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The Effect of ESCHERICHIA COLI Endotoxin on the Cat Periapical Tissues

Thomas G. Dwyer

Abstract

THE EFFECT OF Escherichia coli
ENDOTOXIN ON CAT PERIAPICAL TISSUES

by

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The periapical tissue reaction to three concentrations of endotoxin solutions and to a sterile saline control solution was examined in six adult cats. The maxillary and mandibular cuspids were isolated and the pulps extirpated with barbed broaches. The four solutions (1, 10, 100 µg/ml endotoxin, and saline) were then injected into the four prepared teeth for each cat and the access cavities sealed with IRM.

At two week intervals, two cats were sacrificed and the specimens removed, while the remaining animals were retreated as previously described. The block tissue sections were radiographed, fixed in 10% buffered formalin, and prepared for histopathologic examination.

The radiographic lesions were ranked according to size and the ordinal data used in the Friedman and Wilcoxon statistical tests. The histologic sections were examined for type of inflammatory cell infiltrate, degree of inflammation, and osteoclastic activity. The histologic observations were used in a descriptive manner, while the radiographic findings were used for comparison of treatment results.

The radiographic results indicated that there was a significant difference between treatments, when measured by

ranking radiolucent lesion size ($P < 0.025$). There was also a significant difference between the effects of the three endotoxin solutions when each was compared to saline ($P=0.01$). However there were no significant differences in effects when the endotoxin solutions were compared to each other.

LOMA LINDA UNIVERSITY

Graduate School

THE EFFECT OF *Escherichia coli* ENDOTOXIN ON THE CAT PERIAPICAL TISSUES

by

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A Thesis in Partial Fulfillment of
the Requirements for the Degree
Master of Science
in the Field of Endodontics

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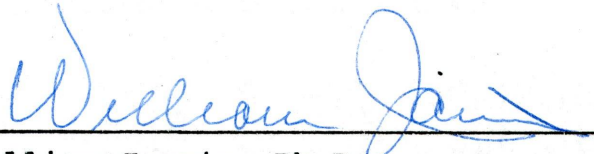

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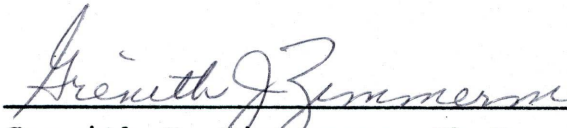

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LIST OF ABBREVIATIONS

ACIF - Anti-Complement Immunofluorescent Test

BHI - Brain-Heart Infusion Broth

BSA - Bovine Serum Albumin

Et - Endotoxin

H and E - Hematoxylin and Eosin

IRM - Intermediate Restorative Material

LPS - Lipopolysaccharide

µg - Microgram

N.S. - Not Significant

PDL - Periodontal Ligament

PMN - Polymorphonuclear Leukocyte

TSA - Trypticase Soy Broth

VPI - Virginia Polytechnic Institute

INTRODUCTION

The possibility that bacterial endotoxins are involved in the production of periapical inflammatory lesions has received little attention in endodontic research. Bacterial endotoxins are produced when the cell walls of gram negative bacteria undergo lysis. These lipopolysaccharide complexes are structural components of the cell wall and are released when the cell integrity is interrupted.

In an effort to measure the endotoxin content of endodontically involved teeth, Schein and Schilder (1975) aspirated fluid from 40 teeth and analyzed it with the limulus lysate test. They found that teeth with necrotic pulps contained greater concentrations of endotoxin than those with vital pulps, and that symptomatic teeth contained more endotoxin than asymptomatic teeth. Further, teeth with radiolucent periapical areas contained higher levels of endotoxin than teeth without such areas. The authors concluded that bacterial endotoxins may be factors in producing pulpal and periapical disease.

Wesselink and associates (1978) investigated the possible role of endotoxin in the pathogenesis of periapical inflammation with an experimental model simulating the root canal. Polyethylene tubes were filled with varying amounts of E. coli endotoxin and implanted into the subcutaneous tissue of 27 rabbits. The results led these investigators to speculate

that the primary toxicity of endotoxin has no major part in the initiation or maintenance of chronic periapical inflammation.

Pitts (1977) studied the role of endotoxin in causing periapical inflammatory lesions in dogs and cats. Endotoxin preparations were introduced directly into the root canal systems of three dogs and four cats. The results supported the hypothesis that endotoxin may be a factor in producing periapical hard and soft tissue destruction.

The purpose of this study was to test the effect of Escherichia coli endotoxin on the cat periapical tissues.

LITERATURE REVIEW

I Microbial Flora of the Mouth

The oral cavity supports one of the most concentrated and varied microbial populations of any area of the human body. During birth the child is inoculated with the normal flora of the genital tract which include lactobacilli, corynebacteria, micrococci, coliforms, streptococci, yeasts and protozoa. At the end of three months all mouths support a microbiota. After one year, streptococci, staphylococci, veillonella and neisseria can be isolated from all mouths; actinomyces, nocardiae, lactobacilli and fusobacteria can be cultured from one-half of the mouths; and bacteroides, leptotrichiae, corynebacteria, and coliforms are isolated in less than half the mouths (Burnett and Schuster, 1978a).

Throughout childhood the bacterial populations increase and in adolescence spirochetes and Bacteroides melaninogenicus are present in all mouths. In the adult, gram positive facultative and anaerobic cocci account for 59% of the cultivable organisms present in the saliva. Gram negative facultative and anaerobic cocci make up 17% of the population, while gram negative facultative and anaerobic rods account for 7%. The gram positive rods, both facultative and anaerobic, compose 17% of the organisms located in saliva (Sokransky, 1971). The interactions between these microbes, the environment and the host produce a wide range of effects varying from "health" to different forms of oral disease.

II Importance of Bacteria in Pulpal and Periapical Disease

Several investigations have demonstrated the importance of bacteria as etiologic agents in pulpal and periapical disease. Kakahashi and associates (1965) compared the pathologic changes resulting from untreated experimental pulp exposures in germ-free and conventional rats. They found that the presence of microorganisms in pulp tissue caused pulpal necrosis and granulomas compared to no such pathology in the germ-free rats. They concluded that the presence or absence of a microbial flora is the major determinant in the healing of exposed rodent pulps.

In an attempt to correlate bacteriologic findings with radiographic evidence of periapical pathology, Smith (1958) concluded that many of the organisms present in root canals are potent pathogens and are capable of producing periapical lesions.

Korzen, Krakow and Green (1974) also studied the effect of microorganisms on the periapical region by infecting gnotobiotic and conventional rats with Streptococcus mutans. After sacrificing the animals and examining the histologic specimens, they concluded that the periapical tissue reactions could be related directly to the bacterial invasion of the root canal. The severity of the periapical inflammatory response was greatest in the canals containing the most microorganisms.

III Entrance of Bacteria into the Root Canal System

Since it has been determined that oral bacteria play a

role in producing pulpal and periapical disease, the manner in which the microbes gain access to the root canal system is important. Morse (1976) states that the most common way in which bacteria reach the pulp is through direct extension of dental caries. Gibbons (1964) in studies with germ-free animals demonstrates that bacteria are essential for the production of caries.

The experimental passage of microorganism through the dentinal tubules to the pulp has been described by Bender, Seltzer and Kaufman (1959). Chirnside (1961) has also demonstrated that bacteria were capable of invading dentin in pulpless teeth exposed to the oral environment for three weeks and reaching the pulp chamber. He concluded that if odontoblastic processes succumb following pulpal necrosis and are exposed to the oral environment, the dentinal tubules are liable to bacterial invasion.

A third mode of bacterial entrance into the root canal system is through the periodontal ligament. In a study designed to isolate spirochetes from the root canal systems of intact but pulpally-involved teeth, Hampp (1957) speculated that the only plausible explanation of the presence of spirochetes in such cases would be by spread from the gingival sulci or extension through soft tissues.

In a study of intact teeth with necrotic pulps, MacDonald, Hare and Wood (1957) obtained anaerobic and aerobic cultures in 38 of 46 teeth examined. The authors postulated that most

of these organisms reached the pulp from the oral cavity via the lymphatics and blood vessels of the periodontium. Grossman (1967) placed a readily identifiable test microorganism into the gingival sulcus of dog and monkey teeth, traumatized the teeth and later recovered the same organism from the pulp. He concluded that during trauma, blood vessels in the periodontal ligament were torn and opened, providing a pathway through which the microorganisms could reach the pulp.

Another way in which the pulp may be invaded is through the localization of blood-borne bacteria in areas of inflammation. Transient bacteremias can localize in an injured pulp which cannot eliminate the invading microorganisms. Robinson and Boling (1941) reported on this effect and concluded that idiopathic post-operative pulpitis may be a result of anachoresis. The work of Burke and Knighton (1960) confirmed this pathway of microbial entrance into the pulp.

IV Flora of the Root Canal System

Among the early investigators of bacteriology relating to the dental pulp were Henrici and Hartzell (1919). They examined healthy teeth, periodontally involved teeth, cariously involved teeth, and a combined group. Table 1 lists their results and compares them to later similar studies.

The findings of Henrici and Hartzell confirmed reports of other contemporary investigators including Goadby (1903) and Collins and Lyne (1919).

Grossman and Christian (1952) reported on a survey of the microorganisms recovered in their culturing of over 1,000 pulpless teeth. They found 77.4% were gram positive organisms, 5.7% gram negative microbes and 16.89% yeasts. A detailed breakdown is given in Table 1.

A study of the microorganisms isolated from pulp canals of pulpless teeth was done by Mazarella, Hedman and Brown (1955). They studied 50 intact teeth with no deep caries, periodontal involvement or history of trauma. Bacterial suspensions prepared from paper points exposed to the contents of the root canal systems were used for direct microscopic studies and culture techniques. The suspensions were inoculated into several different types of culture media, and grown aerobically, anaerobically and in 10% CO_2. Table 1 lists the percentages of bacteria isolated.

Brown and Rudolph (1957) also identified microorganisms from unexposed canals of pulpally-involved teeth. In the 70 patients they studied, mixed infections were prevalent and the high incidence of streptococci, diptheroids and micrococci was confirmed. When classified as to oxygen requirements they found 51% facultative anaerobes, 24% anaerobes, 24% aerobes and 1% requiring CO_2.

Differences in the bacterial flora of root canals as disclosed by two culture media was shown by Leavitt, Naidorff and Shugaevsky (1958). They compared root canal cultures incubated in a trypticase soy broth with 0.1% agar (TSA) to cultures incubated in a dextrose broth. The TSA medium was

found to be sensitive to anaerobic as well as aerobic growth, with the bacterial flora disclosed by the TSA presenting a much wider variety than that disclosed by the dextrose broth. This study showed a 33% incidence of anaerobes in the root canal by use of the TSA medium.

Winkler and Van Amerongen published the bacteriologic results of over 4,000 root canal cultures in 1959. Their cultures were incubated in brain-heart infusion broth (BHI) with 0.05% agar. They identified the microorganisms and their results are listed in Table 1. The conclusion drawn from their results was that streptococci were the most serious pathogens and all other organisms were chance contaminants.

Sciaky and Sultzenu (1961) in their examination of the bacterial flora of the root canal found that 11.1% of the positive cases were made up of purely anaerobic or a combination of anaerobic and aerobic microorganisms, including anaerobic streptococci, actinomyces, gram positive rods, and veillonella.

Microscopic and cultural methods were used to study samples of unexposed root canals of 101 teeth by Sulitzeneau, Beutner and Epstein (1964). They demonstrated bacteria in 84% of the cases, with streptococci occurring as the most frequent infecting agent. Gram positive rods and filaments and gram negative forms occurred less frequently. The authors also found obligate anaerobic bacteria in 25% of the cases studied.

In 1974, Kantz and Henry obtained samples from 24 intact teeth with necrotic pulps and cultured these using a new method of maintaining anaerobiosis during collection and transportation.

These investigators were concerned with isolating and identifying strict anaerobes from the root canal system. They were successful in identifying actinomyces, bacteroides, campylobacter, eubacteria, fusobacteria, peptococcus, and veillonella. One hundred four of the 377 bacterial colonies or 27.2% were strict anaerobes.

Wittgow and Sabiston (1975) also examined the flora of intact teeth with necrotic pulps. They cultured incisors using the anaerobic technique developed by the Virginia Polytechnic Institute Anaerobic Laboratory. They found 31 of 40 teeth to contain obligate anaerobes. Gram negative rods were evident in 67% of all teeth sampled, in 75% of all teeth with necrotic pulps and in 84% of all teeth with positive cultures. The organisms identified were similar to those found by Kantz and Henry.

Keudell and associates (1976) utilized the VPI technique in sampling vital and necrotic pulps. They found 64% of the necrotic pulps sampled contained anaerobic bacteria. They suggested anaerobes were more frequent in necrotic pulps of traumatically injured teeth than in necrotic pulps of teeth with carious lesions.

Goodman (1977) used a simplified transport system to isolate anaerobic bacteria from teeth with necrotic pulps. All 55 of the teeth sampled contained at least one anaerobe and as many as four. Over half of the total number of organisms isolated were anaerobes and of these almost half were gram negative. The genera and species of the anaerobes

identified were similar to the results previously reported.

V Significance and Structure of Endotoxins

Recent investigations examining the root canal flora have disclosed the presence of a significant number of gram negative bacteria capable of releasing lipopolysaccharides (LPS) or endotoxin upon cell lysis. The results of the work done by Schein and Schilder (1975) do show a significant level of endotoxin present in the root canal system. Their results are listed in Table 2. The authors also found that teeth with radiolucent areas contained a higher level of endotoxin than teeth without these areas (0.717 compared to 0.057 µg Et/ml).

Structurally the LPS molecule is quite large and has been characterized as consisting of three principal regions: the lipid A region, the O polysaccharide region and the R core polysaccharide. The lipid A portion is thought to be responsible for the biologically active properties. Hausman (1975) has shown that the lipid A region is primarily responsible for bone resorptive activities of LPS. The O polysaccharide portion is related to antigen specificity (Westphal, 1974). The R core is the middle portion linking the lipid A and O polysaccharide (Elin and Wolff, 1976). Figure 1 illustrates these structural units.

Endotoxins have a wide variety of potent biological properties and it has been stated that man is the most sensitive animal to these biologic effects (Wolff, 1977). As described by Burnett and Schuster (1978b), these bacterial toxins elicit

fever indirectly by causing the release of an endogenous pyrogen from polymorphonuclear leukocytes. A more characteristic pharmacological activity of endotoxins is that they cause an increase in capillary permeability, together with hemorrhages. Another characteristic pharmacological reaction is to elicit inflammation when injected intradermally.

It has been established that in addition to toxic properties, the LPS complexes are strongly antigenic. The O polysaccharide portion is a potent antigen that can elicit antibody formation in submicrogram quantities. Although the initial antibody response is predominantly IgM, antibodies of IgG and IgA classes are present within a few days (Elin and Wolff, 1978).

VI Root Canal as Pathway for Antigen Release

Melville and Birch (1967) cultured both intracanal and periapical areas of 194 teeth treated by apicoectomy. The results of this study led them to conclude that the periapical area may be sterile even when bacteria are isolated within the root canal system. When they did obtain bacteria from the periapical area they were identical with the root canal flora. It is likely that the bacteria were released through the root canal system into the periapical areas.

Other investigations have shown that the root canal system can act as a pathway for release of microbes into the periapical area. Kennedy, Hamilton and Sylverton (1957) inoculated the root canals of eight Macacus monkeys with streptococci. They observed transient bactermias following such inoculations and

recovered the inoculated organism from the root canal at terminal examination. Rosengren (1962) did a similar experiment with cats and found that injection of streptococci into the root canal resulted in radiographically visible bone resorption and raised the anti-streptolysin O titers of the serum considerably. Rosengren and Winblad (1975) also inoculated rat root canals with Streptococcus mutans and had very similar results. Destruction of the alveolar bone could be demonstrated radiographically and histopathologically. The pulpally inoculated bacteria could also be recovered from the systemic blood. These experiments seem to leave little question that bacteria can utilize the root canal as a pathway of invasion into the circulation. Other investigators have placed non-microbial antigens in the root canal to ascertain whether it can release antigens into the periapical area (Block et al., 1977; Oswald and Cohn, 1975; Block et al., 1978).

Barnes and Langeland (1966) placed sheep erythrocytes and bovine serum albumin (BSA) in the chamber of extirpated monkey teeth. They tested for antibody formation by the Ouchterlony (agar gel diffusion) method. Their results showed that the root canal route of sensitizing was effective in stimulating the formation of precipitating antibodies to BSA. Okada and associates (1967) placed lyophilized horse serum into the maxillary molar pulp chambers of rabbits after pulp extirpation. The antigen was placed repeatedly at intervals of 7 to 10 days. They found that the repeated administration of antigen through the dental pulp canals

caused both local and systemic morphological changes which were of allergic nature. Pirila and Rantanen (1960) report a case of allergic reaction resulting from the use of bacitracin-neomycin applied topically in the root canals. Fox and Moodnik (1964) also report on the systemic reaction to polyantibiotic root canal dressings. These studies indicate that when an antigen is placed in the root canal system it can eggress into the periapical area and can cause systemic sensitization, antibody formation and allergic reactions.

VII Bacterial Status of Periapical Area

Since it has been shown that the root canal system can act as a pathway for microbes to reach the periapical area, several investigators have examined the bacterial status of this area.

Hedman (1951) utilized a cannula and culture wire to obtain bacterial cultures from the periapical area. The teeth sampled were opened, cultured and debrided. The cannula and culture wire were then inserted through the tooth to reach the periapex. His results indicated that 68.5% of the cases had viable bacteria in both the pulp canals and periapical areas before treatment. At that time 8.5% had bacteria in the root canal system but not in the periapical areas. Twenty-three per cent showed no bacteria in either location. However, after cleaning and shaping none of the 56 cases showed bacteria in the periapical area. Hedman concluded that in a large percentage of cases of infected pulp-involved teeth with

periapical pathosis the periapical tissues are infected. However, due to the experimental method utilized in the study, these conclusions have been challenged by other investigators.

Grossman (1959) obtained cultures of the periapical area during root resection. He took four or five cultures during each procedure and incubated them in BHI broth aerobically. Reliable data were available in 104 of 150 cases, and of these 85.3% were negative for bacterial growth.

Shindell (1961) used a similar technique of cannula and culture wire to examine 63 cases. His results showed only 5% of the cases to be positive for bacterial contamination in the periapical area. Shindell therefore concluded that most of the periapical areas are sterile, and that the few cases that contain bacteria are of little statistical significance.

Winkler, Mitchell and Healey (1972) studied the periapical area by using a modified-gram tissue stain. Fifteen teeth were extracted along with the attached periapical lesion. The tissues were stained with hematoxylin and eosin (H and E) as well as the Johns Hopkins modified bacterial tissue stain. These investigators found bacteria dispersed uniformly throughout the lesions in slight to moderate concentrations in most cases. They state that inflammatory cells engulfed or surrounded the bacteria but no determination of the viability of the bacteria was made.

Block and associates (1976) studied 230 biopsy specimens obtained during endodontic surgery. The specimens were fixed and stained with H and E, and Brown and Brenn stains. These

investigators found only one case where bacteria were located in the disintegrating pulp tissue and in the periapical tissue.

Langeland, Block and Grossman (1977) report on a histopathologic and histobacteriologic study of 35 periapical endodontic surgical specimens. These investigators used H and E and Brown and Brenn stains on the fixed tissue specimens. They observed one case where bacteria were in the necrotic tissue of the root canal and in the periapical area. The authors state that "a granuloma is not an area in which bacteria live, but in which they are destroyed."

VIII Formation of Inflammatory Periapical Lesion

Fish (1939) discussed the role of microbes in causing periapical pathology. He stated that the resorption of periapical bone around a pulpless tooth is due to infection, but explained that the infecting bacteria were present in the root canal system. Only the soluble toxins invaded the periapical region causing bone resorption and attraction of lymphocytes and polymorphonuclear leukocytes.

Four zones were recognized by Fish as occurring in an inflammatory lesion of bacterial origin. First there is a zone of infection which in the case of a pulpless tooth is limited to the root canal system and its necrotic debris. At the apical foramen the bacterial toxins are concentrated with polymorphonuclear leukocytes (PMN's) present to remove debris.

Next, in the zone of contamination the toxins become diluted. Fish describes this area microscopically as consisting

of round cells infiltrate with lymphocytes and plasma cells predominating.

Still further from the source the toxins become more dilute, and may act as a mild irritant. In this zone of irritation osteoclasts may be found resorbing the bone. Also active phagocytosis may occur.

In the fourth zone, the toxins are so dilute that they act as a stimulant to osteoblasts and fibroblasts whose function is to build new bone and fibrous tissue. This peripheral area is called the zone of stimulation.

These zones, as described by Fish, are illustrated in Figure 2.

Fish's original concepts have been only slightly modified to this time. Simon (1976) states that periapical inflammation is an extension of the pulpal process and its first stage may be chronic in nature. Then as bacteria and their toxins, and cellular breakdown products reach the apex, PMN's are attracted and an acute inflammatory response ensues. As this response progresses the fluid exerts pressure on the surrounding bone causing resorption.

Seltzer (1971) describes the periapical changes which follow total pulpitis. Resorption of the surrounding bone occurs resulting in widening of the periodontal ligament (PDL) space. The fibroblasts of the apical pulp and of the PDL undergo mitoses resulting in daughter cells. These fibroblasts elaborate ground substance and new reticular and collagen fibers. At the same time the blood vessels begin to show

changes. The endothelial cells undergo mitoses and form a large number of new capillaries. The apical pulp tissue becomes transformed from fibrous to granulomatous tissue with the purpose of attempting to encapsulate and neutralize the irritants of the diseased pulp.

According to Shafer, Hine and Levy (1974) the periapical inflammatory lesion begins with edema and hyperemia of the periodontal ligament with infiltration of chronic inflammatory cells. The inflammation and increased vascularity induces bone resorption with proliferation of both fibroblasts and endothelial cells. They state that connective tissue activity is most prominent on the periphery of the granuloma and that a collagenous capsule separates granulation tissue from bone.

In 1972 Naidorf, Nygaard-Ostby and Schilder recognized the possibility of immunological responses playing a role in the initiation and perpetuation of apical inflammatory lesions. They suggested that this area be investigated further.

Morse (1977) proposed an immunologic mechanism of periapical disease. He stated that since microbes, microbial products and components are capable of acting as antigens, specific antibodies or sensitized T-lymphocytes could then be formed which would react with these antigens. The periapical response that forms could be one or a combination of protective or allergic reactions. Allergic reactions may then be a causative factor in "flare-ups" (pain, swelling, bone resorption).

In a recent article, Torabinejad and Bakland (1978) discussed the immunopathogenesis of chronic periapical lesions.

They state that bacteria, bacterial products and altered host tissue can be antigenic. The reaction to these antigenic stimuli from the root canal system can take two forms: antibody mediated and cell-mediated forms of immunity. The antigen-antibody complex could initiate preliminary changes in the periapical tissue, with the cell-mediated response participating in the progression of periapical disease. The authors suggest that although immune responses are important for localization and destruction of antigenic materials emerging from the root canal system, they may lead to a local destruction of the periapical tissues.

IX Evidence of Immunologic Reactions in Periapical Areas

There is increasing evidence that immunologic reactions are factors in the pathogenesis of periapical lesions. Morse, Lasater and White (1975) examined sections from periapical lesions using pyronin-methyl green staining. This stain shows the presence of messenger RNA granules in the cytoplasm of protein-producing plasma cells. Since the only proteins plasma cells produce are antibodies, the significance of staining granules is that antibody production is occurring. In this study, the authors found a 62% incidence of extensive production of pyroninophilic cells in sections diagnosed as periapical cyst compared to a 30% incidence of extensive production of these cells in those lesions diagnosed as granulomas. They conclude that serum antibodies or immunoglobulins are produced in periapical cysts and granulomas.

Naidorf (1975) presented a method for the isolation and identification of immunoglobulins in periapical granulomas. Two lesions examined contained lymphocytes and plasma cells. These were diagnosed as granulomas and were found to have produced immunoglobulins of the IgA, IgG and IgM categories. A periapical scar showed no such activity. Kuntz (1977) and associates examined the localization of immunoglobulins and complement components in periapical lesions. They observed IgG, IgA, and IgM extracellularly as well as in plasma cells. One half of the lesions showed C3 stained small vessel-like structures. The authors suggest that reactions involving complement (C3) play a role in some periapical lesions.

Another recent study by Pulver, Taubman and Smith (1978) examined periapical lesions for immunoglobulins. Using an immunofluorescent technique they found that in periapical granulomas IgG, IgA, IgM, and IgE represented 70, 14, 4 and 10 per cent, respectively, of the immunoglobulin-containing cells observed. In radicular cyst specimens, IgG and IgA each represented 45% and IgM and IgE each represented 5% of the immunoglobulin-containing cells. Thus the authors concluded that periapical tissues contain the components necessary for host immunopathologic responses.

Morton, Clagett and Yavorsky (1977) examined periapical tissue specimens for the presence of antigen-antibody or immune complexes. Through use of immunofluorescence microscopy they found such complexes in one specimen out of 25 examined. In a recent study, however, Torabinejad and Kettering (1978)

employed an anti-complement immunoflourescent (ACIF) test to determine the presence of immune complexes. Since antigen-antibody complexes can bind complement, the presence of C3 in this test indicates the existence of immune complexes at the sites of fluorescence. In this study 23 of the 25 periapical specimens were positive for C3. No staining was noted in the two specimens diagnosed as scars. The authors state that these findings strongly indicate that soluble immune complexes are present in periapical lesions.

The effects of such antigen-antibody complexes in periapical lesions may partially explain how these lesions evolve and enlarge. Simulated immune complexes (heat aggretated IgG) were injected via the root canal into the periapical tissues of cat maxillary cuspid teeth by Torabinejad, Clagett and Engel (1979). They found radiographic and histologic evidence of bone resorption periapically within seven days. The bone loss was accompanied by severe inflammation of the surrounding connective tissue. The investigators state that these findings provide direct evidence that immune complexes may induce bone resorption in vivo. In similar experimental work done by Torabinejad and Kiger (1978), cats were immunized with subcutaneous injections of a known antigen. After determining that circulating antibodies to the antigen had developed, challenge doses of the antigen were administered via the root canal system. The radiographic and histologic evidence suggests that antigen-antibody complex reactions can occur in periapical tissue and that they can play a role in the pathogenesis of periapical lesions.

MATERIALS AND METHOD

I Materials

Animals. Other experiments have indicated that the cat (Felis catus) is a suitable experimental animal for the study of immunopathogenesis of periapical lesions (Torabinejad and Bakland, 1978). Six cats, each weighing 2.5 to 3.5 kg, were obtained from a commercial source, housed in individual cages, and maintained on Purina cat chow and water ad libitum.

Lipopolysaccharides (LPS). The bacterial lipopolysaccharides used in this study were extracted from Escherichia coli 0111:B4 and were supplied in a lyophilized form. Subsequently this LPS was dissolved in sterile saline to a concentration of 100 µg/ml. This solution was diluted to make 10 µg/ml and 1 µg/ml concentrations of endotoxin.

Irrigation saline. The saline (sterile sodium chloride irrigation USP, McGaw Laboratories) used as a control solution and to dissolve the lyophilized LPS was obtained from a commercial source.

II Method

Each cat was anesthetized with an intramuscular injection of Ketamine hydrochloride (Bristol Laboratories) 20 mg/lb, combined with Xylazine (Haver-Lockhart Laboratories) 1 mg/lb. Pre-operative radiographs of maxillary and mandibular cuspids were taken. Using an aseptic technique and rubber dam, the incisal portion of the teeth was removed exposing the pulp.

With successively larger Hedstrom files, the access opening was enlarged to the extent that a medium-sized broach could be inserted easily into the root canal. The pulp was extirpated with the broach, and the canal was irrigated with sterile saline and dried with paper points. No filing was done to enlarge the canal. Using a 1 ml sterile disposable syringe and a 1.25 inch 23 gauge needle approximately 0.1 ml of endotoxin or saline was injected into the root canals of both maxillary and both mandibular cuspids of the cats. The needle was inserted until it was binding inside the canal, but the needle never penetrated apically beyond the middle of the canal. The openings to the root canals were sealed with Intermediate Restorative Material (IRM, Caulk). Figures 3 through 9 illustrate the operative technique.

Two cats were sacrificed after two weeks and the remaining cats were retreated following the same procedure as described previously. After four weeks two additional cats were sacrificed and the remaining cats again retreated. At the end of six weeks the final two cats were sacrificed. Table 3 summarizes the treatment sequence for all the animals.

The specimens were examined radiographically to determine the presence and size of the periapical lesions, and histologically to describe the periapical pathology. Block sections containing the maxillary or mandibular cuspids and the surrounding tissues were removed and radiographed. The specimens were placed in 10% buffered formalin, decalcified in 20% formic acid, dehydrated and embedded in paraffin. Sections at six

microns were prepared, and stained with hematoxylin and eosin for histopathologic examination. The sections were submitted to and read by a veterinary pathologist. The sections were examined for the type of cellular inflammatory infiltrate, the degree of inflammation and the presence of osteoclastic activity. The histologic observations were used in a descriptive manner and no statistical comparisons were made between treatment groups.

The radiographs for each cat were placed in a projector-viewer and a clear plastic sheet was placed over the viewer screen. On this clear sheet the outline of the radiographic lesion was traced independently by two observers on different days. A clear plastic grid containing 3.5 mm squares was then placed over the drawing, and the grid intersections inside the representations of the lesions were counted. This data was averaged, resulting in a figure which allowed ranking of the radiolucent areas according to size for each cat. Rank one indicated the smallest radiolucent area, with the ranking increasing up to four for the largest. When there was less than two intersections difference between two specimens the rank was averaged.

The Friedman test and the Wilcoxon test were used to analyze the ordinal data obtained through the ranking procedure. By using these tests, statistical differences between treatments could be determined.

RESULTS

I Radiographic Findings

Relatively large apical radiolucencies were noted in the teeth injected with endotoxin when examined after two weeks, and these changes continued to be present in the specimens examined after four and six weeks. The radiographic interpretation of the specimens showed that all of the teeth in which saline was injected had no apical radiolucency or a smaller radiolucent area compared to the other teeth in the same cat. In three of the six cats the teeth in which 100 µg/ml Et solution was placed showed the largest radiolucency of the four teeth.

Figures 10, 11 and 12 are radiographs of typical specimens, and a summary of the radiographic observations is presented in Table 8.

II Statistical Results

The Friedman test was performed on the ranked data to determine differences between the reaction to treatments of each cat. The null hypothesis (Ho) is, in this case, that each ranking of the random variables within a block is equally likely to occur (i.e., the treatments have identical effects). The alternate hypothesis (Ha) is that at least one of the treatments tends to yield larger observed values than at least one other treatment.

When the calculations are carried out the null hypothesis is rejected with $P < 0.025$. The results of the Friedman test

indicate that there is a tendency for one treatment to produce larger apical radiolucencies than the others.

The Wilcoxon test is used to analyze differences between two types of treatments using differences in lesion size. These differences are determined for each animal and the difference is ranked. In this test, saline was compared to each of the three endotoxin solutions, and the endotoxin solutions compared to each other. The results of the Wilcoxon test, listed in Table 9, indicate that there are significant differences (P=0.01) between the effects of the treatments when comparing saline to all three endotoxin solutions. However, there are no significant differences in effects when comparing the different endotoxin treatments.

III Histologic Observations

To determine the histological characteristics of the radiographically apparent lesions, the sections taken at two, four, and six weeks were examined microscopically.

In general, the cellular inflammatory infiltrate consisted primarily of polymorphonuclear leukocytes, although some macrophages, plasma cells and lymphocytes were also present. Multinucleated cells having the morphologic appearance of osteoclasts were frequently seen at the periphery of the surrounding bone and areas of bone resorption were associated with these cells. Figures 13, 14 and 15 represent the specimen histology. The histologic observations are described in Tables 4, 5 and 6 and summarized in Table 7.

A relatively consistent histologic pattern developed in

the periapical tissues in the teeth treated with the endotoxin solutions, when compared to the teeth injected with saline. The degree of inflammation was generally more intense (more inflammatory cells per unit area) in the endotoxin-treated teeth and this group demonstrated more osteoclastic activity.

DISCUSSION

Endotoxin derived from Escherichia coli was selected for use of this investigation even though the coliform bacilli are not principle constituents of the oral flora in man. Mergenhagen (1967) states that the lipopolysaccharide endotoxins of oral bacteria compare favorably in toxicity tests with endotoxins derived from E. coli. Three concentrations of the E. coli endotoxin were prepared for intracanal administration to provide information regarding the effective range of endotoxin.

The experimental design of this project allows for comparison of the effects of the endotoxin and saline treatments, recognizing and allowing for variation between cats and for varying lengths of time for treatment. For each cat the only difference in the experiment was the type of solution placed in the four teeth. The biologic variation between cats and the differing time periods are not variable factors for one cat. Therefore the effects of the treatments can be compared and ranked for each cat.

There are variables which could affect the experiment other than the effects of the instrumentation and the injection of saline or endotoxin. The increasing number of treatments performed on the cats increased the chance for contamination of the root canal during the procedures. Therefore, it may be that the specimens from cats A and B taken after one treatment and the specimens from cats C and D removed after two treatments

may be more reliable than those from cats E and F which were treated three times.

Another variable is the presence of an apical remnant of pulp tissue in the teeth of some of the cats. Histologic examination in cat B and in cat D revealed that pulp extirpation was incomplete. It is uncertain therefore that the injected endotoxin diffused through the tissue into the periapical area. It would seem that the apical pulp stump would have allowed less endotoxin into the periapex and possibly diminished its effect in these two specimens.

In analyzing the raw data used for ranking the radiographic lesions, it appears that the procedure used was quite consistent and repeatable. In only one specimen cat C UR was there a marked difference in radiographic interpretation of the lesion boundaries. In the other twenty-three specimens there was a fairly close agreement between examiners in interpretation. In the case where there was disagreement, it did not affect the ranking. Although the lesion size data was averaged, if each observer's data was ranked independently, the order would not change for the specimens.

When the ranking procedure was carried out and the calculations for the Friedman test completed, it was determined that the effects of the treatments were not equal. One can conclude that at least one of the treatments yielded larger apical radiolucencies than at least one other treatment. In order to determine differences between two treatments the Wilcoxon test was utilized. As the results indicate, there was a significant

difference between the effects of the endotoxin solutions compared to saline. The effect of the endotoxin solutions on the periapical tissues, when measured by determining lesion size, appears to be greater than that of saline.

This observation also appears to be true when one examines the histologic findings. The teeth injected with saline tend to have milder inflammatory reactions periapically when compared to those injected with endotoxin.

There are three basic mechanisms which could support the role of endotoxin in producing periapical lesions. First, the biologic properties of endotoxin make it a potent inflammatory agent, and when introduced locally into a number of species, including man, they induce a dramatic acute inflammatory response (Snyderman, 1973). Simon, et al. (1970) found a statistically significant correlation between the quantity of endotoxin in gingival exudate and the clinical degree of gingival inflammation. Mergenhagen (1970) states that intradermal injection of small amounts of endotoxin leads to the rapid accumulation of PMN's. Also, the interaction of endotoxin and serum results in the degranulation of mast cells which are sources of histamine and heparin (Hook, Snyderman and Mergenhagen, 1970). Histamine is an inflammatory agent which can mediate inflammatory changes in tissues. Norton, Proffit and Moore (1970) also showed that histamine is capable of inhibiting rat bone growth <u>in vitro</u> and that a combination of endotoxin plus histamine synergistically increased the inhibition of bone growth.

In addition to being an inflammatory agent, endotoxin has biologic properties that produce bone resorption. Hausman, Weinfield and Miller (1972) demonstrated that the LPS of bacterial endotoxin stimulated osteoclastic activity in tissue cultures of fetal rat bones. They also state that since Fusobacterium, Veillonella and Bacteroides are prevalent in dental plaque, bacterial LPS may be implicated in bone resorption in periodontal disease. It has also been demonstrated that as little as 1 µg/ml of endotoxin from an oral Bacteroides caused significant bone resorption of fetal rat bones in tissue culture (Hausman, Raist and Miller, 1970). The authors report that morphologically endotoxin caused a proliferation of osteoclasts and removal of matrix. Rizzo and Mergenhagen (1964) inoculated the palatal gingiva of rabbits with endotoxin from an oral Veillonella. A single injection of 50 µg of this endotoxin produced an acute inflammatory lesion accompanied by bone resorption. The inflammatory and biologic properties of endotoxin could therefore be one mechanism in periapical inflammatory lesions.

A second possible mechanism for the production of periapical pathology would be the activation of complement by endotoxin. Morrison and Kline (1977) have demonstrated the activation of the classical and alternate pathways of complement by bacterial LPS. They state that the lipid A region of the LPS is responsible for classical pathway activation and the polysaccharide region is responsible for properdin (alternate) pathway activation. Classical pathway activation by lipid A does not depend upon

antibody to the lipid A and properdin pathway activation proceeds by a lipid A-independent mechanism.

Fine (1974) also showed that E. coli endotoxin can activate both pathways. Frank, May and Kane (1973) state that endotoxins are considered potent activators of the alternate complement pathway, which can opsonize bacteria and participate in bactericidal reactions. C3, a component of complement activated in the alternative pathway, has been isolated in human periapical lesions (Kuntz, et. al., 1977). When the complement components are activated and cleaved by endotoxin, biologically-active peptides are released which mediate a number of inflammatory reactions including vascular permeability, chemotatic attraction of PMN's and macrophages, degranulation of mast cells and cell lysis (Snyderman, 1973). Complement activation by endotoxin results in release of biologic fragments which may result in inflammatory changes and possibly bone resorption in the periapical region.

The third mechanism would also involve the activation of the complement system, but through the formation of antigen-antibody complexes as activating agents. Rudbach (1976) described endotoxins as "super-antigens" in that nanogram amounts sensitized animals for specific secondary responses. Ball and Trott (1971) found that intragingival injections of 1.0 µg of E. coli endotoxin in rabbits could produce a significant antibody response in three days. Specific serum antibody titers have been produced in monkeys by introducing antigens through the root canals. Dahlen and Fabricus (1977)

experimentally infected monkey root canal systems with a gram negative oral bacteria and demonstrated serum antibodies to LPS extracted from the same animal six months later. In the human, serum antibodies to gram negative oral bacteria have been demonstrated (Evans, et. al., 1966).

Landy (1970) stated that the persisting IgM antibody response to stimulation by endotoxin has been noted in all animal species examined. IgM, in addition to IgG and IgA, has been demonstrated in periapical lesions. The binding of endotoxin to these immunoglobulins would result in the formation of antigen-antibody complexes capable of activating complement and generating mediators of inflammation and tissue destruction. Biologically active peptides are released when certain complement components are cleaved. C5A or anaphylatoxin is released from C5 when activated by endotoxin or an antigen-antibody complex and C5A mediates a number of important events in the inflammatory process. These include enhancement of vascular permeability and the chemotactic attraction of PMN's and macrophages.

Mergenhagen (1967) states that endotoxins from gram negative bacteria could have considerable significance in the host-parasite relationship in oral disease because of the array of physiological and immunological effects they have on the host. From the results of this study, these characteristics of endotoxin appear to have induced inflammatory changes and bone resorption in the periapical area of cat teeth. However it is impossible to state that a particular mechanism or characteristic of endotoxin is responsible for the observed effects on the periapical tissues.

The experimental observations may be significant when applied to the human, since endotoxins have been isolated in human endodontically involved teeth. The concentration of endotoxin reported in human necrotic symptomatic teeth was approximately 1 µg/ml. In this study 1 µg/ml of endotoxin appeared to have induced greater periapical pathology in the cat than did saline. This result provides indirect evidence that endotoxins may be factors in initiating and perpetuating periapical disease in the human.

SUMMARY

The cat animal model was utilized in this study to determine the effects of endotoxin on the periapical tissues. Radiographic and histologic observations were used in determining the effects of three concentrations of endotoxin and a saline control solution. The results indicate that bacterial endotoxin placed in the root canal system of the cat caused greater pathologic periapical changes than did saline. The results support the conclusion that bacterial endotoxin may play a role in the production of periapical inflammatory lesions.

TABLE 1

FLORA OF THE ROOT CANAL SYSTEM

	Henrici Hartzell 1919	Grossman Christian 1952	Mazzarella Hedman, Brown 1955	Brown Rudolph 1957	Winkler Van Amerongen 1959
Streptococci (all)	65.2	51.8	27.8	28.1	62.9
Micrococci (Staphylococci)	19.6	16.6	14.4	16.7	16.3
Lactobacilli	-	0.3	-	-	6.9
Corynebacteria (Diptheroids)	6.5	0.5	22.2	24.6	4.0
Gram negative Rods*	4.3	5.5	6.7	8.8	2.0
Yeasts	4.3	16.9	3.3		1.6
Spriochetes	-	-	8.9	12.3	-
Bacilli (+)	-	6.7	-	-	1.4
Actinomyces	-		-	-	1.4
Gram positive Rods	-	1.4	4.4	-	1.4
Neisseria (-)	-	0.2	3.3	2.6	1.0
Unidentified	-	-	7.7	6.1	-
TOTAL	99.9	99.9	98.7	99.2	98.9

*Bacteroides, Fusobacterium, Aerobacter, Pseudomonas, Coliforms

TABLE 2

CONCENTRATION OF ENDOTOXIN IN SAMPLED TEETH

Group	# of Samples	Mean µg Et/ml	Range
Vital, Asymptomatic	10	0.007	0.000-0.032
Vital, Symptomatic	10	0.075	0.001-0.256
Non-vital, Asymptomatic	10	0.192	0.064-0.512
Non-vital, Symptomatic	10	1.070	0.256-2.048

Et = Endotoxin

TABLE 3

TREATMENT SEQUENCE FOR EXPERIMENTAL ANIMALS

A and B	1. Initial instrumentation, solutions injected. 2. After two weeks, animals sacrificed, specimens removed.
C and D	1. Initial instrumentation, solutions injected. 2. After two weeks, teeth re-injected with solutions. 3. After four weeks, animals sacrificed, specimens removed.
E and F	1. Initial instrumentation, solutions injected. 2. After two weeks, teeth re-injected with solutions. 3. After four weeks, teeth re-injected with solutions. 4. After six weeks, animals sacrificed, specimens removed.

TABLE 4

HISTOLOGY OF TWO-WEEK SPECIMENS

Cat	Tooth	Treatment	Histologic Observations
A	LL	Saline	Small area of moderate inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	LR	1 µg/ml Et	Small area of mild inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	UR	10 µg/ml Et	Small area of moderate inflammatory cell infiltrate consisting primarily of PMN's. Some osteoclastic activity noted.
	UL	100 µg/ml Et	Large area of intense inflammatory cell infiltrate consisting primarily of PMN's. Osteoclastic and osteoblastic activity.
B	UL	Saline	PDL intact. No evidence of inflammatory cells present.
	LL	1 µg/ml Et	PDL intact, pulp tissue present in apical area. Inflammatory cell infiltrate present near coronal surface of pulp remnant.
	LR	10 µg/ml Et	Small area of intense inflammatory cell infiltrate consisting primarily of PMN's. Inflammatory cells invading root canal. Osteoclastic and osteoblastic activity noted with necrotic bone spicules present.
	UR	100 µg/ml Et	Large area of intense inflammatory cell infiltrate consisting primarily of PMN's. Lesion extends proximally to PDL. Osteoclastic and osteoblastic activity noted with necrotic bone spicules present.

TABLE 5

HISTOLOGY OF FOUR-WEEK SPECIMENS

Cat	Tooth	Treatment	Histologic Observations
C	UL	Saline	Small area of minimal inflammation. No osteoblastic or osteoclastic activity noted.
	LL	1 µg/ml Et	Small area of mild inflammation consisting of PMN's, plasma cells and lymphocytes. Occasional osteoclasts noted.
	LR	10 µg/ml Et	(Section cut tangential to apex.) Localized inflammatory response consisting primarily of PMN's with some macrophages, plasma cells and lymphocytes. Necrotic bone spicules present with osteoclastic activity noted.
	UR	100 µg/ml Et	Large area of intense inflammatory cell infiltrate consisting primarily of PMN's. Increased osteoblastic and osteoclastic activity noted with necrotic bone spicules present.
D	LL	Saline	Normal pulp tissue present at apex. No specific evidence of inflammation in periapical tissues. No osteoblastic or osteoclastic activity.
	LR	1 µg/ml Et	Small area of mild inflammation. Minimal osteoblastic and osteoclastic activity present.
	UR	10 µg/ml Et	No specific evidence of inflammation in periapical tissues. No osteoblastic or osteoclastic activity.
	UL	100 µg/ml Et	Marked inflammation of pulpal tissue remnant in root canal. Minimal inflammation in periapical tissue. No osteoblastic or osteoclastic activity.

TABLE 6

HISTOLOGY OF SIX-WEEK SPECIMENS

Cat	Tooth	Treatment	Histologic Observations
E	UR	Saline	Small area of moderate inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	UL	1 µg/ml Et	Small area of intense inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	LL	10 µg/ml Et	Large area of intense inflammatory cell infiltrate consisting primarily of PMN's Osteoclastic activity noted with necrotic bone spicules present.
	LR	100 µg/ml Et	Large area of intense inflammatory cell infiltrate consisting primarily of PMN's. Osteoclastic activity noted with necrotic bone spicules present.
F	LR	Saline	PDL disrupted apically. No evidence of inflammatory cells present. No osteoclastic activity noted.
	UR	1 µg/ml Et	Small area of intense inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	UL	10 µg/ml Et	Small area of intense inflammatory cell infiltrate consisting primarily of PMN's. No osteoclastic activity noted.
	LL	100 µg/ml Et	(Section cut tangentially to apex.)

TABLE 7

SUMMARY OF HISTOLOGIC FINDINGS

Cat	Tooth	Treatment	Histologic Response	Presence of Osteoclasts
A	LL	Saline	++	0
	LR	1 µg/ml Et	+	0
	UR	10 µg/ml Et	++	+
	UL	100 µg/ml Et	+++	+
B	UL	Saline	0	0
	LL	1 µg/ml Et	0	0
	LR	10 µg/ml Et	+++	+
	UR	100 µg/ml Et	+++	+
C	UL	Saline	+	0
	LL	1 µg/ml Et	+	+
	LR	10 µg/ml Et	unavailable	
	UR	100 µg/ml Et	+++	+
D	LL	Saline	0	0
	LR	1 µg/ml Et	+	+
	UR	10 µg/ml Et	0	0
	UL	100 µg/ml Et	+	0
E	UR	Saline	++	0
	UL	1 µg/ml Et	+++	0
	LL	10 µg/ml Et	+++	+
	LR	100 µg/ml Et	+++	+
F	LR	Saline	0	0
	UR	1 µg/ml Et	+++	0
	UL	10 µg/ml Et	+++	0
	LL	100 µg/ml Et	unavailable	

0 = none
+ = mild
++ = moderate
+++ = severe

0 = absence
+ = presence

TABLE 8

RADIOGRAPHIC FINDINGS AND RANKING RESULTS

Cat	Length of Time	Tooth	Treatment	Lesion Size	Rank
A	2 weeks	LL	Saline	15,21 = 18	1
		LR	1 µg/ml Et	17,24 = 20.5	2
		UR	10 µg/ml Et	22,25 = 23.5	3
		UL	100 µg/ml Et	35,38 = 36.5	4
B	2 weeks	UL	Saline	0,1 = 0.5	1
		LL	1 µg/ml Et	6,6 = 6	2
		LR	10 µg/ml Et	12,9 = 10.5	3
		UR	100 µg/ml Et	39,34 = 36.5	4
C	4 weeks	UL	Saline	0,3 = 1.5	1.5
		LL	1 µg/ml Et	0,5 = 2.5	1.5
		LR	10 µg/ml Et	16,19 = 17.5	3
		UR	100 µg/ml Et	17,44 = 30.5	4
D	4 weeks	LL	Saline	0,0 = 0	1
		UR	10 µg/ml Et	0,8 = 4	2
		UL	100 µg/ml Et	5,15 = 10	3
		LR	1 µg/ml Et	8,21 = 14.5	4
E	6 weeks	UR	Saline	22,21 = 21.5	1
		UL	1 µg/ml Et	38,40 = 39	2
		LR	100 µg/ml Et	82,92 = 87	3
		LL	10 µg/ml Et	103,113 = 108	4
F	6 weeks	LR	Saline	7,7 = 7	1.5
		LL	100 µg/ml Et	8,7 = 7.5	1.5
		UR	1 µg/ml Et	21,20 = 20.5	3
		UL	10 µg/ml Et	36,27 = 31.5	4

x,x = average

TABLE 9

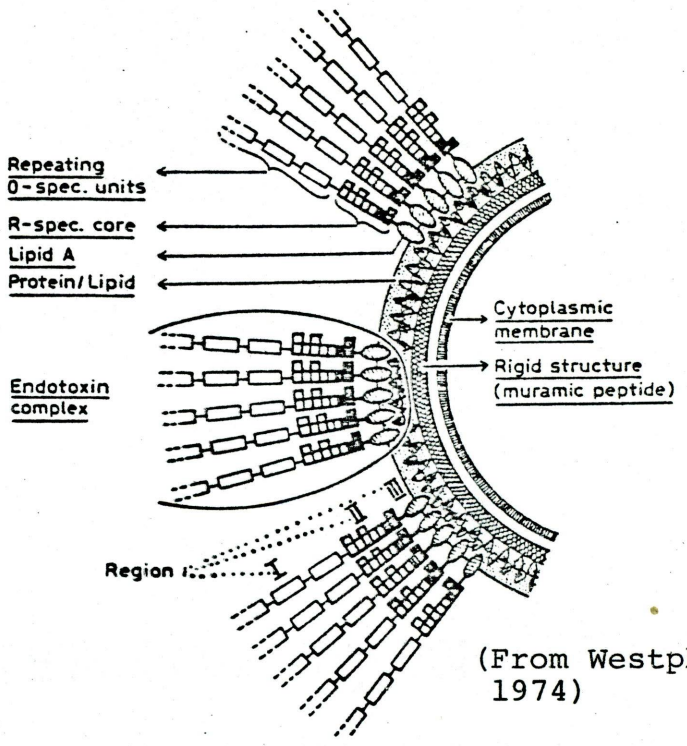
STATISTICAL RESULTS OF TREATMENT COMPARISON

Treatments	P Value
Saline - 1 µg/ml Et	0.01
Saline - 10 µg/ml Et	0.01
Saline - 100 µg/ml Et	0.01
1 ug/ml Et - 10 µg/ml Et	N.S.
1 ug/ml Et - 100 µg/ml Et	N.S.
10 ug/ml Et - 100 µg/ml Et	N.S.

N.S. = not significant

FIGURE 1

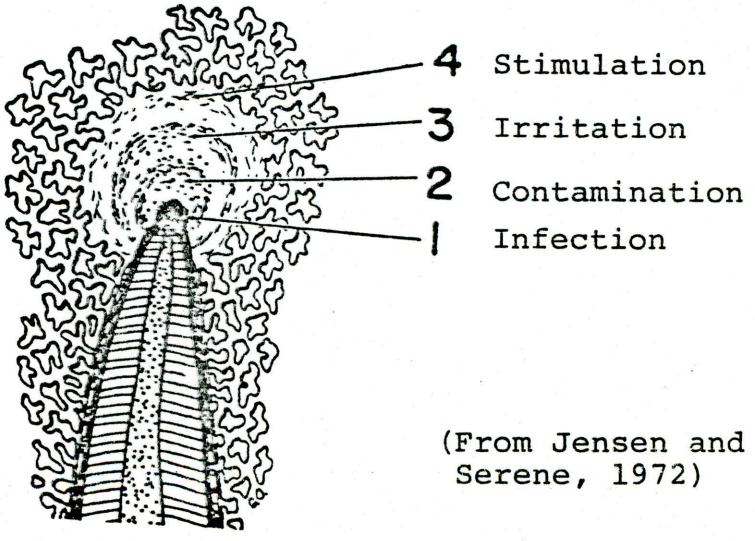
STRUCTURE OF ENDOTOXIN COMPLEX



(From Westphal, 1974)

FIGURE 2

ZONES OF AN INFLAMMATORY LESION



(From Jensen and Serene, 1972)

FIGURE 3

MAXILLARY PRE-OPERATIVE RADIOGRAPHIC TECHNIQUE

FIGURE 4

MANDIBULAR PRE-OPERATIVE RADIOGRAPHIC TECHNIQUE

FIGURE 5

MAXILLARY PRE-OPERATIVE RADIOGRAPH

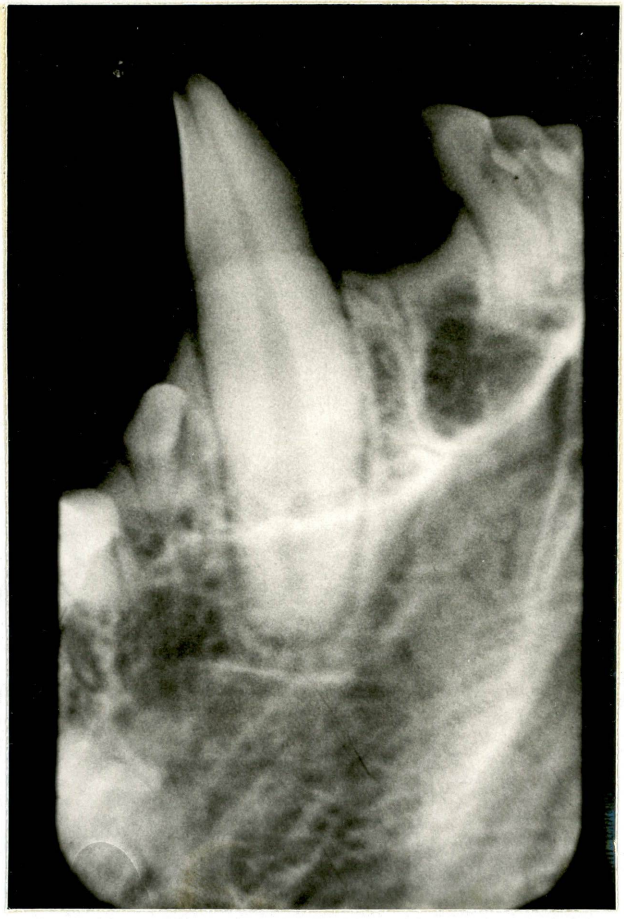


FIGURE 6

MANDIBULAR PRE-OPERATIVE RADIOGRAPH

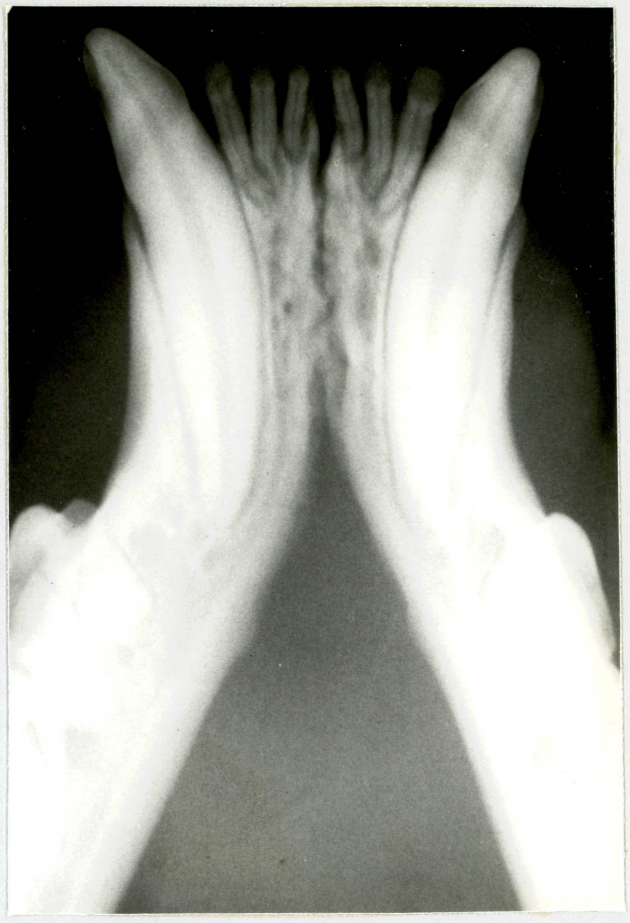


FIGURE 7

ISOLATION OF TOOTH WITH RUBBER DAM

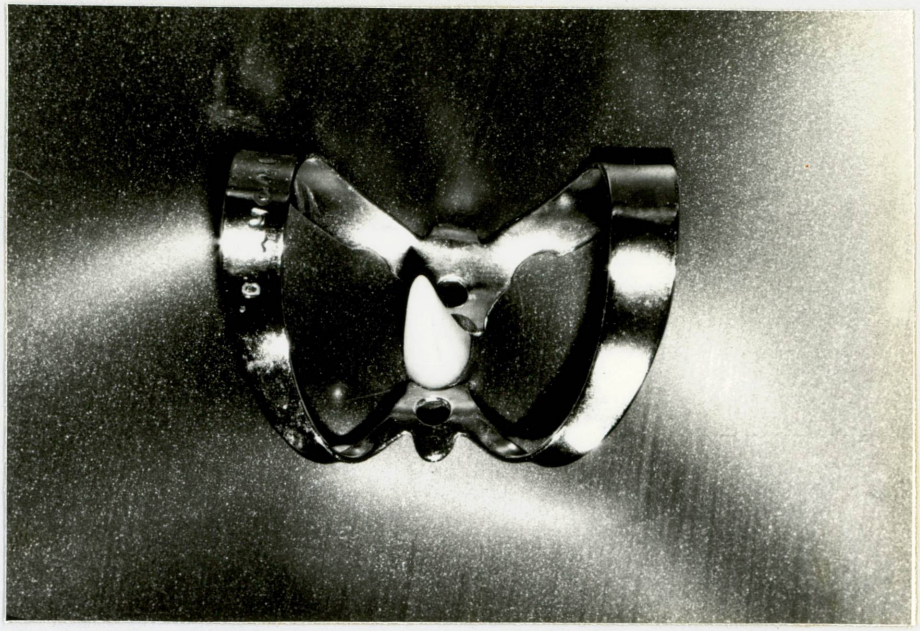


FIGURE 8

EXTIRPATION OF PULP WITH BARBED BROACH

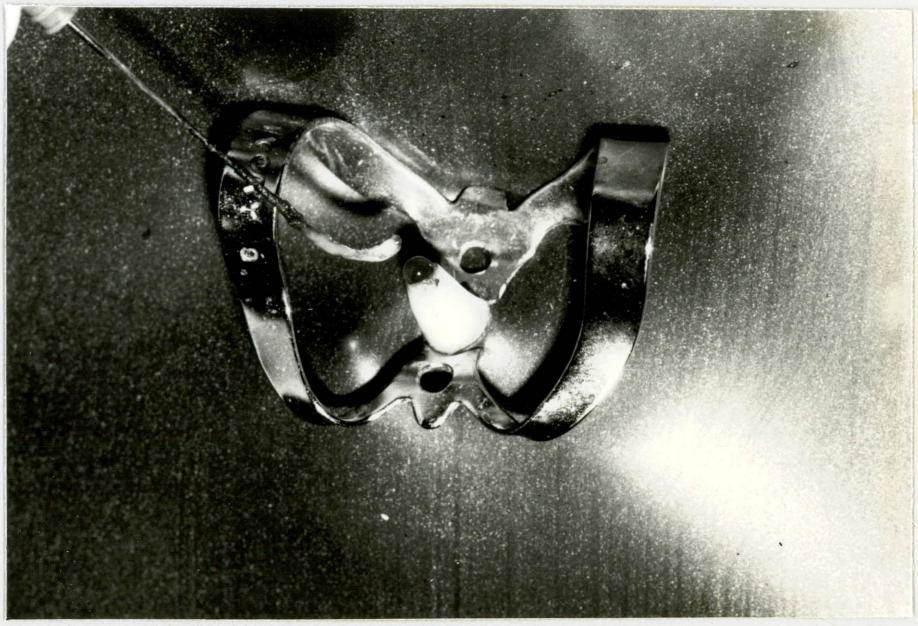


FIGURE 9

INJECTION OF SOLUTION

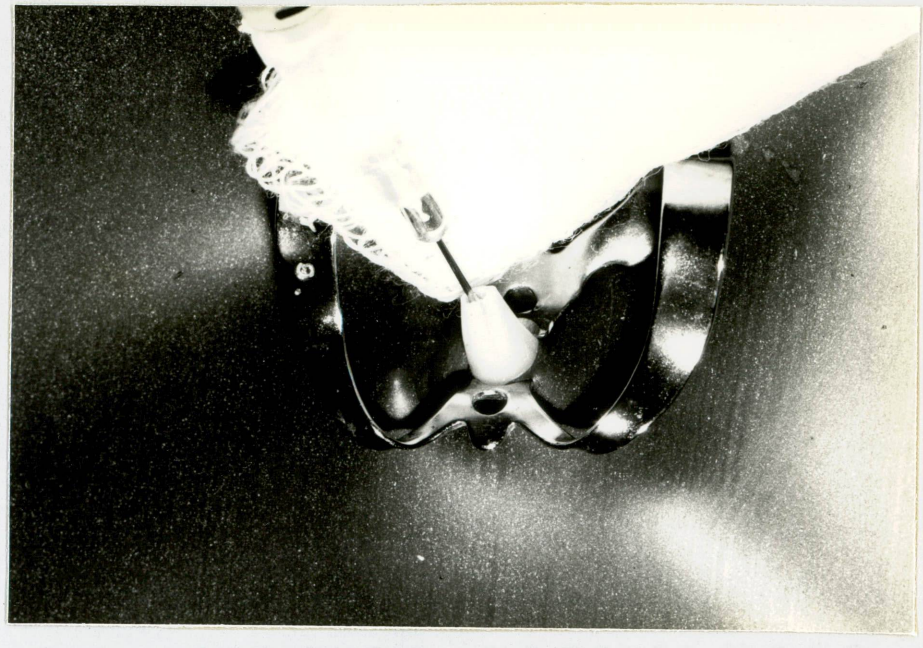
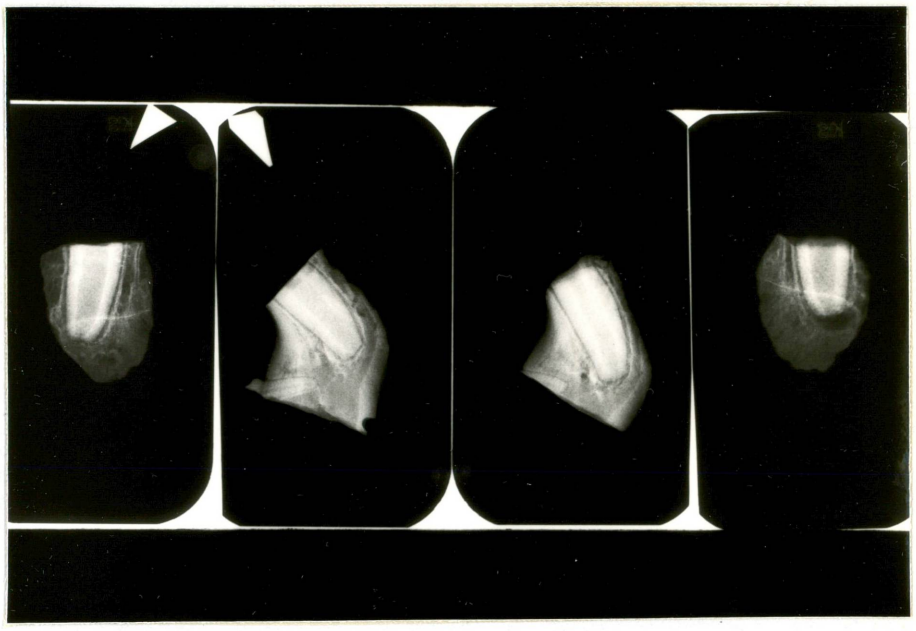


FIGURE 10

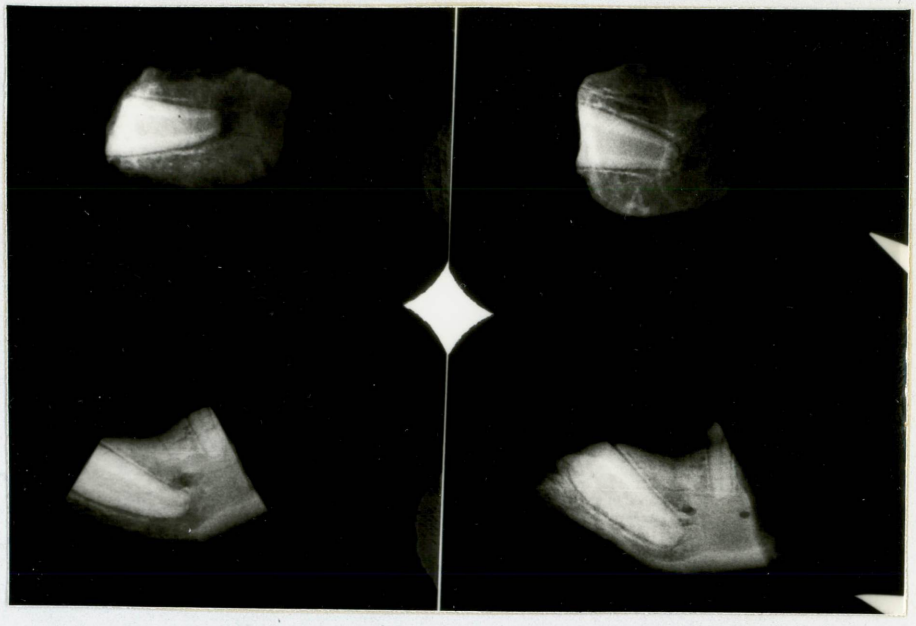
RADIOGRAPHS OF TWO-WEEK SPECIMENS (CAT B)



Left to right; UL Saline, LL 1 µg/ml Et,
LR 10 µg/ml Et, UR 100 µg/ml Et

FIGURE 11

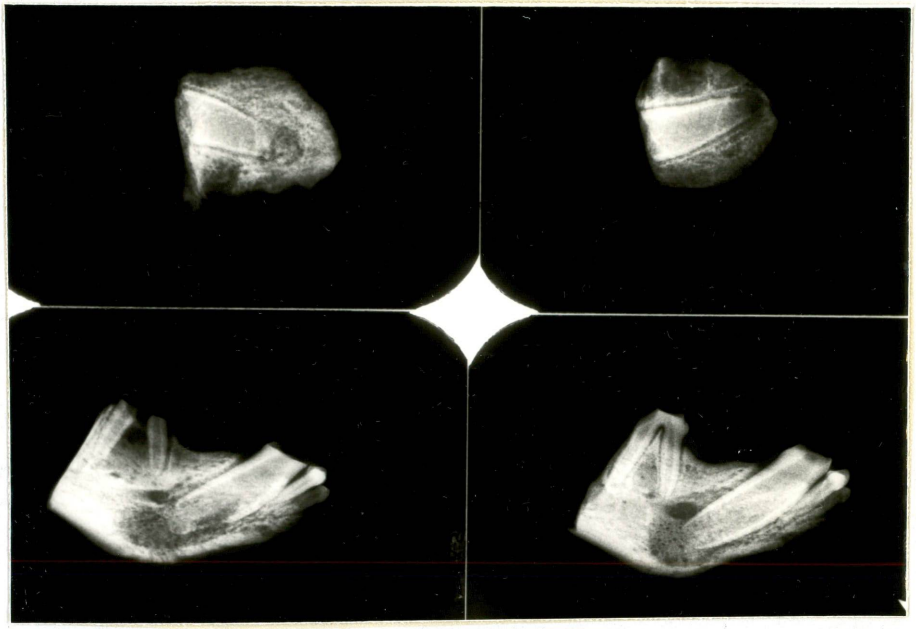
RADIOGRAPHS OF FOUR-WEEK SPECIMENS (CAT C)



Clockwise from upper left; UR 100 µg/ml Et,
UL Saline, LL 1 µg/ml Et, LR 10 µg/ml Et

FIGURE 12

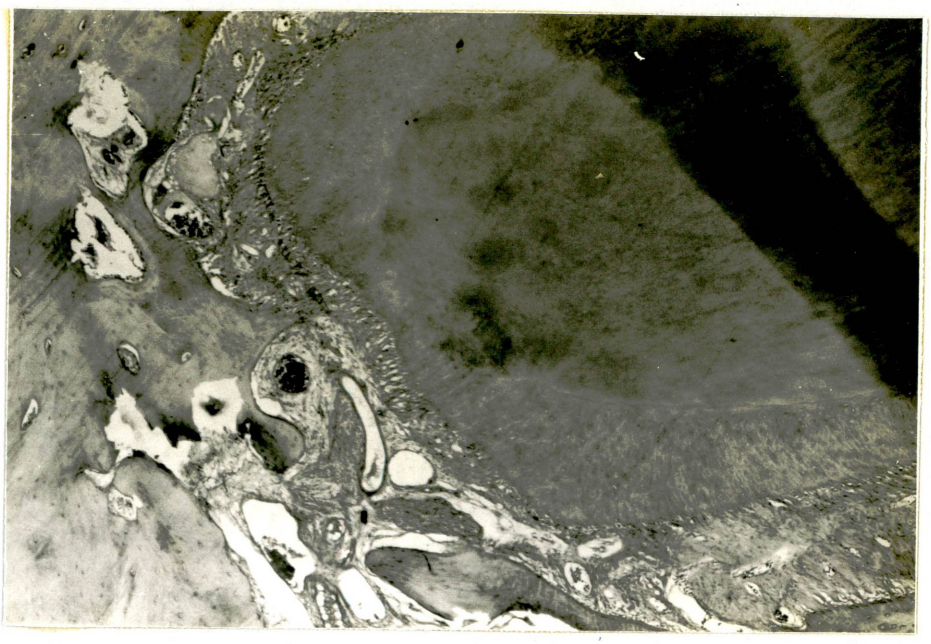
RADIOGRAPHS OF SIX-WEEK SPECIMENS (CAT E)



Clockwise from upper left; UL 1 µg/ml Et,
UR Saline, LL 10 µg/ml Et, LR 100 µg/ml Et

FIGURE 13

HISTOLOGY OF SALINE-TREATED SPECIMEN



H & E Stain, 40x

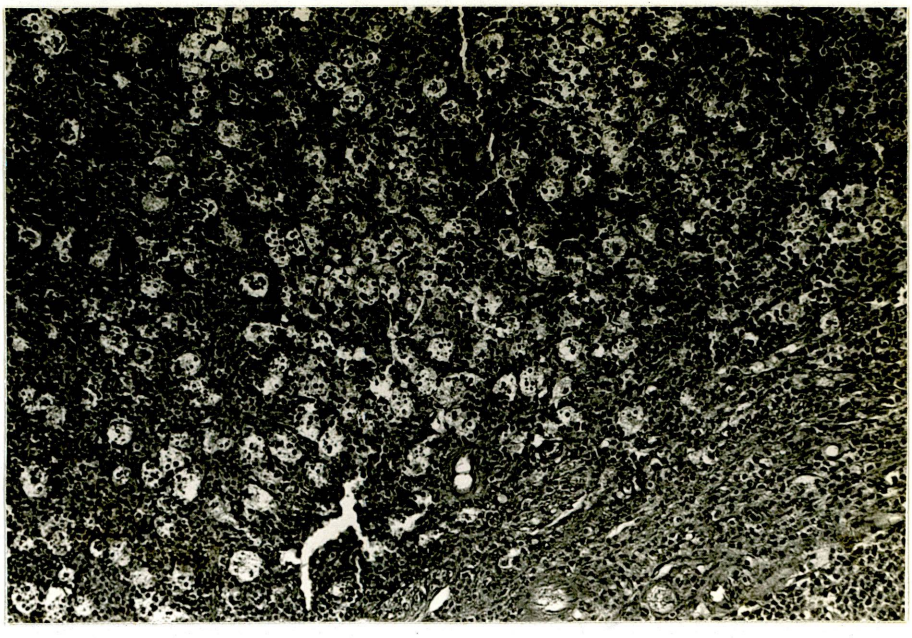
FIGURE 14

HISTOLOGY OF ENDOTOXIN-TREATED SPECIMEN

H & E Stain, 40x

FIGURE 15

HISTOLOGY OF ENDOTOXIN-TREATED SPECIMEN



H & E Stain, 250x

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