plasma cells and

macrophages is rapidly filling the root canal (D). A layer of necrotic cells appears on the coronal portion of the granulation tissue. Some spicules of bone (E) are being formed among the granulation tissue in the root canal. A foreign brown substance is present in the dentin lining the root canal.

SERIES II

Operative Controls

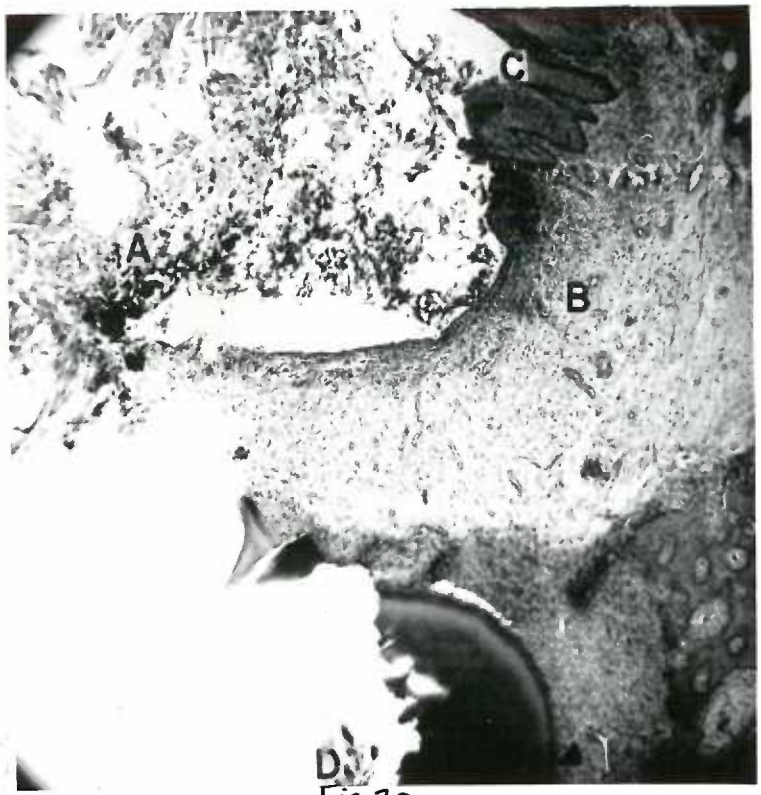


Fig. 20

Dog. No. 6. Tooth No. 1. 56 days. A massive area of severe acute inflammation (A) with cystic formation is present at the apex of the root surrounded by an area of severe chronic inflammation (B). This cystic area is lined with epithelial cells and surrounded with connective tissue (C) infiltrated with lymphocytes, plasma cells, granulocytes and macrophages. A fistulous tract is not demonstrable. Dentinal resorption is absent. Osteoclastic resorption of the surrounding bone is present. The root canal contains viable and degenerating granulocytes, fragments of dentin and colonies of bacteria (D) with absence of granulation tissue.

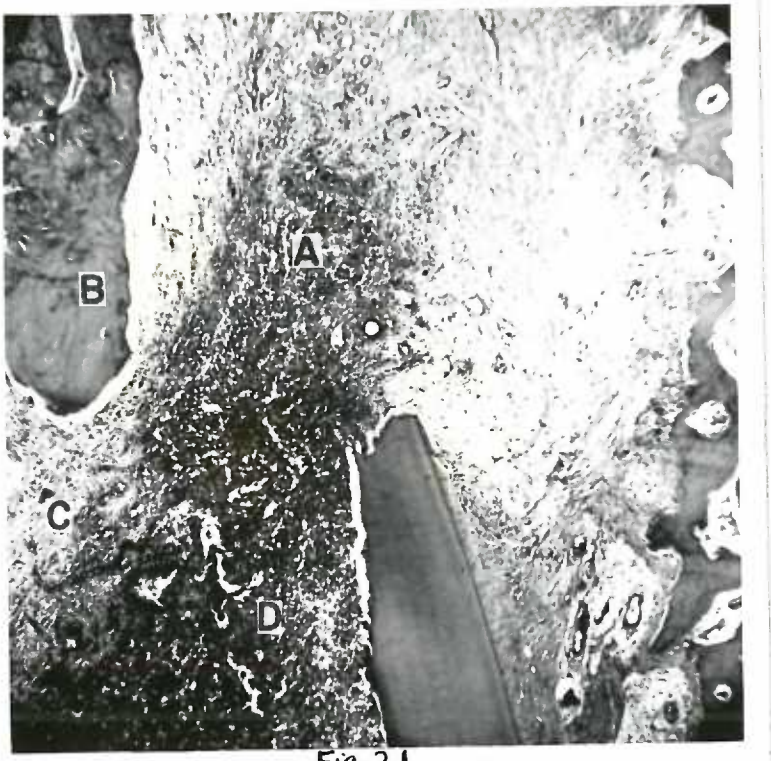


Fig. 21

Dog No. 7. Tooth No. 1. 56 days. Minimal acute and chronic inflammation is present at the apex of the root (A), which consists of a sprinkling of granulocytes and lymphocytes enmeshed in connective tissue. Some osteod type tissue (B) has formed just within the apex of the root. Dentinal resorption is absent. Epithelial proliferation and the presence of a fistulous tract is not demonstrable. Much vascular granulation tissue densely infiltrated with lymphocytes (C), and preceded by viable and degenerating granulocytes (D), has moved upwards into the root canal.

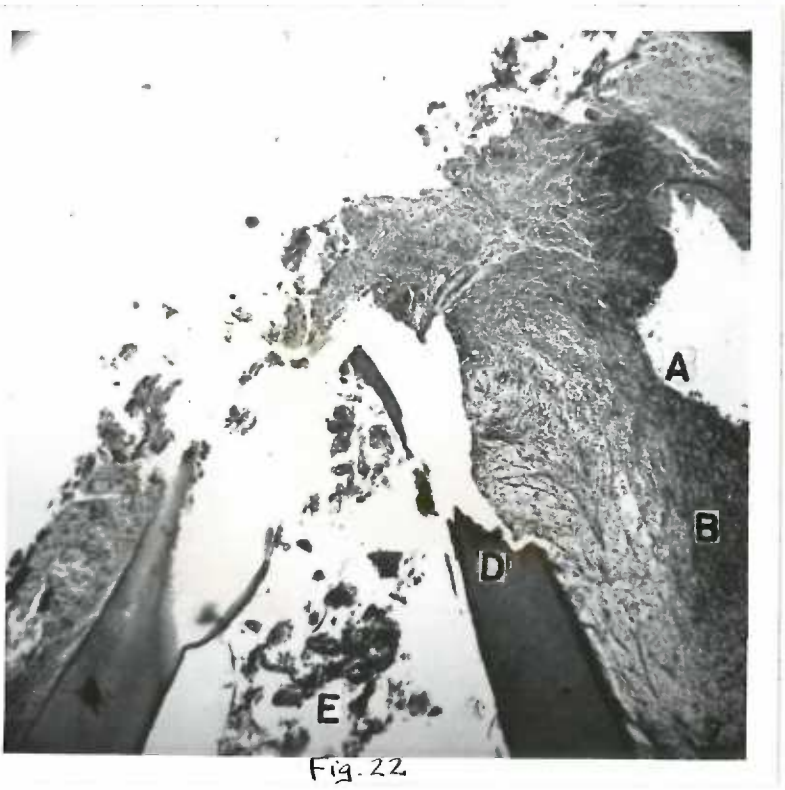


Fig. 22

Dog No. 9. Tooth No. 6. 42 days. Beyond the apex of the root is an area of severe acute inflammation with a necrotic center partially lined with squamous epithelium (A), and with a zone of moderate chronic inflammation surrounding it (B). Encapsulation of this inflamed area with connective tissue has occurred. Epithelial formation with microcystic areas are present. Osteoclastic activity is present in the surrounding bone (C). Dentinal resorption is present (D). No fistulous tract is demonstrable. The root canal is essentially empty except for a small amount of viable and degenerating granulocytes, colonies of bacteria and with absence of granulation tissue.

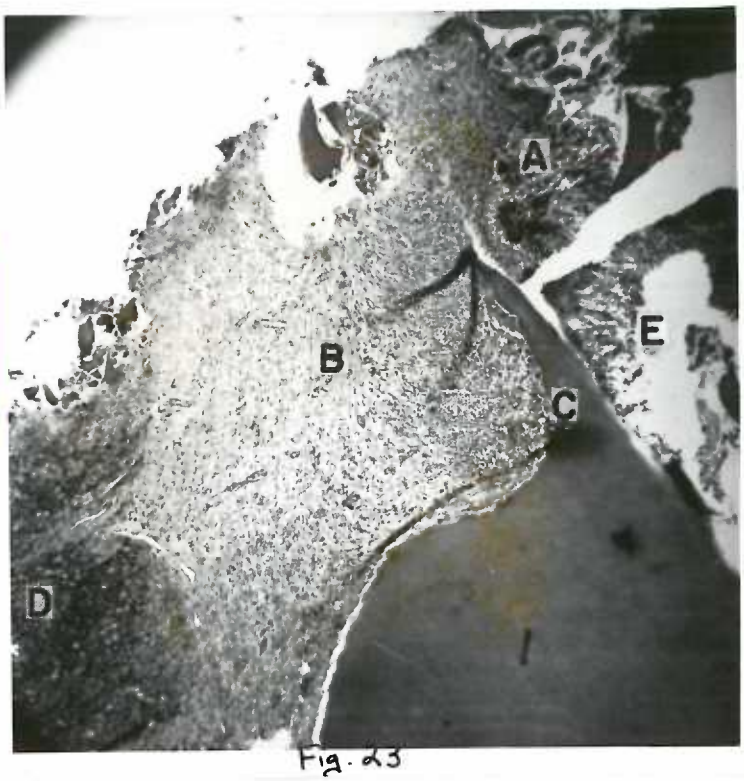


Fig. 23

Dog No. 10. Tooth No. 1. 35 days. An area of severe acute and moderate chronic inflammation (A) is present at the apex of the root surrounded by connective tissue (B) densely infiltrated with granulocytes with a minimal amount of lymphocytes, and a sprinkling of plasma cells. Epithelial proliferation or presence of a fistulous tract is absent. Active dentinal resorption (C) is present at the apex of the root. Acute osteomyelitis is present in the surrounding bone (D) with these areas infiltrated with numerous macrophages filled with ingested material. The root canal contains viable and degenerating granulocytes, dentinal fragments and colonies of bacteria with absence of granulation tissue.

Prednisolone Sodium Succinate

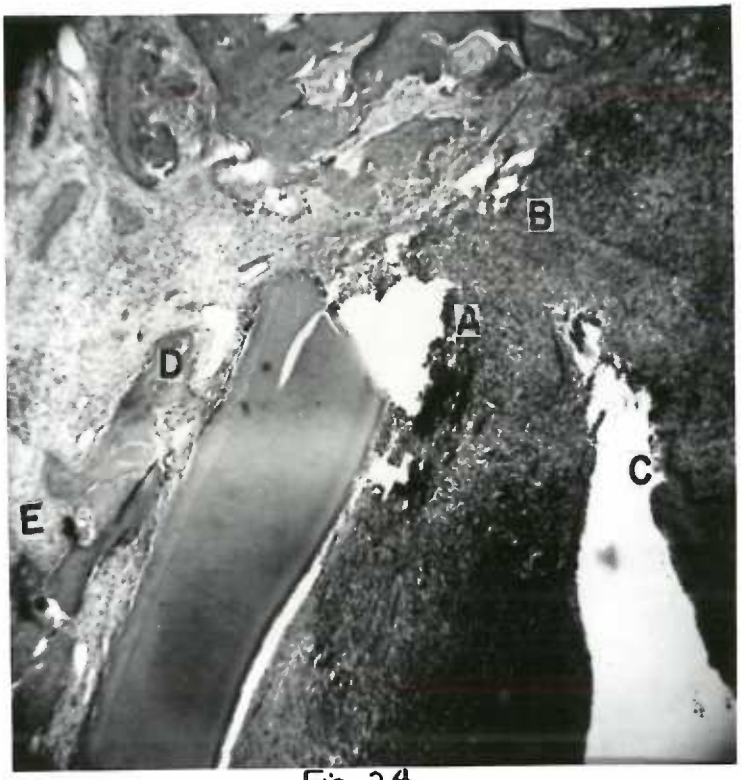


Fig. 24

Dog No. 6. Tooth No. 6. 56 days. An area of moderate acute inflammation (A) with abscess formation is present lateral to the apical opening of the root canal surrounded by a region of moderate chronic inflammation (B). An epithelialized fistula extends from the abscess area to the oral mucosa, (C). At the apex of the root are particles of osteod tissue (D) which also extend into the root canal a short distance. Periodontal fibers have moved into the root canal along one wall. Dentinal resorption is absent. Much granulation tissue (E) has moved into the root canal preceded in the coronal portion by a mass of degenerating granulocytes. Some pulpal remnants are present along one wall of the

root near the apex. Colonies of bacteria are present in the coronal portion of the root canal.

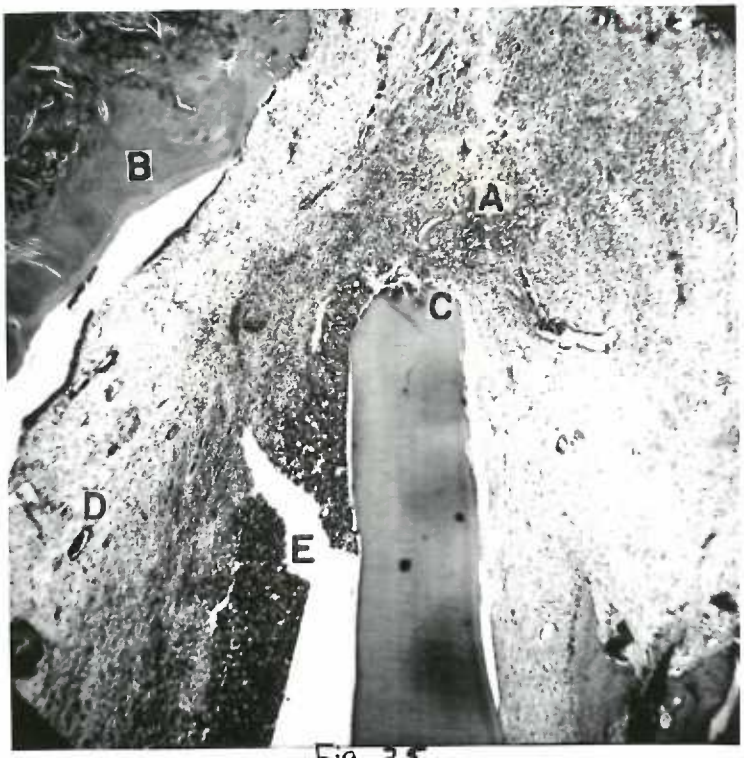


Fig. 25

Dog No. 7. Tooth No. 6. 56 days. The root of the tooth has been perforated laterally near the apex. A small area of severe acute and moderate chronic inflammation is being encapsulated with connective tissue (A). Osteodentin has formed along the lateral opening of the apex of the root, (B). Some odontoblasts are present along one wall of the root canal near the apex, apparently from incomplete reaming of the root canal. Epithelial proliferation and presence of a fistulous tract are not demonstrable. Dentinal resorption is present near the apex of the root (C). A moderate amount of vascular granulation tissue (D) has moved upwards

into the root canal along with viable and degenerating granulocytes (E), which appear laterally and coronally to the granulation tissue. Colonies of bacteria are present at the coronal portion of the granulation tissue.

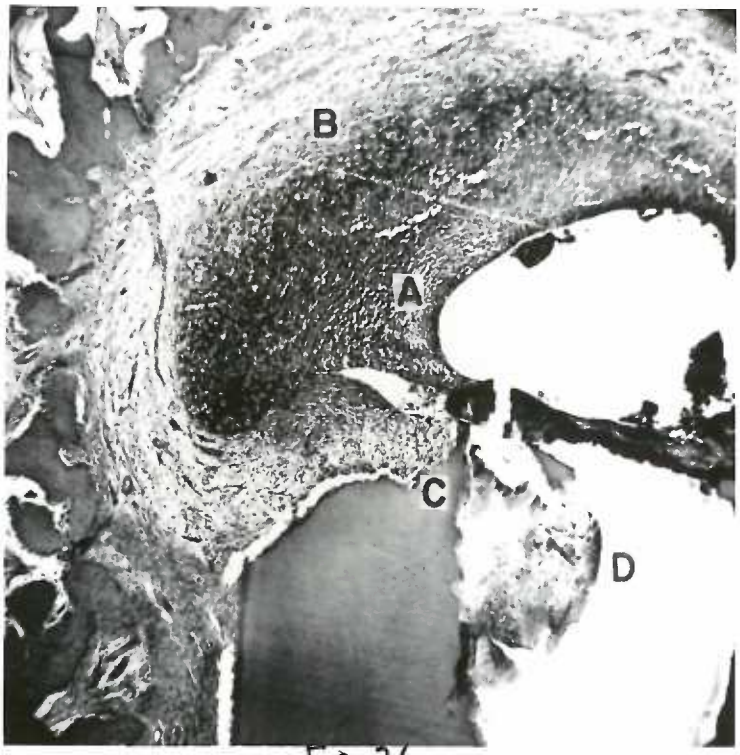


Fig. 26

Dog No. 9. Tooth No. 3. 42 days. Beyond the apex of the root is an area of severe acute inflammation with a necrotic mass of granulocytes with a cystic center surrounded by an area of moderate chronic inflammation. Connective tissue infiltrated with debris filled macrophages surrounds this inflammatory area (B). Dentinal resorption is present both on the outer and apical surface of the root (C). Epithelial proliferation is absent and a fistulous tract is not demonstrable. The root canal is essentially empty except

for a few viable and degenerating granulocytes, colonies of bacteria and dentinal fragments (D).

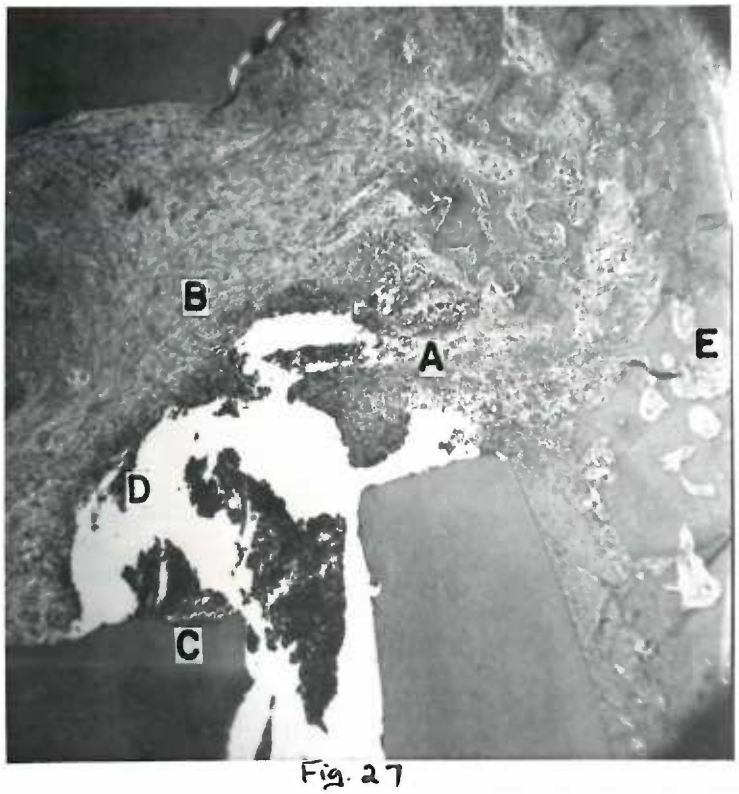


Fig. 27

Dog No. 10. Tooth No. 3. 35 days. A small area of minimal acute and chronic inflammation (A) is present at the lateral opening of the root near the apex. A moderate amount of granulation tissue containing engorged and thrombosed blood vessels has moved into the root canal (B). The region outside the root canal contains a sprinkling of granulocytes with an almost complete absence of lymphocytes in the bone and surrounding tissues. Within the root canal resorption of dentin has occurred (C). Epithelial proliferation is absent and no fistulous tract is demonstrable. A dense layer of granulocytes is present on the

coronal portion of the granulation tissue in the root canal (D). A few macrophages are present surrounding the inflammatory area at the opening of the root canal. Osteomyelitis is present in the surrounding bone (E).

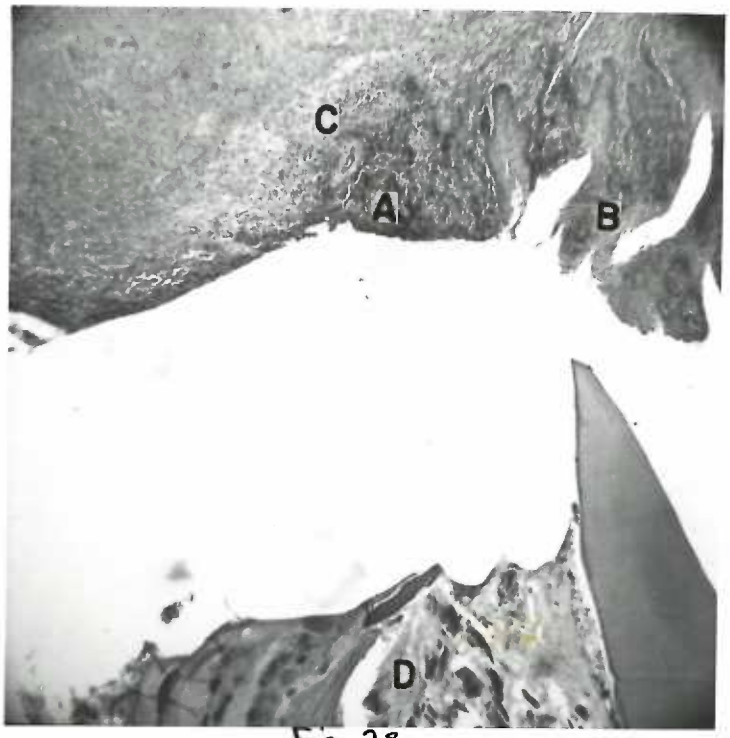


Fig. 28

Dog No. 6. Tooth No. 5. 56 days. The root canal is perforated near the apex. A large area of severe acute inflammation is present at the apex (A), surrounded by an area of moderate chronic inflammation (C). An epithelialized fistula (B) to the oral cavity is present. The root canal contains dentinal fragments, viable and degenerating granulocytes and colonies of bacteria with absence of granulation tissue. Dentinal resorption is absent.

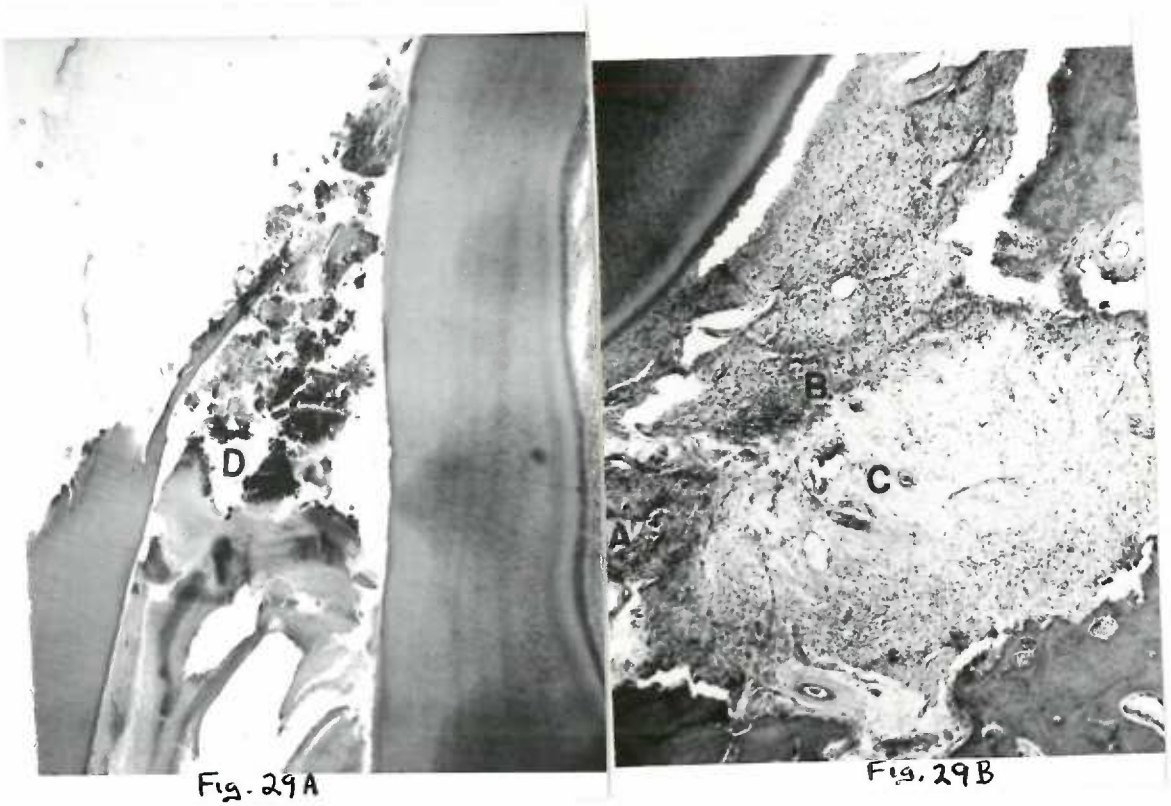


Fig. 29A

Fig. 29B

Dog No. 7. Tooth No. 5. 56 days. The periapical region beyond the apex of the root is not present in this section. Lateral to the root is an area of moderate acute (A) and chronic (B) inflammation with dilated blood vessels in the surrounding area. This inflammatory mass is becoming encapsulated with connective tissue (C). Dentinal resorption is absent. Epithelial proliferation is absent and a fistulous tract is not demonstrable. The root canal is partially filled with dentinal fragments, viable and degenerating granulocytes, colonies of bacteria and with absence of granulation tissue (D).

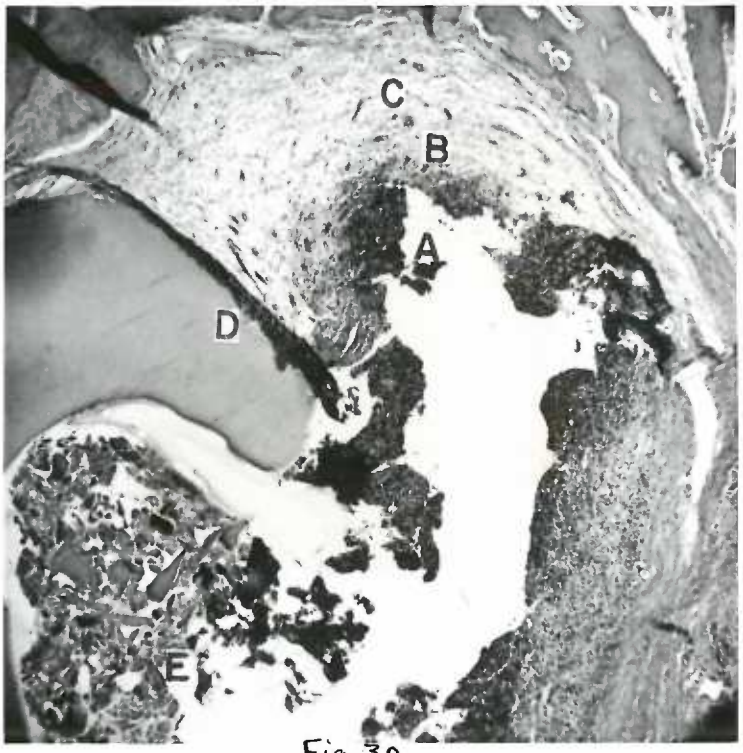


Fig. 30

Dog No. 9. Tooth No. 5. 42 days. A large mass of severe acute inflammation with a necrotic center is present at the apex of the root (A) and is surrounded by a mild chronic inflammatory area (B). Encapsulation of this area with connective tissue infiltrated with macrophages (C) is occurring. A small amount of dentinal resorption is present (D). The root canal is partially filled with viable and degenerating granulocytes, dentinal fragments and colonies of bacteria with an absence of granulation tissue (D). Epithelial proliferation is absent and no fistulous tract is demonstrable.

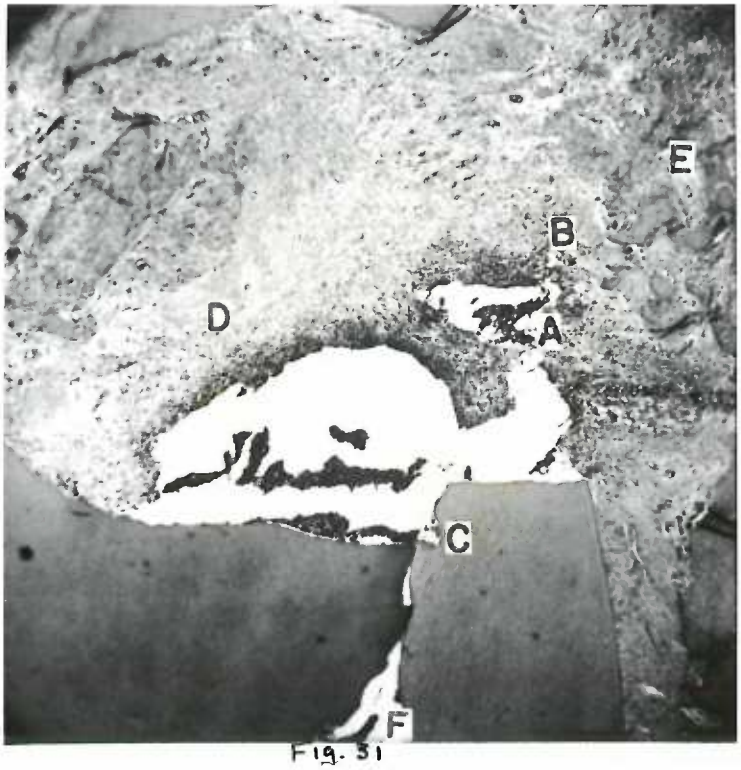


Fig. 31

Dog No. 10. Tooth No. 5. 35 days. At the apex of the root a moderate acute inflammation is present (A) surrounded by mild chronic inflammation (B) which contains few lymphocytes and numerous debris filled macrophages. Epithelial proliferation is absent and no fistulous tract is demonstrable. Dentinal resorption is present near the apex of the root (C). Granulation tissue is present at the apex of the root but not in the root canal (D). Ostemyelitis is present in the surrounding bone (E). The root canal is partially filled with dentinal fragments and viable and degenerating granulocytes (F).

Iodoform Powder

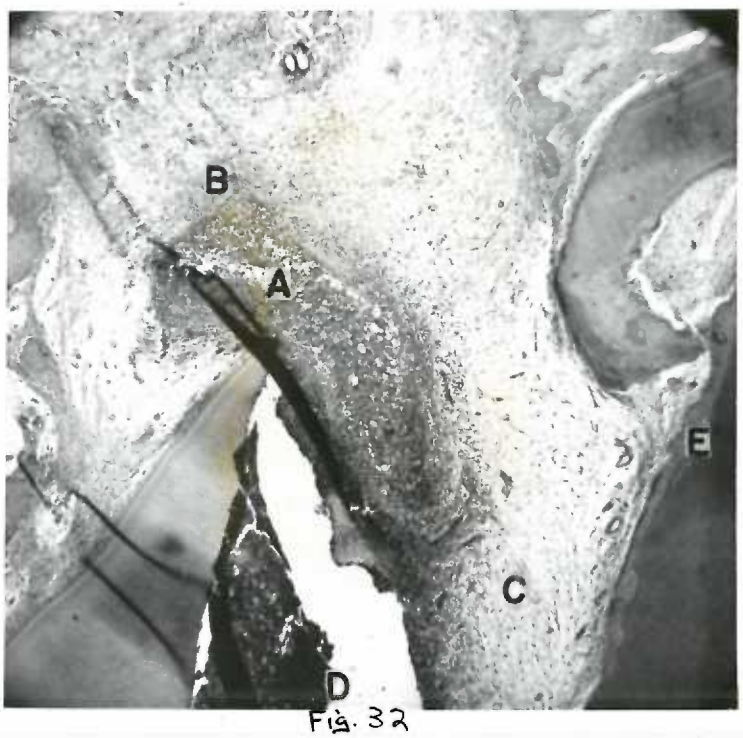


Fig. 32

Dog No. 6. Tooth No. 3. 56 days. The root has been perforated laterally near the apex. A small area of severe acute inflammation (A) is present on one side of the lateral perforation, surrounded by an area of moderate chronic inflammation (B). Fairly mature granulation tissue (C) containing dilated blood vessels has moved upwards into the root canal. Dentinal fragments and viable and degenerating granulocytes are present within the root canal (D). At the apical region of the lateral perforation of the root canal, small fragments of dentin are present acting as false denticles and are attached to the inner surface of the dentin (E).

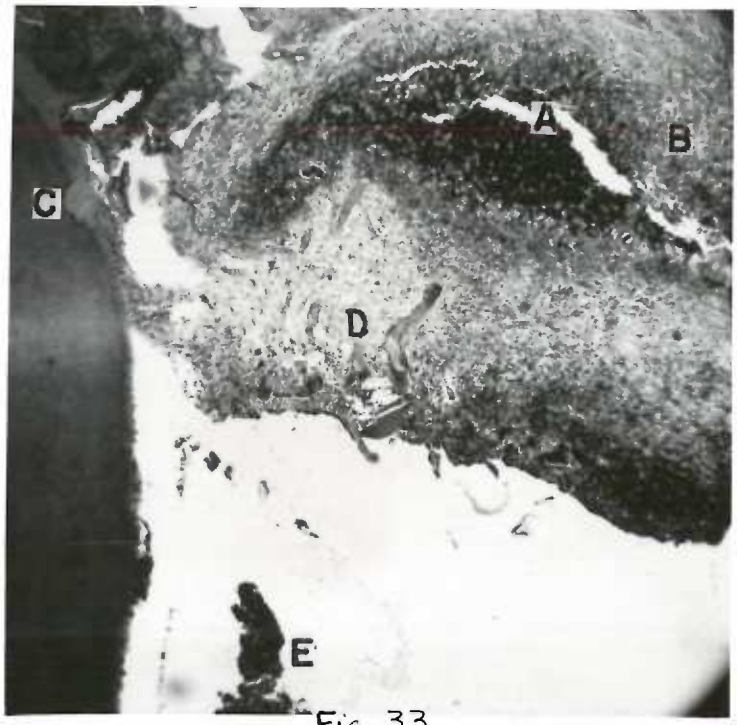
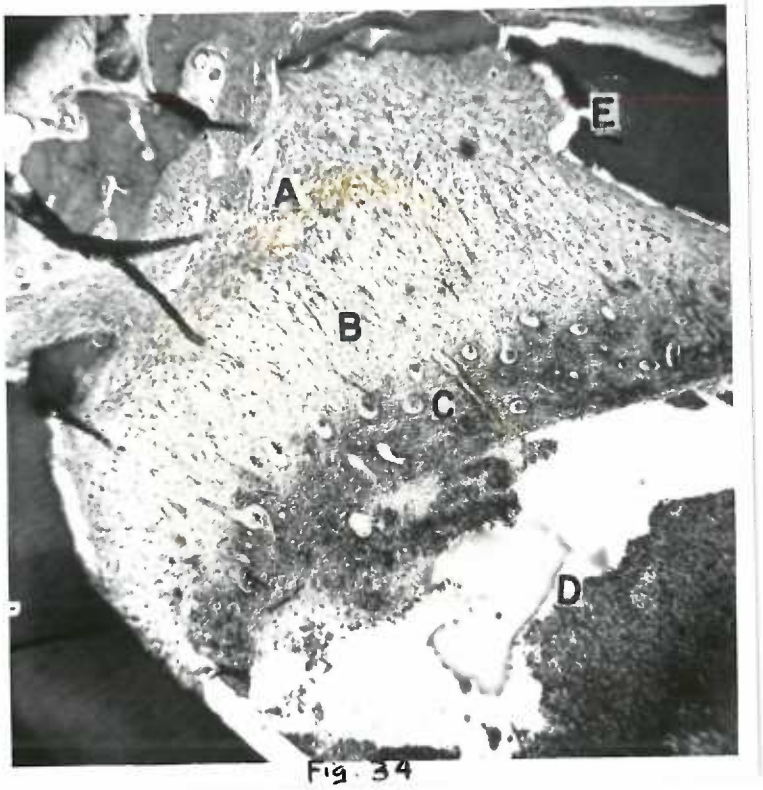


Fig. 33

Dog No. 7. Tooth No. 3. 56 days. The root is perforated laterally very near the apex. A large area of severe acute inflammation (A) containing granulocytes, plasma cells, lymphocytes and large macrophages is present in the apical region surrounded by a moderately chronically inflamed area (B). This inflammatory area is becoming encapsulated with fibrous connective tissue. Dentinal resorption is present (C). No epithelial cells or fistulas are present. Vascular granulation tissue (D) is present at the apex of the tooth and has progressed a short distance into the root canal. The root canal is partially filled with dentinal fragments and viable and degenerating granulocytes (E).



Dog No. 9. Tooth No. 1. 42 days. Directly at the apical region is a band of dense connective tissue (A) covering the apical opening of the root. Mild chronic inflammation is present inward from this dense connective tissue band. Young edematous granulation tissue (B) has penetrated upwards into the root canal which contains a vascular zone of dilated blood vessels (C) in the coronal region. Viable and degenerating granulocytes are present above the granulation tissue (D). Dentinal resorption is occurring at the apical region of the root canal. No.epithelial proliferation or fistulous tracts are demonstrable.

Fig. 35

Dog No. 10. Tooth No. 6. 35 days. At the apex of the root is a massive area of severe acute inflammation (A) which extends outward into the bony spaces. No attempt of encapsulation appears to be present. Mild chronic inflammation surrounds the acutely inflammed area which contains numerous lymphocytes and plasma cells. Osteomyelitis is present in the surrounding bone (B). A minimal amount of granulation tissue (C) is present at the apex of the root. The root canal contains viable and degenerating granulocytes and dentinal fragments. No epithelial proliferation is present and a fistulous tract is not demonstrable. Dentinal resorption is absent.

TABLE I

Comparison of Periapical Changes in Series I

Drugs Used	Dog	Tooth	No. Days	Apical Inflamm.* Type and Degree	Epith. Prolif. at Apex	Fist. Tract to Oral Ca.	Dentinal Resorption	Gran. Tis.** in Root Canal	Bacteria in Root Canal
Operated Controls	1	6	50	Unknown	Unknown	Unknown	None	0	≠
	2	5	91	Acute 2≠ Chronic 3≠	None	None	Present	2 ≠	≠
	3	1	119	Acute 3≠ Chronic 3≠	None	None	Present	0	≠
	5	4	62	Acute 1≠ Chronic 3≠	Present	Present	None	0	≠
Fludrocortisone Acetate Ophthal. Ointment	1	4	50	Acute 3≠ Chronic 2≠	Present	Present	Present	1 ≠	≠
	1	5	50	Acute 3≠ Chronic 3≠	Present	Present	Present	0	≠
	3	2	119	Acute 1≠ Chronic 2≠	Present	Present	Present	0	≠
Fludrocortisone Acetate Ophthal. Oint. With Furacin Sol. Dressing	2	1	76	Acute 3≠ Chronic 1≠	None	None	Present	1 ≠	≠
	2	6	76	Acute 3≠ Chronic 1≠	None	None	Present	2 ≠	-
	4	1	105	Acute 1≠ Chronic 2≠	Present	None	None	0	≠
Furacin Sol. Dress. With Iodoform	4	6	105	Acute 2≠ Chronic 1≠	None	None	None	1 ≠	-
Furacin Sol. Dress. With Vioform	4	4	105	Acute 3≠ Chronic 2≠	None	None	Present	1 ≠	-
Prednisolone Sol. Succ. in Lanolin	5	6	91	Acute 3≠ Chronic 2≠	Present	Question	Present	0	≠
Lanolin	5	2	62	Acute 2≠ Chronic 2≠	Present	Present	None	1 ≠	≠
Tetracycline Hcl.	5	3	91	Acute 2≠ Chronic 1≠	Present	Present	Present	0	-
Iodoform	2	2	91	Acute 1≠ Chronic 1≠	None	None	Present	3 ≠	≠
Hydrocortisone Sodium Succinate	5	5	62	Acute 1≠ Chronic 2≠	Present	None	Present	2 ≠	-

TABLE II

Comparison of Periapical Changes in Series II

Drugs Used	Dog	Tooth	No. Days	Apical Inflamm.* Type and Degree	Epith. Prolif. at Apex	Fist. Tract to Oral Cav.	Dentinal Resorption	Gran. Tis.** in Root Canal	Bacteria in Root Canal
Operated Controls	6	1	56	Acute 3+ Chronic 3+	Present	None	None	0	+
	7	1	56	Acute 2+ Chronic 1+	None	None	None	3 +	0
	9	6	42	Acute 3+ Chronic 2+	Present	None	Present	0	+
	10	1	35	Acute 3+ Chronic 2+	None	None	Present	0	+
Prednisolone Sodium Succinate	6	6	56	Acute 2+ Chronic 1+	Present	Present	None	2 +	+
	7	6	56	Acute 3+ Chronic 1+	None	None	Present	2 +	+
	9	3	42	Acute 3+ Chronic 2+	None	None	Present	0	+
	10	3	35	Acute 1+ Chronic 1+	None	None	Present	2 +	0
Methylprednisolone	6	5	56	Acute 3+ Chronic 2+	Present	Present	None	0	+
	7	5	56	Acute 2+ Chronic 2+	None	None	None	0	+
	9	5	42	Acute 3+ Chronic 1+	None	None	Present	0	+
	10	5	35	Acute 2+ Chronic 1+	None	None	Present	0	0
Iodoform	6	3	56	Acute 2+ Chronic 2+	None	None	Present	2 +	0
	7	3	56	Acute 3+ Chronic 2+	None	None	Present	1 +	0
	9	1	42	Acute 1+ Chronic 1+	None	None	Present	2 +	0
	10	6	35	Acute 3+ Chronic 1+	None	None	None	1 +	0

KEY TO TABLES I AND II

* Signifies the degree of inflammation:

3 / Severe
2 / Moderate
1 / Mild
0 None

** Signifies the amount of granulation tissue in the root canal:

3 / Granulation tissue has moved upward one-fourth the length of the root canal or more.
2 / Granulation tissue has moved upward into the root canal beyond the apical opening but has not progressed beyond one-fourth the length of the root canal.
1 / Granulation tissue is present at the apical opening of the root canal and has just begin to progress upward into the root canal.
0 No granulation tissue is present at the apical opening of the root canal or in the root canal.

DISCUSSION

Proliferation of granulation tissue into the root canal of non-vital teeth whether untreated or mummified after chemical devitalization, has been thought to occur for many years. This method of treatment, used in the early era of dentistry, has been largely discarded in the more recent years. Treatment of a non-vital tooth, by insertion of a root canal filling, the generally accepted method to date, has several disadvantages: 1) The time-consuming process of reaming the root canal, sterilization and the filling precess, 2) The dehydration and resulting brittleness of the remaining tooth structures, 3) Discoloration of the crown and the root of the tooth, 4) In some instances, resorption of the dentin in the periapical region and 5) Lack of a high degree of success of the treatment (by some authors). If these disadvantages could be overcome by a simplified technique in which a single drug or combination of drugs were inserted into the root canal or in the periapical region so as to induce periapical tissues to fill the pulp chamber, a greater service could be rendered to the patient by retaining as essentially vital, non-brittle tooth.

In this study some selected cortico-steroids and anti-infective agents were placed in the periapical region of dog's teeth to observe

their effects on the proliferation of granulation tissue into the pulpless root canals.

The four cortico-steroids in this study cover a wide range of local anti-imflammatory potencies; from the least to the greatest, these are: hydrocortisone sodium succinate, prednisolone sodium succinate, methylprednisolone, in its pure water insoluble micorcrystalline form, and fludrocortisone as its acetate salt in a commercially prepared ophthalmic ointment base.

The choice of anti-infective agents covered a range of organic iodine compounds to antibiotic and antibacterial agents. Iodoform was selected because of its past use in dental practice as a well-known tissue stimulating agent[1], and its effectiveness in "drying up abscess cavities". Iodochlorhydroxyquin (Vioform^{R}), another organic iodine compound was selected because it does not possess the unpleasant iodoform odor and is an effective antibacterial and anti-fungal agent. Tetracycline hydrochloride (Panmycin^{R}) was employed because of its broad spectrum antibiotic activity. A nitrofuran compound, nitrofurazone, prepared in a water soluble ointment base (Furacin^{R} Soluble Dressing), was selected because it is relatively non-irritating to tissues, does not inhibit phagocytosis and has a wide antibacterial spectrum[25,45,53].

SERIES I

The operated controls showed a variety of responses. Acute and chronic inflammation was present in all cases, varying from mild to severe

degrees. The presence of an epithelialized fistulous tract in one case appeared to suppress the severity of the inflammatory response. It appeared that dentinal resorption did not occur where an epithelialized fistulous tract was present to the oral cavity, apparently due to the lack of prolonged contact of the inflammatory and clastic cells with the dentin. Proliferation of granulation tissue into the root canal of one tooth did occur although bacteria were present within the root canal. However, in this instance, the presence of a mild inflammatory reaction may have accounted for the granulation tissue growth into the root canal.

The cortico-steroids combined with an ointment vehicle showed severe to mild periapical inflammation with epithelial proliferation. Dentinal resorption occurred in all instances, which may indicate that acute inflammatory cells had been in contact with the dentin for a period long enough to produce clastic activity. Presence of bacteria in all the root canals indicated that no suppression of bacterial growth was evident by these drugs. Occurrence of a minimal amount of granulation tissue in the root canal in one case, in the presence of severe acute inflammation, is unexplainable. A question may be raised here regarding the effect of the ointment base itself in the production of the acute inflammatory response, however, one should bear in mind that there may be also prolonged anti-inflammatory action of the cortico-steroid in the ointment base itself. It appears that the ointment base would be gradually removed by the phagocytic activity. This may be the reason why a marked decrease of inflammation was observed in one case in which 119

days had intervened between placement of the drug and sacrifice.

The combination of fludrocortisone acetate ophthalmic ointment with Furacin Soluble dressing produced less periapical epithelial proliferation than with the use of fludrocortisone acetate ointment alone. In one instance, of a total of three teeth, a layer of epithelium appeared to surround the apex of the root in a manner which appeared to prevent granulation tissue from entering the root canal. However, in this case, where 105 days intervened between placement of the drug and sacrifice, minimal acute and chronic inflammation was present. In the remaining two cases granulation tissue proliferated into the root canal in minimal and moderate degrees. Presence of bacteria in the root canal of one tooth did not appear to deter proliferation of granulation tissue into the canal. The Furacin Soluble Dressing appeared to be an effective bacteriostatic agent in two out of three instances. The combination of fludrocortisone ointment with Furacin Soluble Dressing appeared to be more effective in inducing proliferation of granulation tissue into the root canal than with the use of fludrocortisone ointment alone. The effectiveness of Furacin in reducing the bacterial growth may have been one of the influencing factors in aiding granulation tissue growth into the root canal.

Furacin Soluble Dressing combined with iodoform or Vioform produced a similar amount of proliferation of granulation tissue into the root canal, although minimal amounts in both instances. Both combinations appeared to be effective anti-infective agents as evidenced by

absence of bacteria within the root canal. Vioform combined with Furacin Soluble Dressing produced a greater inflammatory response periapically than with the Iodoform-Furacin Soluble Dressing combination. Vioform appeared to be a greater tissue irritant than iodoform, however, the possibility of an incompatability between Vioform and Furacin Soluble Dressing must first be ruled out. Absence of periapical epithelial proliferation appeared to be a desireable characteristic with the use of both combinations. The dentinal resorption occurring following the use of Vioform and Furacin Soluble Dressing appeared as one of the undesireable characteristics of this combination.

Lanolin, which is a commonly used ointment base, was employed to observe its compatability with periapical tissues. The periapical inflammatory response was of moderate type in both acute and chronic inflammation. Epithelial proliferation with a fistulous tract to the oral cavity was present and dentinal resorption was absent. A minimal amount of granulation tissue containing colonies of bacteria had proliferated into the root canal. It thus appears that lanolin is tolerated by the periapical tissues to a moderate degree.

Tetracycline hydrochloride, a wide spectrum antibiotic, used alone, produced a moderate amount of periapical inflammation. However, epithelial proliferation did occurr with the presence of a fistulous tract to the oral cavity. Dentinal resorption had occurred and no granulation tissue was present at the apex of the root canal opening. An epithelial layer nearly covering the apical region of the root canal opening may

have accounted for the moderate inflammatory response outermost from this layer and prevented granulation tissue from entering the root canal. Bacteria were absent within the root canal, apparently due to the effective antibiotic activity of tetracycline. If an epithelial layer had not been present partially covering the apical opening of the root canal, it would be interesting to note if growth of granulation tissue would have been depressed by the tetracycline similarly to the depression of skeletal growth in chick embryos, by this same compound, as noted by Bevelander et al[5]. Castagnola and Oraly[7] condemn the use of antibiotics in pulpal and root canal therapy for a similar reason; inhibition of cellular growth.

Hydrocortisone sodium succinate placed in the periapical region, produced a very mild inflammatory response in the periapical tissues. Epithelial proliferation without fistulous tract formation was seen at the root apex. Dentinal resorption was present. Granulation tissue had proliferated upward into a bacteria-free root canal to a moderate degree. It appears that the hydrocortisone in a water soluble form did not remain in the periapical region for any long period of time, but may have remained in that region long enough to suppress the initial inflammatory reaction. Possibly this suppression would be beneficial in view of the fact that a severe acute inflammatory reaction may produce undesireable tissue damage, whereas a lessened inflammatory reaction would be more conservative concerning initial tissue destruction produced by the fluid exudate resulting in edema with a consequent massive cellular infil-

tration.

After the placement of iodofrom in the periapical region, in which 91 days intervened before sacrifice, the periapical inflammatory response, mild in character, was less than after the use of any other drug or in the controls. No epithelial proliferation or fistulous tract was evident periapically; however, dentinal resorption did occur. Proliferation of granulation tissue into the root canal was extensive, however, colonies of bacteria did appear coronal to this tissue. It appears that the iodoform had been present in the apical region for a sufficient length of time to initiate the proliferation of granulation tissue into the root canal. Castognola and Orlay[7] state that a paste of iodofrom, camphor, menthol and monochlorphenol (Kri-3) placed beyond the apex of the root canal "is absorbed in a few days", and does not produce any discomfort to the patient. From their observations it would appear that iodoform is quite compatible with periapical tissues during the relatively short period of time it remains in the area.

SERIES II

Three of the four operated controls showed a severe acute periapical inflammatory response with bacterial growth within the root canal with an absence of granulation tissue in the canal. One operated control showed moderate acute inflammation in the periapical region with absence of epithelial proliferation, a fistulous tract, dentinal resorption and bacterial growth within the root canal. However, in this same

control extensive proliferation of granulation tissue into the root canal has occurred. In the three remaining controls periapical epithelial proliferation and dentinal resorption was present in two instances and absent in one. It appears that a suppression of inflammation is conducive to proliferation of granulation tissue into the root canal chamber.

Prednisolone sodium succinate placed in both the coronal portion and at the apex of the root canal produced a suppression of periapical inflammation in two of the four cases. Inflammation was suppressed to a greater extent in the dog (No. 10) in which the drug had been placed for a shorter interval of time (35 days). In this particular instance both epithelial proliferation at the apex of the root and a fistulous tract to the oral cavity were absent, and granulation tissue had proliferated upward into the bacteria-free canal to a moderate degree, even though osteomyelitis was present in the surrounding periapical bone. In one instance epithelial proliferation at the root apex and presence of a fistulous tract to the oral cavity was noted with an absence of dentinal resorption; however, granulation tissue had progressed upward into the bacteria-contaminated root canal to a moderate degree. Moderate acute inflammation was also present in this case. In the two remaining dogs (No. 7 and 9), severe acute periapical inflammation with dentinal resorption was present. Both showed an absence of epithelial proliferation and a fistulous tract to the oral cavity with presence of bacteria in the root canal. Dog No. 7 showed a moderate proliferation of granu-

lation tissue into the root canal whereas, Dog No. 9 showed none. It is interesting to note the various periapical conditions that prevailed in the three instances in which granulation tissue was present in the root canal. In Dog. No. 6 where epithelial proliferation with an oral fistula was present at the apex of the root, and Dogs. No. 7 and 10 where neither of these conditions were present, granulation tissue proliferated upward into the root canal to the same degree. Also in Dogs., No. 6 and 7, bacteria were present within the root canal whereas, there was an absence of bacteria in the canal in Dog No. 10. These results indicate that granulation tissue does proliferate upward into the root canals with presence or absence of bacteria, following the use of prednisolone sodium succinate.

After the use of methylprednisolone severe acute inflammation was present in two instances and moderate acute inflammation in the other two instances. In Dog. No. 10 in which the time interval from placement of the drug to sacrifice was 35 days, not only was the acute inflammatory response suppressed, but the number of chronic inflammatory cells (lymphocytes) were markedly reduced. Epithelial proliferation and presence of a fistulous tract occurred in one instance, Dog No. 6. Dentinal resorption was present in two instances, Dogs No. 9 and 10. Proliferation of granulation tissue into the root canals was absent in all cases. Bacteria were present in the root canals except Dog No. 10. From the overall picture of the methylprednisolone-treated teeth, it appears that some of the steroid effects were still present in Dog. No.

10, judging from the marked suppression of the in-flammatory response, and may have been present in the other dogs because of the complete absence of granulation tissue in the root canals. The insoluble particles of methylprednisolone may have altered the inflammatory response by producing a foreign body reaction.

The overall response of the periapical tissues to the iodoform-treated teeth was superior to all other drugs in Series II. Severe acute inflammation was present in two instances, Dogs No. 7 and 10, moderate and mild in one instance each, in Dogs No. 6 and 9, respectively. Epithelial proliferation at the apex and a fistulous tract to the oral cavity was absent in all cases. Dentinal resorption had occurred in all instances except Dog No. 10. Moderate proliferation of granulation tissue upward into the root canals had occurred in two instances, Dogs No. 6 and 9, and minimal proliferation of granulation tissue in Dogs No. 7 and 10. It is interesting to note the absence of bacteria in the root canals of this group, which apparently was due to the bacteriostatic properties of iodoform. It has been well demonstrated in this experimental group that iodoform does stimulate production of granulation tissue into a pulpless root canal.

The duration of time these drugs remained in the periapical region is not known. In Series I it is presumed that the drugs used alone, without and ointment vehicle, remained in the area but a short time whereas the drugs incorporated into an ointment base may have remained in the area from several days to a week or more. In Series II, the drugs

placed in both the periapical region and in the coronal portion of the tooth above a blood clot may have remained for a longer period of time. A question remains as how the cortico-steroids affect this blood clot with regard to its dissolution or organization. It is known that iodoform and Vioform can be removed by body fluids and cells from the tissues as their presence in sufficient quanity, can be identified roentgenographically.

A lessened amount of inflammation in the periapical region of the teeth appeared to be conducive to the proliferation of granulation tissue into the root canals of the experimental teeth as well as the operated controls. Presence of a fistulous tract to the oral cavity also appeared to reduce the acuteness of the periapical inflammation but also had a tendency to block granulation tissue from entering the root canal.

Dentinal resorption occurred with an incidence of 70.6 per cent in Series I and 62.5 per cent in Series II. This resorption occurred in both instances wheither fistulous tratts to the oral cavity were present or absent and wheither granulation was present or absent in the root canal.

There is a suppression by the cortico-steroids, of the initial inflammatory penomenon resulting from trauma of surgery. Whether this suppression of inflammation, with the accompanying decreased loss of fluid exudate and cells from the capillaries, can markedly affect the production of granulation tissue and to what extent, is yet to be determined. It has been pointed out by Edwards and Dunphy[3] that there is a

close relationship between acute inflammation and subsequent connective tissue proliferation. They found that mild injury invokes a mild inflammation followed by a small amount of lessened connective tissue proliferation and repair. The more severe the injury of trauma, the greater is the inflammatory response and the greater the connective tissue proliferation. This appears to relate the effect of cortisone on wound healing to its well-known suppression of the initial inflammatory reaction and supports the concept of an intimate connection between acute inflammation and the initiation of repair. The healing response produced by tissue stimulation may explain the considerable amount of granulation tissue which is generally produced in areas where iodoform has been used; it is primarily the result of the irritant action of this compound.

It is however, generally known that proliferation of granulation tissue occurs after the inflammatory process has subsided. Also, it is known that during the inflammatory process tissues are destroyed and proteins are broken down into substances which produce irritation, to the surrounding tissues. These protein breakdwon products are removed by the macrophages entering the area during the healing process.

A unique situation occurs during the inflammatory process at the apical region of a pulpless tooth which is not duplicated elsewhere in the body. The pulpless root canal, during the inflammatory process, acts as a reservoir not only for bacterial contamination but also for the irritant protein breakdown products. With the presence of bacteria and/or irritant protein breakdown products in the root canal, granulation tissue

does not enter the root canal. Further because of the adverse diffusion gradient and inaccessibility, vascular elements and macrophages do not enter the root canal to remove this debris. Consequently the empty root canal with its contaminents, becomes walled off by a defensive tissue barrier, the granuloma. This may explain the reason why teeth will root canal fillings which do not extend to or very near the apex result in incomplete healing in the periapical region.

The therapeutic approach to this problem by the use of the cortico-steroids is chiefly by suppressing or preventing the inflammatory reaction from occurring in the periapical region, fewer irritating protein breakdown products are allowed to forme; and with the prevention of bacterial contamination, granulation tissue has a better opportunity of proliferating into the root canal. The addition of a mild tissue stimulant and bacteriostatic agent such as iodoform to the cortico-steroid, would further assist in the movement granulation tissue to enter into the root canal. It would appear, that a soluble cortico-steroid should be used in the periapical region as evidenced by the undesireable effect of the relatively insoluble methylprednisolone. It is unknown whether the methylprednisolone place petiapically prevented granulation tissue formation by being retained for an extended period of time, thus producing a longer and more profound depression of inflammation than the other cortico-steroids, and by producing a foreign body reaction, or by alowing relatively uninhibited bacterial invasion of the area. This may explain the difference between the periapical effects between

the very slightly soluble methylprednisolone and the very soluble prednisolone sodium succinate.

The degree of success of attempting to fill the root canal with granulation tissue was only partially realized in this investigation. However, it did show that with the suppression of the inflammatory response with the use of cortico-steroids, granulation tissue did proliferate upward into the root canal. It also showed the advantage of incorporating a tissue stimulant and bacteriostatic agent, such as iodoform, along with the cortico-steroid in such future experimental work.

SUMMARY AND CONCLUSION

The local effects of cortico-steroids and anti-infective agents on the proliferation of granulation tissue into 33 pulpectomized and apicoectomized maxillary incisor teeth in nine dogs was studied. The cortico-steroids used were hydrocortisone sodium succinate, prednisolone sodium succinate, methylprednisolone and fludrocortisone acetate. The anti-infective drugs used were iodoform, Furacin Soluble Dressing, Vioform and tetracycline hydrochloride. One tooth per dog (with the exception of one dog in the first series) served as the operated, untreated control. In the first series (five dogs) the drugs, individually or in mixture were placed in the surgically produced apicoectomy defect either in the pure state or in combination with an ointment base. The drugs in the second series (four dogs) were placed individually in the coronal portion of the tooth as well as in the surgically prepared apicoectomy defect. The coronal opening of all teeth were sealed after treatment with soft paraffin wax or zinc-oxide and eugenol cement followed by a silver amalgam filling. Histological sections of the teeth and periapical tissues were made in Series I after intervals of time ranging from 50 days to 119 days after drug placement; in Series II after intervals of 35 to 56 days. Periapical features observed microscopically were: 1) Inflammation 2) Epithelial proliferation, 3) Dentinal

resorption, 4) Fistulous tracts to the oral cavity, 5) Presence of bacteria in the root canal and 6) Granulation tissue in the root canal. Microscopic findings revealed: 1) All drugs produced periapical inflammation, the greatest amount being produced by drugs incorporated in ointment bases, and the insoluble methylprednisolone, 2) Periapical epithelial proliferation appeared to prevent proliferation of granulation tissues into the root canal, 3) Dentinal, resorption occurred both in areas where granulation tissue was present or absent in the root canal, 4) Fistulous tracts to the oral cavity were present in eight instances, four in which the ointment base had been used and once each with the use of prednisolone, methylprednisolone, tetracycline and in one control, 5) Granulation tissue movement into the root canal was greatest with the use of hydrocortisone, prednisolone and iodoform powder, 6) Presence of bacteria in the root canal did not appear to affect the proliferation of granulation tissue into the root canal of teeth treated with the cortico-steroids. A lessened inflammatory process in the periapical region, where a fistula to the oral cavity was not present, appeared to allow greater proliferation of granulation tissue into the root canal.

Perhaps a combination of iodoform and a soluble cortico-steroid may prove to be more effective in stimulating proliferation of granulation tissue into a pulpless root canal - a possibility to be tested in the future.

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