PULPAL AND PERIODONTAL TISSUE EFFECTS OF THE PERIODONTAL LIGAMENT INJECTION

An Experimental Histologic Study

by

James O. Roahen, D.D.S.

A Thesis

Presented to the Department of Endodontics
and the Graduate Education Committee of the
Oregon Health Sciences University
School of Dentistry
in partial fulfillment
of the requirement for the degree of
Master of Science
September 29, 1983

APPROVED

[redacted]

F. James Marshall, D.M.D., M.S.
Professor and Chairman
Department of Endodontology

[redacted]

Robert Quinton-Cox, Ph.D.
Chairman
Advanced Education Committee

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ACKNOWLEDGMENTS

The completion of this thesis would have been impossible without the assistance, support and encouragement of numerous individuals.

To my wife, Kathy. Through the endless hours - days - weeks - months during which I was cloistered away in the lab or den, you endured with patience, understanding, and love. I give you a lifetime of thanks.

To Dr. F. James Marshall - mentor, counselor, and confidant. A gifted scholar with the rare ability to educate without intimidation. Although your pen (red) was indeed mightier than the sword, your skill at the helm of my education was masterful.

To Dr. Robert Quinton-Cox, a skilled anatomist and scholar. I will always remember your eloquence, even when discussing garden slugs. No cream in the tea, though. . .thanks.

To Dr. Donald Adams. Yes, Virginia, there is a periodontium. . .and nobody knows it better than Dr. A. You expanded my knowledge and broadened my skills.

To Mr. Dick Tacke and his staff. You made my first animal research project instructive and rewarding.

To Fran Miller, Ethel Hauser, Barbara Holcomb, Flo Ellison, Mary Davis and Ann Colvin. Fran - your technical assistance in preparing the histologic specimens was great and greatly

appreciated. Ethel and Barbara - for being so accomodating on those last minute photographic requests. Flo - for your friendliness and willingness to help. Mary - I always looked forward to your cheerful smile and greeting. Thanks for the superb typing. Ann - my surrogate mother - you took care of me and you cared - I'll never forget you.

To the Healthco Corporation, for timely financial support.

To the U.S. Navy, for allowing me the opportunity to obtain this fine education.

DEDICATION

This project is dedicated to my daughter, Jenny Ann, and to my son, David Charles

INTRODUCTION

The achievement of adequate anesthesia is one of the hallmarks of modern dentistry. Although great advancements have been made since the first introduction of anesthesia by Wells and Morton in the 1840s, there is, in fact, no guarantee that every dental procedure can be completely painless. A plethora of techniques, devices, and medicaments have evolved, been introduced and promoted often without the requisite research, only to fail when they could not stand the test of time.

The periodontal ligament (PDL) injection of local anesthesia, also known as the intraperiodontal or intraligamentary injection, may be one such technique. This procedure has been promoted both as an adjunct to, and as a replacement for conventional conduction and infiltration local anesthesia. It entails direct injection of the anesthetic solution under pressure into the space between the tooth and alveolar socket, the PDL space, and is especially promoted by the manufacturers of syringe-like devices specifically designed for the technique, most notable of the several devices available are the Peri-Press(TM)* and the Ligmajet(TM)**. These instruments, developed in the mid 1970s, are capable of injecting the anesthetic

*Universal Dental Implements, Inc., Edison, N.J.
**Healthco, Inc., Boston, Massachusetts

fluid into the PDL space under high pressure. Since their introduction and acceptance by dental clinicians, several other manufacturers have introduced similar devices.

A review of the literature reveals ambivalent opinions concerning the implications of injecting fluid under pressure into this narrow tissue space. Recent studies have investigated, to a limited extent, the effects of this procedure on the local tissue in the PDL. However, to this date there has been no analysis of the potential of this technique to affect the pulp of the tooth injected.

This study was designed primarily to investigate the possible adverse effects of the PDL injection on pulpal tissues. If the injected anesthetic solution causes closure or constriction of entering pulpal blood vessels, then the resultant stasis could lead to inflammation and possible necrosis of the pulp. The combination of the PDL injection plus an operative procedure, liable to be traumatic to the pulp, was also evaluated for a possible additive effect.

The extent and reversibility of local tissue damage due to passage of the needle and anesthetic fluid was evaluated, as was the spread of the injected solution in the surrounding tissues.

LITERATURE REVIEW

Clinical Aspects

Pain control during dental treatment is considered to be a primary requisite for successful patient management. As stated by Travin (1978): "Traditionally, our concern has always been directed toward the elimination of pain." This remains true today. However, the elimination of pain is a sometimes elusive goal and often creates a major source of frustration for the practitioner and patient alike.

Local anesthesia is routinely attempted for dental treatment by the injection into soft tissues of one to several milliliters of anesthetic solution. If a nerve block is attempted, as in the case of the inferior alveolar nerve supplying the mandibular teeth, the anesthetic fluid is usually deposited near the mandibular foramen, where the nerve enters the ramus of the mandible. In the case of infiltration anesthesia, the solution is deposited in the soft tissues, often subperiosteally, adjacent to the apex of the tooth to be treated. When the injection is successful, the anesthetic fluid acts directly on the nerve, interfering with the passage of sodium and potassium through the membrane, thus preventing nerve depolarization and impulse conduction. The anesthetic must reach the nerve in sufficient concentration

and without alteration of its form to exert the desired effect on the membrane (Bennett, 1974).

Achieving the desired effect is not always an easy task. Mandibular molar teeth are particularly difficult to anesthetize routinely. This difficulty relates to the anatomy of the mandibular nerve and/or area where injection is done, the required volume and concentration of the anesthetic solution, or the reduced potency of supplementary infiltration anesthetic in the presence of tissue inflammation (Rood, 1977-1978). The mandible has been shown to be supplied by a plexus of nerves (Carter and Keen, 1971) rather than the single main nerve trunk. Failure to achieve adequate anesthesia, or achieving only partial anesthesia, with a mandibular block alone is thus anatomically understandable. One study (Petersen, 1971) reported the frequency of mandibular molar anesthesia with this technique to be 43.5%, although at times he deliberately inserted the needle "wrongly"--something that practitioners may do inadvertently. Rood (1976) stated that it may be necessary to supplement an inferior alveolar nerve block about 20% of the time to achieve anesthesia for even routine restorative treatment. Another study (Northrup, 1949) reported that there would be a 15% failure to achieve anesthesia solely on an anatomical basis.

In an attempt to improve upon this relatively poor rate of success, alternative approaches to the traditional

mandibular nerve block have been proposed. The Gow-Gates method (Gow-Gates, 1973) utilizes extraoral landmarks and claims a 96-98% success coefficient (Gow-Gates and Watson, 1977, and Levy, 1981). Akinosi (1977) proposed an injection made with the mouth closed. This technique was thought to be superior to the Gow-Gates method, by some, because it was easy to learn, simple to administer, and did not require wide opening of the mouth (Gustainis and Peterson, 1981). Complete extraoral approaches have also been proposed (Topazian, 1962). Besides studying the various approaches, the needle itself has come under scrutiny. Menke and Gowgeil (1979) recommended a short needle rather than the standard long needle for inferior alveolar block anesthesia.

Some dental procedures require more profound anesthesia than others. This is often more difficult to achieve, especially in the mandible, with success rates reported variously at 76.2% (Rood, 1976), and 84.2% with the Gow-Gates technique (Gow-Gates and Watson, 1977). With local complications such as the presence of infection or inflammation, the effectiveness of anesthesia is reduced even farther (Cramer and Mitton, 1973; Najjan, 1977). Supplemental anesthesia is thus recommended in cases where inadequate block anesthesia occurs, and some practitioners routinely administer supplemental anesthesia when making any inferior alveolar nerve block.

Supplemental anesthetic techniques recommended to enhance the profoundness of mandibular nerve anesthesia include: the long buccal block, lingual infiltration, and the mental nerve block. Techniques used to supplement maxillary nerve infiltration include the posterior superior alveolar block and palatal infiltration. Additional techniques useful for any tooth include: interseptal, intraosseous, and intrapulpal injections (Cohen and Burns, 1980). All of these methods have inherent difficulties, disadvantages, and varying rates of success.

The periodontal ligament injection is another supplemental technique which, although not particularly new, has been previously discounted as a viable clinical tool. Phillips (1945), in a review of anesthetic techniques, mentioned the PDL injection, but stated that it was "contraindicated" (although he gave no reasons for his opinion). Morse (1974) recommended using it only as a last resort because of the possibility of periodontal ligament damage. The advent of new syringe-like devices specifically designed for this procedure in the mid to late 1970s has, however, generated a resurgence of interest in the PDL injection. These instruments are advertised as being "effective," "simple and convenient to use," and a "means of delivering anesthesia without the many drawbacks associated with the inferior alveolar

nerve block" (Ligmaject(TM), 1981). Further, they provide "immediate. . .profound. . .safe anesthesia" (Peripress(TM), 1982), allowing for "selective anesthesia of individual teeth," and the method is "virtually painless" (Ligmaject(TM), 1982). The PDL injection technique is being promoted not only as a supplement to but also as a replacement for the traditional approach inferior alveolar nerve block.

The clinical research reported seems to support many of these claims. Lafargue (1973) researched the technique for his doctorate thesis and favorably reported on his clinical experiences. Chenaux, Castagrola, and Colombo (1976) also reported favorably on their empirical clinical experience with the PDL injection utilizing the Peri-Press(TM) syringe. deShazer and Coffey (1981) stated that this technique rendered the mandibular block "obsolete" in their report of 1,000 "documented" injections and also stated that, although it took a few days of experience to perfect the technique, it could be "98% effective after two to three hundred injections." Walton and Abbott (1981) reported a clinical study consisting of 120 PDL injections given with a standard syringe and a variety of needle sizes. The injections were given only after block or infiltration anesthesia had failed. The authors noted a first injection success rate of 63% and a reinjection rate of 71%, for an overall success rate of 92%. They stated that the length and gauge of needle was

unimportant in attaining anesthesia, but that injections must be made with a strong back pressure to achieve success. Malamed (1982) reported a clinical study of 100 cases using both a conventional syringe and a PDL syringe for PDL injections. He noted that the PDL technique may be specifically indicated for patients in whom nerve blocks are contraindicated, including the hemophiliac or those with other blood disorders, young children, and the mentally or physically handicapped. Utilizing the PDL injection technique alone, he achieved Grade A anesthesia (Dobbs and DeVier, 1950) in over 85% of his patients. The PDL syringe achieved 88.5% success while the conventional syringe measured 82%, a difference Malamed thought was insignificant. A study by Kaufman (1983) utilized the Peri-Press(TM) syringe on 258 teeth and achieved 84% overall success, with highest rates on extractions (95%) and lowest on vital pulpectomy (73%). Canine teeth presented the lowest degree of success (46%), while incisors, premolars, and molars were anesthetized with equal effectiveness. A study by Faulkner (1983), with undergraduate operators using PDL syringes, found a success rate of 97% in 100 extraction cases and 76% in 100 "conservative" treatment cases. He reported that the 81% overall success rate was relevant since the injections were "applied by relatively inexperienced undergraduates previously unfamiliar with the procedure."

The PDL injection has also been used as a diagnostic aid in locating teeth with acute pulpitis. Littner et al. (1983) noted an 86% success rate in locating fifty "difficult cases of acute pulpitis" and concluded that the technique was a useful aid for locating and diagnosing acute pulpitis after all other methods had proven inconclusive. Simon et al. (1982) reported on fifty cases in which intraligamentary anesthesia not only provided single tooth anesthesia sufficient to render a positive diagnosis of "pulpalgia," but also eliminated the patient's pain, which was their chief complaint, and allowed immediate endodontic therapy.

According to the American Dental Association's Status Report on the PDL injection technique (Giovannitti and Nique, 1983), the advantages and disadvantages of the technique were summarized as follows:

> "Advantages:
>
> - . . .permits single-tooth anesthesia and avoids numbness to the lip, tongue, and other soft tissues, thereby facilitating treatment in different quadrants during the same appointment.
>
> - A controlled, metered dose of anesthetic solution is administered (one-eighth cartridge or approximately 0.2 ml per injection with Peri-Press(TM) and Ligmaject(TM) syringes) that may be of value in instances where the total dose of anesthetic solution or vasoconstrictor is of concern.
>
> - It represents an alternative injection technique whenever anesthesia is difficult to obtain with conventional methods or when conventional techniques are contraindicated.

- Profound anesthesia can be obtained with onset ranging from immediate to thirty seconds, and a duration of 45 to 55 minutes.

- Administration of the injection is less painful for most patients than conventional techniques.

- The technique is well suited to certain procedures such as treatment of children, multiple quadrant procedures, single tooth procedures, endodontic and periodontal therapy."

Two advantages not noted in this report are: the ability to achieve anesthesia with a very minimal dose of anesthetic agent (solution), and the advantage of utilizing this technique as a diagnostic tool as noted by Simon et al. (1982) and Littner et al. (1983).

"Disadvantages:

- Proper needle placement is difficult in some areas such as the distal aspects of molars.

- Leakage of anesthetic solution into the patient's mouth produces an unpleasant, bitter taste.

- Excessive pressure because of rapid injection can result in breakage of the anesthetic cartridge, which presents a problem if a conventional syringe is being used for this procedure.

- A special syringe may be required.

- Excessive pressures required for success of the technique may produce focal tissue damage.

- Although there is little or no pain during injection, post-injection pain for several days is not uncommon." (This does not seem to be well supported by the literature.)

> " - Although not well documented, there is a potential for extrusion or avulsion of teeth with excessive volumes or pressures. Furthermore, even slight extrusion of the anesthetized teeth can complicate establishing proper occlusion of just-placed restorations."

To this list should be added the note that even with the PDL injection there is difficulty in achieving adequate anesthesia for cuspid teeth.

The literature based upon clinical studies or experience supports most of these statements. Faulkner (1983) reported five cases of post-injection pain out of 200 injections, for a rate of 2.5%. Three of these problems resolved quickly with warm saline rinses and two were treated with antibiotics for resolution. He recommended that a tissue-tolerated disinfecting agent, such as hexetidine, be applied to the injection site routinely before injection to possibly circumvent this complication. Malamed (1982) also reported the PDL injection to be well tolerated, and noted that the overwhelming majority of patients preferred the PDL injection to the inferior alveolar block. Kaufman et al. (1983) noted five cases of slight post-operative localized pain following 258 injections, for a rate of 1.9%. They attributed this to pre-existing "periodontitis." They also noted that carpule breakage can occur, although this does not present a problem with specialized PDL syringes since the carpule is enclosed in a protective sheath. deShazer and Coffey (1981) noted

that the most common patient complain was a bitter taste. They observed post-operative pain in "about 4% of the cases," and also noted difficulty in anesthetizing cuspid teeth with this technique. Perhaps the most serious sequela of a PDL injection was noted by Nelson (1981). He reported the case of a mandibular premolar which "floated" out of the socket after a PDL injection to the extent that the patient could extract the tooth. Since the tooth was "healthy, with no caries or periodontal involvement," he attributed this phenomenon to the "hydrostatic pressure" of the injection. Overall, however, the literature clinically supports the PDL injection technique as being relatively safe, simple, and effective.

Biological Aspects

The acceptance or popularity of a clinical treatment modality is not, unfortunately, a reflection of its biological compatibility. A method of treatment is too often, in fact, accepted on the basis of its "clinical success" in spite of its potentially harmful results. The PDL injection technique is a case in point. Empirically, it is obvious that wedging a sharp needle into the narrow, tissue-filled periodontal ligament space and forcing a fluid into that space under relatively heavy pressure must have some deliterious effect

on tissues and cells. The American Dental Association, in its recent status report on the PDL injection technique (Giovannitti and Nique, 1983), advised against the routine use of the technique until additional research determines the effect of the method on the periodontal ligament and adjacent structures, and on the dental pulp tissue.

A few studies have been performed investigating the effects of the technique on the PDL space. Walton and Garnick (1982) used a conventional syringe with a 30-gauge needle on monkey teeth in their histological evaluation of the method. With 0, 10, and 25 day post-injection examinations, they noted "minimal" tissue damage in the PDL, which was followed by rapid repair. They reported that this was "valid evidence that this injection technique is safe to use in patients with respect to the periodontium," but they also noted that patented PDL syringes generate over two times the pressure of the standard syringe, and thus may cause more damage. They also noted that the effect of the technique on the pulp warranted investigation. Brannstrom et al. (1982) made a similar study using the Peri-Press(TM) syringe on monkey teeth. They, too, reported damage in the periodontium similar to that reported by Walton and Garnick: immediate disruption of collagen bundles, lesions on the adjacent cementum-dentin and alveolar walls, extravasated erythrocytes

and, in 24 hours, acellularity in both the PDL and the adjacent bone lacunae and occasional necrotic material and inflammatory cells. In no instance, however, did this extend more than 1.5 mm from the area of the needle. Within a week, cell-rich granulation tissue appeared and osteoclasts apparently were remodeling the damaged alveolar bone. In two weeks the tissues were "resuming a normal appearance." They cautioned against injecting on both sides (buccal and lingual) of an alveolar crest, however, as they noted "a necrotic-like condition" at the top of the crest when this was done. Fuhs et al. (1983) injected anesthetic solution, normal saline, or dye in the PDLs of dogs with a Peri-Press(TM) syringe. They found no evidence of tissue disruption or inflammation at 2, 7, 10, 14, 21 or 28 day intervals. These are the only histologic studies to date that have reported on the effects of the PDL injection on the tissues.

Areas of concern remain unanswered. Where does the solution go when injected? How is anesthesia obtained with use of this technique? What is its effect on the pulp of the tooth injected?

Smith and Walton (1983), using a conventional syringe, injected colloidal carbon and a radiopaque solution into the PDL of dogs to examine the spread of the solution in the tissues. They found a generally unpredictable, though

frequently widespread distribution of the solution through the tissues, including spread into the PDL, periapex, medullary bone, pulp, and often into the same tissues of adjacent teeth. They concluded that the PDL injection was actually an intraosseous injection. Brannstrom et al. (1982) used radiopaque dye to study the distribution of solutions injected with the Peri-Press(TM) syringe. They, too, found a rather widespread dispersion of the fluid. They noted that since anesthetic drugs are rapidly taken up in developing enamel, with possible interference of amelogenesis (Hammarstrom, 1970), then perhaps this technique should not be used on primary teeth to prevent damage to succedaneous tooth germs near the injection site. Fuhs et al. (1983) injected an India ink-saline solution in the PDLs of a dog and also found a widespread dispersion of the solution in the PDL and the medullary spaces.

The mechanism of action of the PDL injection is also a matter of debate. Instantaneous onset of anesthesia is frequently noted--this may indicate that anesthesia is due to the pressure of the injection solution alone. Birchfield and Rosenberg (1975) studied the role of the anesthetic solution in intrapulpal anesthesia and concluded that it was the pressure created on the pulp and not the anesthetic solution per se which rendered profound and immediate anesthesia.

They reported no difference in the success of anesthesia when either sterile saline or lidocaine with epinephrine was used. The exact mechanism that produces these findings is not fully understood. Monheim, quoted by Bennett (1978), suggested that prolonged pressure may lead to degeneration of nerve fibers; however, Birchfield and Rosenberg (1975) stated that if all the pulp tissue is not removed after an intrapulpal injection, the remaining tissue will be vital on subsequent appointments. The role of pressure in PDL injections remains to be studied.

Pashley et al. (1981) noted that the pressure exerted during an injection would vary based on the pressure exerted on the syringe by the operator, the tissue resistance and distensibility, the rate of the injection, and the needle size. They noted that the periodontal tissues were the least distensible tissues of those tested, and PDL injections, using a standard syringe, produced pressures ranging from 200 to 460 psi, depending on how firmly the needle was wedged in the PDL. The average was 340 psi, compared to 219 psi for hard palate injections and 172 psi for intrapulpal injections. They theorized that elevated tissue pressure could play a role in local anesthesia, possibly by preventing local microcirculation which, in turn, might inhibit sensory nerve activity. It could also, they added, be due to direct tissue injury and/or compression of nerves.

A study measuring fluid pressures in the PDL of dog canines (Walker et al., 1978) noted, however, numerous fenestrations in the alveolar wall, especially in the apical area, through which fluid would be free to flow. This low fluid resistance would, they theorized, rapidly attenuate PDL pressure changes. Walton and Abbott (1981) found that the most critical factor in achieving anesthesia was to inject under strong resistance. This, as shown by Pashley et al. (1981), would, in turn, generate the most periodontal pressure.

Another theory of the mechanism of anesthesia is related to the vasoconstriction effects of epinephrine in the anesthetic solution. A study using microspheres to measure blood flow in dog pulps (Kim et al., 1982) found a temporary cessation of this flow within four minutes of a PDL injection using lidocaine and 1:100,000 epinephrine. This flow cessation does not occur when using lidocaine without epinephrine. In a related clinical study, they achieved 81% successful anesthesia with the PDL injection on "painful" molar teeth while using lidocaine with epinephrine, but had 70% of the patients report no anesthesia at all when using anesthetic without vasoconstrictor. He thus concluded that it is the pulpal ischemia which produces the resultant anesthesia. On this basis, he recommended

only using the technique for dental procedures in which irreversible pulp damage would be of no consequence, i.e., endodontia and exodontia.

Sticht et al. (1966) measured the effects of epinephrine on the blood pressure within the pulp of dog teeth. They found that intravenous administration of epinephrine resulted in a transient rise, followed by a "profound" fall in pressure, which "far exceeded in duration the effect on the systemic arterial pressure." Kraintz and Conroy (1960) noted the small volume in the pulps of dog teeth, ranging from 0.0004 ml in the central incisor to 0.0414 ml in the canine, and Neidle and Liebman (1964) noted that both systemic and gingival injections of epinephrine resulted in a significant and persistant reduction of blood flow in cat pulps.

Takahashi et al. (1982) fabricated vascular casts of dog pulps using low-viscosity synthetic resin and analyzed them under scanning electron microscopy. They found numerous apical foramina, varying in size from 20 to 150 microns, and that each foramen housed one vessel--arterioles in the small ones and venules in the larger. Tonder (1980) noted that no arteries, only terminal arterioles, enter the pulp, and collateral circulation is distinctly limited. She also reported an unexplained higher pulpal blood flow (0.15-0.17

ml/min · g) compared to other tissues, which seemed to be in excess of the pulpal metabolic requirements. Tonder theorized that this could be related to thermal homeostasis, or perhaps is necessary for repair of local insults to which the pulp is frequently exposed. For the most part, however, the metabolic or functional requirements of this high flow cannot be fully explained.

If, in fact, the pulp does require a high fluid exchange to maintain homeostasis and if, as demonstrated by Kim and others (1982), PDL injections can interrupt or stop this flow, then perhaps irreversible pulpal damage could ensue. This may be further aggravated in the presence of simultaneous external trauma. Olgart and Gazelius (1977) found that adrenaline injections, whether alone or with lidocaine, supraperiosteally in the apical area of teeth, caused almost complete inhibition of blood flow in the pulp within a few minutes. This was followed by a total inhibition of sensory nerve activity. This finding agrees with Edwall and Scott (1971) who found similar concomitant reductions in blood flow and sensory nerve activity of cat pulps following vaso-constrictor injection, and thus concluded that tooth sensory neuron excitability is strongly modulated by changes in blood flow. Olgart and Gazelius (1977) stated that the anesthetic effect was due to constriction of the arterioles

proximal to the pulp itself. They noted that lidocaine alone had no effect on the intradental sensory nerve activity. Although they noted that after three to four hours there was a return to control values of blood flow and nerve activity, they cautioned that it was unknown whether the adrenaline-induced pulpal ischemia could generate latent serious consequences. Additional pulpal insults may be additive to the effects of epinephrine and result in irreversible alterations of pulpal function. This was supported by a study by Pohto and Scheinin (1960) who found that when pulpal blood flow was reduced by a mandibular block injection of adrenaline, the added insult of heat on exposed dentin caused a higher incidence of irreversible changes in pulpal circulation than in the controls without adrenaline. Skoglund et al. (1978), in a study of vascular changes in replanted and autotransplanted dog teeth, found that revascularization can occur in most cases after the tooth is replanted. This would seem to indicate that the loss of intrapulpal blood vessels could be overcome without permanent pulpal necrosis. This was done in young teeth with wide-open apices, however, which differ significantly from the closed apices with minute foramina present on mature dog teeth.

Research Aspects

It would appear from the research to date, that the PDL injection technique is clinically effective, though potential complications and harmful side effects may result; that the tissue damage in the PDL space generated from the needle and/or the injected fluid may be minimal, tolerated, and even reversible; that the technique may work by increasing local tissue pressure, by cessation of blood flow in the pulp, and/or just by the action of the anesthetic agent alone on the nerves as with other injections, and that there is definitely a need for further investigation of this technique. Probably the most serious deleterious effect of this technique would be the potential for pulpal anoxia from reduced blood flow leading to total pulpal necrosis and the death of an otherwise viable tooth, especially when added to the trauma of restorative procedures. Numerous authors (Kim 1982, Malamed 1983, Olgart and Gazelius 1978, Walton and Garnick 1982, and Giovannitti and Nique 1983) have recognized this potential and the need for a pulp study. Accordingly, this research was designed primarily to investigate histologically the effects of the PDL injection on the pulp, both with and without additional operative procedures. Secondly, it was designed to examine histologically the effect of the injection on the PDL and adjacent tissues, and the spread of the injectate in these tissues.

MATERIALS AND METHODS

Animal Preparation

Three male Basenji dogs, ranging in age from five to six years, were utilized in this study. The animals were housed in the research facility for two weeks prior to surgical procedures for acclimation. They each received a complete blood count and a SMAC* blood chemistry screening to ensure that they were healthy. The maxillary and mandibular incisors and premolars were selected for injections (Figures 1 and 2). These teeth were radiographed to verify apical closure and absence of root or periodontal abnormalities or pathology. Cuspids and some molars were utilized, along with other teeth as controls. The selection of teeth was based on a description of root morphology by Evans and Christensen (1979). The extremely long, curved root of the cuspid and the diverse, large roots of molars were considered unsuitable for this anesthetic technique.

Instrumentation

The Ligmaject(TM)** periodontal ligament syringe was employed (Figure 3) with 30-gauge extra short (15 mm)

*SMAC - Sequential, Multiple, Analyzer, Computerized
**Healthco, Inc., Boston, Massachusetts

needles (Figure 4). These needles are specifically designed for the PDL injection technique.

Three solutions were injected: (1) Lidocaine 2% with 1:100,000 Epinephrine*, (2) India Ink**, of approximately the same viscosity as the anesthetic solution, and (3) a prepolymerized solution of methyl methacrylate***. The acrylic solution was developed by mixing two drops each of Universal and Catalyst with 0.1 cc of monomer. Pilot studies, in vitro, showed that this solution polymerized in seven minutes. The solution was mixed, placed into an empty 1.8 cc anesthetic carpule and injected in the same manner as was the anesthetic and India Ink solutions.

The primary difference between the Ligmaject(TM) or other PDL injection syringes and conventional anesthetic syringes is that PDL syringes achieve a mechanical advantage that significantly increases the force of injection. Conventional syringes have a 1:1 thumb/finger grip to plunger ratio, while the PDL syringes have a lever which moves approximately two inches to advance the plunger 1/10th the length of the carpule. Each complete travel

*Xylocaine, Astra Pharmaceutical Products, Inc.

**Higgins Black India Drawing Ink, A. W. Faber-Castell Co., USA

***Delton Pit and Fissure Sealant, Johnson and Johnson, Inc., USA

of the lever injects approximately 0.2 ml of anesthetic solution. This added mechanical advantage also increases the pressure of injected fluid two to three times that of conventional syringes.

Surgical Approach

Prior to the injections, the dogs were placed under general anesthesia. Intravenous access and sedation was first established via the cephalic vein of the left or right foreleg with a 21-gauge butterfly intravenous needle. One ml of Atropine sulfate* (0.5 mg/ml) was injected, followed by Thiamyl sodium** given to effect, and then oral-tracheal intubation. Surgical anesthesia was achieved and maintained by closed circuit Enfleurane***-nitrous oxide-oxygen inhalation for the duration of each procedure.

Injection of the teeth with the PDL syringe was accomplished by wedging the needle into the PDL space between tooth and alveolar bone at the mesiobuccal or distobuccal aspect of each root, until it would advance no farther

*Burroughs Wellcome Co., Research Triangle Park, North Carolina

*Surital, Parke-Davis, Detroit, Michigan

***Ethrane, Ohio Medical Products, Madison, Wisconsin

(Figures 5, 6 and 7). The lever was initially depressed a few millimeters. If significant back pressure was not felt, or if the injectate was observed welling up out of the gingival crevice, the injection was stopped and the needle repositioned until significant back pressure was obtained. When this was achieved, the lever was advanced to its full traverse in approximately twenty seconds (as per manufacturer's recommendations), thus depositing 0.2 ml of solution into the PDL space. This procedure was carried out for each root of each tooth designated for injection.

Following the experimental procedures, the anesthesia machine was switched to deliver 100% oxygen. This was continued until it was ascertained that the dog was breathing adequately on its own. When this status was assured, the intubation was removed and the animal placed into a protected cage, wrapped in a blanket, and closely monitored until full recovery was confirmed.

Aseptic surgical techniques were adopted to the fullest extent possible for all procedures.

Experimental Groups

In this study 58 teeth were treated and 12 of the remaining untreated teeth were selected as controls. The teeth were divided into nine experimental groups:

Group 1: Twelve teeth received no treatment and were designated as controls.

Group 2: Twelve teeth were injected with 0.2 ml of 2% Lidocaine with 1:100,000 Epinephrine per root thirty days before killing the animals and gathering the specimens.

Group 3: Twelve teeth were injected as above, then one or two Class Five amalgam restorations were placed in the crown of each tooth. A #33½ inverted cone bur in a highspeed handpiece was utilized to prepare round cavities approximately 2 mm in diameter. The bur was advanced approximately 2 mm, just past the dentoenamel junction into the dentin, and circumferential retentive grooves were added. The cavities were placed buccally and approximately 1 mm coronal to the gingival crest above each root. Single rooted incisors and first premolars received one restoration each, two-rooted premolars received two restorations. Dispersalloy* amalgam, mixed as per manufacturer's instructions, was inserted, condensed, and burnished to place in each cavity using hand instruments (Figure 8). The dogs were killed thirty days later.

Group 4: Six teeth were not injected but did receive the same amalgam restorations as in Group 3. These teeth

*Western Metallurgical Ltd., Edmonton, Canada

served as secondary controls to examine the effect on the pulp of the preparation alone. These dogs were also killed thirty days later.

Group 5: Eight teeth received PDL injections of 2% Lidocaine with 1:100,000 Epinephrine (0.2 ml per root) fifteen days before termination of the experiment.

Group 6: Six teeth received PDL injections of 2% Lidocaine with 1:100,000 Epinephrine (0.2 ml per root) two days before termination.

Group 7: Eight teeth received PDL injections of 2% Lidocaine with 1:100,000 Epinephrine (0.2 ml per root) approximately ten minutes prior to killing the animal.

Group 8: Three teeth were injected with the India Ink solution approximately ten minutes prior to termination. Unlike the anesthetic injections, these injections of 0.2 ml of India Ink were made mesially and distally for each tooth, regardless of the number of roots. These teeth were in a quadrant which had received no previous treatment.

Group 9: Three teeth were injected with pre-polymerized methyl methacrylate solution approximately twenty minutes before killing. Unlike the anesthetic injections, these injections of 0.2 ml of the solution were made mesially

and distally for each tooth, regardless of the number of roots. These teeth were also in a quadrant which had received no previous treatment.

Tissue Preparation

Thirty days following initial treatment, surgical anesthesia was obtained, the thoracic cavity was opened, the heart exposed and the dogs were killed by intrathecal perfusion. The perfusion was delivered by a cannula sutured into the left ventricle. Normal saline was introduced first and continued until a clear flow was observed, then it was followed by 10% buffered (pH 7.2) formalin, with drainage taking place through the opened right atrium. Adequate perfusion was judged to have taken place when blanching of the gingival tissues and extremities was followed by a yellowish tinge. At this stage the heads were disarticulated and stored in 10% buffered formalin for ten days.

Block sections were cut from the mandibles and maxillae at designated points that preserved the pulps and injection sites. Some teeth were split at the bifurcation. Tissues to be stained were immersed in Kristensen's Decalcification Solution (1 liter 8/N formic acid and 1 liter 1/N sodium formate - Sheehan and Hrapchak, 1980) for decalcification.

The end-point of decalcification was verified radiographically and took from 14 to 30 days, depending upon the size of the specimen.

The tissues were then washed in running tap water for several hours, neutralized in a saturated solution of lithium carbonate, and washed again in tap water. They were dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate, infiltrated at 58°C and embedded in 56-57°C melting-point Paraplast(TM)* wax.

The tissues were sectioned when possible parallel to the long axis of the tooth at seven microns, forming continuous ribbons. Serial sections were selected at the mesial, distal, and pulpal aspects of each tooth and mounted on glass slides. Representative sections were stained with each of the following methods: (1) Harris' Hematoxylin and Eosin, to show general microanatomical detail, (2) Masson's Trichrome, modified for staining bone (Ponceau de Xylidine, diluted 1:10 with 1% acetic acid for three minutes - Drury and Wallington, 1980), and (3) Gomori's Stain for reticular fibers (Drury and Wallington, 1980).

The India Ink specimens were decalcified and dehydrated with the other tissues, then immersed in methyl salicylate until clear, requiring about 24 hours. These blocks were

*Paraplast, Sherwood Medical Industries, St. Louis, MO 63103

observed and photographed macroscopically to show the infiltration of India Ink through the jaw. They were stored in methyl salicylate solution to retain transparency.

The methyl methacrylate specimens were also decalcified and dehydrated, then photographed to record their outline and dimensions. One was immersed in methyl salicylate for clearing, then all blocks were returned to water via absolute alcohol and the tissues were macerated in 10% Potassium Hydroxide at 20°C to reveal the solid plastic cast of the injectate.

Specimen Evaluation

The mounted slides were labelled and organized according to tooth and procedure. These specimens were initially examined histologically using a light microscope at various magnifications and a method was developed for evaluating the status of the pulp and the PDL tissues. The evaluation was based on the variations from the normal. The "normal" pulp and PDL tissues were taken to have the following characteristics:

(1) "Normal" PDL and adjacent structures

A "normal" periodontal ligament space should demonstrate a uniform array of collagenous fiber bundles from the

cemento-enamel junction to the apex. The fibers are anchored evenly in the cementum of the tooth and traverse the PDL space to where they are embedded in the alveolar bone or to the gingiva where they end as free fibers. The alveolar bone demonstrates normal physiologic structural changes characterized by areas of resorption with osteoclasts, or areas of deposition of new bone with osteoblasts and resting and reversal lines.

In addition to the fibers, the connective tissue in the PDL space contains a cellular element of fibroblasts, osteoblasts and osteoclasts, and cementoblasts, as well as interstitial spaces of loose connective tissue with blood vessels, lymphatics and nerves. Epithelial rests in either elliptical form or long strands may also be seen.

The cementum covers the root dentin from the cemento-enamel junction to the apex and would be expected to be thicker in the apical portion than coronally. Both acellular and cellular cementum may be observed, the latter containing cementocytes embedded in lacunae. Incremental lines may divide layers of cementum, indicating periodic formation.

(2) "Normal" pulp and adjacent tissues

The dentinal tubules appear in a uniform, regular pattern from the dentinoenamel junction to and through the

predentin. In some areas, especially in the coronal portion, a more amorphous layer of secondary dentin may appear between the tubular dentin and odontoblastic layer.

The odontoblasts are evenly aligned and covered the less dense predentin. In the coronal aspect, they form a thick, tall columnar layer, becoming less dense and more low columnar in the mid-root, and changing to a cuboidal and even flattened fibroblast-like appearance as the apex is approached. Central to the layer of odontoblasts, cells, fibers and ground substance appear in varying degrees. Coronally, there are few fibers with many stellate shaped fibroblasts scattered throughout. More apically, the interstitial cells decrease and the fibers increase significantly, filling the lumen with a pink-staining (in H and E specimens) mass of tissue aligned parallel to the long axis. More blood vessels should be seen in the apical segments. With adequate perfusion fixation, vessels are devoid of erythrocytes. Nerve bundles are more demonstrable in the apical aspect, while random miscellaneous cells such as histiocytes and undifferentiated mesenchymal cells are observed throughout.

Comparative Evaluation

A blind study was conducted of selected, representative sections of the pulp of each tooth. The slides were arranged

in random order and were examined and evaluated by three independent, qualified observers for evidence of the pulpal tissue state of each specimen. The observers examined three sections of each root: one stained with H and E, one with Masson's Trichrome, and one with Gomori's Stain. The sections were not identified. Each section was evaluated microscopically and the pulpal tissue for each root was graded on a scale of one to four:

Grade One:

No evidence of inflammation. Grade one pulps demonstrated the appearance of a "normal" pulp, as described previously. There were pallisading odontoblasts with a moderate amount of cells, fibers, blood vessels, and nerves, and few, if any inflammatory cells.

Grade Two:

Mild inflammation. Grade Two pulps showed an odontoblastic layer with reduced palisading, with some disruption of the pulpodentinal membrane and disorientation of the odontoblasts. Hyperemia, edema, and/or mild intrapulpal hemorrhage were sometimes seen. A few odontoblastic nuclei were seen aspirated into the dentinal tubules. Scattered inflammatory cells were present, especially polymorphonuclear leukocytes along the predentin. Amorphous tertiary, or reparative, dentin might be observed, especially subjacent to cavity preparation.

Grade Three:

Severe inflammation. Grade Three pulps were characterized by areas showing complete loss of the odontoblastic layer and small, localized abscesses forming in the pulp. Granulation tissue was present with lymphocytes, plasma cells, and macrophages in areas of attempted repair. Granulomatous tissue may also be present, either within the pulp or in the periodontium apical to the pulp. There may be evidence of internal resorption and/or significant intrapulpal hemorrhage.

Grade Four:

Necrosis. These pulps would be devoid of viable tissue. Complete cellular disruption, a purulent exudate, and amorphous ill-defined tissue elements were seen. There may be an accompanying periapical granuloma.

Statistical Analysis

The results of these studies were evaluated for statistical significance in three ways.

First, the data were analyzed for inter-examiner reliability. This was done by comparing how frequently the individual observations of each examiner were within one grade of each other.

Second, the data were analyzed to determine if the degree of pulpal inflammation reported for each group varied significantly. The mean pulpal evaluation score of each observer in each group was computed and these figures were subjected to an analysis of variance (ANOVA). The null hypothesis stated that there was no difference between the means of all the groups.

Finally, a Scheffe' test was computed to analyze the mean of each group against the mean of the control group. The null hypothesis stated that there was no difference between the mean of each group and the mean of the control group.

RESULTS

Group 1

(Controls)

Pulp

Eleven of the twelve control teeth were judged to have no or mild pulpal inflammation (Figures 9-11, Table 1). One tooth was evaluated by all three observers as being necrotic. The pulp chamber and canal of this tooth was devoid of recognizable tissue; rather, there was a loose stroma of amorphous substance resembling tissue and cellular remnants (Figure 14). The mean pulp score for this group was 1.50.

PDL

All twelve teeth exhibited the traits of a "normal" PDL and adjacent tissues, as described in Methods and Materials.

Group 2

(30 day specimens--injection only)

Pulp

The twelve teeth in this group produced sixteen roots with pulps suitable for evaluation. These were all judged to be either "normal" or mildly inflamed (Table 2). The

mean pulp score for this group was 1.25.

PDL

Pathologic changes likely to be caused by the needle and/or injection were seen in six of the specimens and possible pathology was noted in three others. Three specimens showed no evidence of the injection.

The most evident change was a localized area of external resorption on the buccal root surface of the injected tooth. The resorption was in the vicinity of the alveolar crest or just apical to it, where the needle point probably came to rest (Figures 15 and 16). This resorptive process showed an eroded, scalloped lesion into the dentin, and was characterized by numerous multinucleated giant cells, fibroblasts, and a few inflammatory cells. In most of the cases, there was no evidence of cementoblasts or reversal lines in the resorbed areas (Figure 17). However, in one case, the resorptive area appeared less active than in the other specimens, with a darker-staining border, indicating the possible existence of cementoid formation (Figure 18).

In some cases the PDL tissues appeared intact, while in others there was an apparent disruption of the tissues which had not completely resolved.

Group 3

(30 day specimens--anesthesia injection plus buccal amalgam)

Pulp

The twelve teeth in this group produced 15 roots with pulps suitable for analysis. Twelve of these pulps were judged by the three observers to have little or no inflammation, while three had mild to moderate inflammation (Table 3). In some cases there was a disruption of the regular pallisading odontoblastic layer subjacent to the cavity preparation which was not seen in other areas of the pulp. The mean pulp score for this group was 1.49.

PDL

Two specimens in this group displayed definite evidence of tissue disruption in the vicinity of needle penetration while three showed possible disruption. The tissue alterations were similar to those described for Group 2.

Group 4

(30 day specimens--buccal amalgam only)

Pulp

The six teeth in this group produced eight pulps for analysis. Seven of these were judged to be "normal" or mildly inflamed while one was judged to be mildly inflamed

by one observer and severely inflamed by the other two observers (Table 4). The mean pulp score for this group was 1.54. The disruption of the odontoblastic layer noted in Group 3 pulps was also evident here (Figures 19 and 20).

PDL

There were no pathologic changes noted in the PDL spaces of these teeth.

Group 5

(15 day specimens--anesthesia injection only)

Pulp

The eight teeth in this group resulted in ten pulps for analysis. Eight were judged to be uninflamed or mildly inflamed by the three observers. Two were judged mildly inflamed by two observers and these two were judged as mild to severely inflamed and severely inflamed to necrotic by the third observer (Table 5). One tooth (2ULI1) had an abundance of intravascular erythrocytes throughout the pulp tissue. Mean pulp score for this group was 1.53.

PDL

Three teeth demonstrated definite pathologic changes in the PDL, while two teeth had possible changes. One tooth showed ankylosis throughout most of the lingual aspect of

the root, but not the buccal aspect. This tooth was also judged to have an inflamed pulp and resorptive areas were noted in the dentin and in the bone at the level of the alveolar crest (Figures 21 and 22).

Group 6

(2 day specimens--anesthesia injection only)

Pulp

The six teeth in this group yielded nine pulps suitable for examination. Two were judged to be mildly inflamed while the other seven were judged to have either no or little inflammation (Table 6). One tooth had extensive secondary dentin formation, filling most of the pulp canal. The mean pulp grade for this group was 1.57.

PDL

Three teeth showed possible periodontal pathology from needle penetration. This was characterized by a localized acute inflammatory reaction with PMNs, lymphocytes, macrophages and tissue remnants (Figures 23 and 24).

Group 7

(Ten minute specimens--anesthetic injection only)

Pulp

The eight teeth in this group produced nine pulps for examination. All were rated to be without inflammatory changes or only mildly inflamed by all observers (Table 7). Varying degrees of pulpal hyperemia were noted, however, with one pulp being extremely engorged with intravascular erythrocytes (Figure 25). The mean pulp grade for this group was 1.28.

PDL

Of the eight teeth in this section, five showed definite evidence of tissue pathology from needle penetration and three showed possible pathology. An obvious separation of periodontal tissues was often evident, sometimes from the gingival crevice into the PDL space between alveolar crest and root surface (Figure 26). The connective tissue was torn apart and the cementum stripped from the root surface where the needle apparently gouged the root. Extravasated RBCs were evident. In some cases the needle point ended in bone, with gouging and/or fracturing evident (Figure 27).

Group 8

(Ten minute specimens--India Ink Injection)

After hard tissue decalcification, and prior to clearing, evidence of the injected India Ink was present in the gingival

tissues (Figure 28) and in cross-sections of the mandible (Figure 29). After clearing, the spread of the injectate was revealed to extend beyond the confines of the alveolar socket. The black ink could be seen extending laterally to the vicinity of adjacent teeth and, in the apical region, close to the mandibular canal (Figure 36). The ink appeared to pool in intramedullary spaces, especially in the apical regions (Figure 30).

Group 9

(Twenty minute specimens--methyl methacrylate injections)

The methyl methacrylate was not visible in the gingival tissues prior to, or after, clearing the tissues with methyl salicylate. The tissues were macerated slowly in potassium hydroxide over a period of several days in an attempt to retain a solid methyl methacrylate matrix. However, small (1-2 mm) amorphic clumps of solid methyl methacrylate were all that remained after the tissues dissolved. These were not connected to each other and thus could not be related to the tissues in any way.

Statistical Analysis

The amount of agreement between the observers in judging the degree of inflammation in the pulps was measured

by comparing the pulp grade of each observer for each specimen with that of each other observer for that specimen. If the grades were within plus or minus one of each other, that was considered to be in agreement and was counted. This was reflected as a percentage of agreements for each group. Thus, as seen in Table 8, observer 1 agreed with observer 2 and observer 3 100% of the time in evaluating the specimens of Group 1, and so on. Of 237 total comparisons between observers, they disagreed eight times, for an overall agreement rate of 97%.

The analysis of variance computed between the mean pulp grades of all the groups was evaluated at the $p = .05$ level. The table value for this, with 6 and 14 degrees of freedom, is 2.85. The F-test computed value for these measurements was 1.152. This is lower than 2.85, therefore the null hypothesis was accepted: there was no statistical difference between the degree of inflammation in the pulps of all the teeth.

Summary Table For ANOVA

Source	SS	df	MS	F
Total	0.97	20		
Between	0.32	6	0.053	1.152
Within	0.65	14	0.046	

The Scheffe' test was computed for the mean pulp grade of each group against the mean pulp grade of the controls (1.5). In all cases the F value was lower than the table value at $p = .05$, thus the null hypothesis was accepted in all cases: there was no statistical difference between the mean pulp grades of any of the groups in comparison with the mean pulp grade of the controls.

DISCUSSION

Methodology

Eighty-eight individual PDL injections were administered during the course of this project. The Ligmaject(TM) PDL syringe performed in a satisfactory manner throughout. One difficulty noted, however, was frequent bending of the 30-gauge extra short needle. The needle often bent at several points, making manual straightening impossible and requiring a replacement needle. The cause of this problem was basically twofold. First, the crowns on many of these teeth demonstrated a marked mesial and distal cervical bulge. This overhang sheltered the PDL space and required that the needle enter at a marked angle to the long axis of the root (Figure 7b). Second, the wedging force on the needle and the heavy back-pressure that was required to achieve anesthesia, combined with an awkward angle of penetration, often resulted in lateral forces that bent the needle. The incisors, where access was good and the crowns had little cervical bulging, were less of a problem than the premolars. This difficulty is also found in the treatment of human molars. At times correct needle placement required scaling to remove supra-gingival calculus deposits. Routine calculus removal should precede the injections since a healthy gingival condition is considered a prerequisite to the technique, Faulkner (1983) and Kaufman et al. (1983).

This study could not directly assess the adequacy of anesthesia or the spread of the injected anesthetic solution in the tissues. However, India Ink solution was injected using the same technique and had a similar viscosity. The observed widespread distribution of the India Ink solution around all three (cleared) injected teeth suggests that the anesthetic solution would also spread sufficiently to reach the apex.

The perfusion fixation technique used in this study was considered to be the best methodology available to produce the desired results. First, and perhaps foremost, it provided for rapid, thorough fixation of all the tissues. This was especially necessary in a pulp study, where routine fixation techniques delay the fixation because of poor access to the pulp. Delayed fixation produces artifactual pulpal changes which could be incorrectly attributed to experimental variables. Perfusion fixation also clears erythrocytes and leukocytes from all vascular tissues accessible to the fixatives. Thus RBCs noted in this study were most likely either extravasated cells and the result of treatment, or intravascular in vessels closed to the fixative flow.

Specimen preparation and sectioning for histologic examination were without major difficulties. The severe angulation of incisor roots made longitudinal pulp specimens

difficult to obtain routinely for each root, but sufficient oblique or cross-sections were obtained with pulp tissue intact, and these were adequate for pulpal analysis. Trial sectioning determined that seven microns thickness produced the longest continuous ribbons and allowed for selection of the best sections.

Clearing of the India Ink specimens was rapid and very satisfactory. It provided a very good three-dimensional view of the injectate while still showing enough hard and soft tissue landmarks for orientation. Photography of these specimens suspended in clearing solution was most satisfactory when a fluorescent backlight and front flash were used.

Clearing the tissues injected with methyl methacrylate failed to demonstrate the injectate spread. The unstained methyl methacrylate was a pale yellow upon polymerization and it blended in too closely with the cleared tissues to be visualized. Pilot studies adding a red dye to the methyl methacrylate were attempted, but when the solution was colored, the altered viscosity and polymerization time were unsatisfactory for injection. Further study should persue this technique to find a dye which will allow adequate visualization of the injectate prior to tissue maceration and not interfere with polymerization.

When the plastic injected tissues were dissolved with 10% potassium hydroxide solution, small, 1-2 mm,

unidentifiable separate clumps of polymerized methyl methacrylate were all that remained. Inadequate polymerization may have occurred, or the polymerization time may have been altered in such a way that the injectate disseminated too readily in the tissues. Natural spread of the injectate through alveolar fenestrations and into intramedullary spaces may have broken it up into segments too small to remain connected with each other. Other studies using similar techniques in analyzing vasculature have proven quite successful. Perhaps this methodology can be improved and used for future studies of the PDL injection.

The Pulp Study

General

Separation of the pulp tissue from the predentin was noted in some sections, but this artifact did not affect the evaluations of the treated tissues. This separation may have been a result of the quality of the vacuum embedding of the specimens in paraffin, the sharpness of the microtome knives, the thickness of the sections, or a combination of technical factors. The great disparity of tissue density between dentin and the thin, delicate pulpal stroma certainly contributed to this effect. More separation was noted in coronal pulp than in apical areas where the pulp tissue

was more fibrotic. Future studies should note the difficulty in obtaining "ideal" pulp specimens, and plan the technique accordingly.

Vacuoles were noted in the odontoblastic layer throughout the samples, regardless of group. Langeland (1957) noted "vacuoles and other deviations from the normal in the odontoblasts" and stated that when they were observed in both the experimental and the control teeth, they could not be used as a criteria in judging the experimental material. He postulated that they could be a result of the fixation technique, or the result of normal physiologic aging processes. Langeland believed that vacuolization in the odontoblastic layer was not necessarily a manifestation of pathologic changes. In view of the apparent adequacy of the perfusion fixation technique in this study, and the maturity of the dogs, such cavities were probably physiologic and not pathologic. Some fatty degeneration in the odontoblasts of five to six year old dogs is probably within normal limits. Nevertheless, the results were considered to cancel each other out and were not considered to be due to experimental procedures.

The observers were very consistent in their pulpal analyses. In comparing the grade per specimen of each observer with the grade of each other observer, the grades were within one level of each other 97% of the time (see Table 8). The lack of marked variance in evaluation between the observers was accepted as indicating satisfactory observer reliability.

The Pulps

Eleven of the twelve control teeth demonstrated "normal pulp characteristics" for dog teeth. One control tooth had an obviously necrotic pulp. This was a lower right first premolar. The reason for necrosis of this pulp is not known, but most likely was due to poor lab procedures or pulp death due to unknown reasons and not the experiment itself. This tooth received no injections or cavity preparations, nor did the tooth anterior or posterior to it.

In the thirty day specimens, the pulp tissues were unremarkable except for some of the tissue subjacent to the cavity preparations. As noted in studies by Stanley (1968), pulpal tissues below cavity preparations can exhibit a variety of pathologic alterations. These include cellular displacement, inflammatory infiltrate, and abscess formation or "foci of necrosis." He also noted that reparative dentin formation in human pulp tissue sections infrequently occurs sooner than 30 days following an experimental procedure.

Samples of pulp tissue subjacent to the cavity preparations in this study showed mild inflammatory changes in most cases with no evidence of necrotic foci. The odontoblastic layer was thinner than the layer elsewhere, and contained fewer cells, with occasional instances of odontoblastic nuclei displaced into dentinal tubules. A localized layer

of amorphous, irregular reparative dentin formation was observed, and the predentin layer was thicker than in untreated areas of the pulp. Many studies have reported a direct relationship between deep cavity preparations and marked pulpal inflammation. If the preparations in this study had been cut deeper, impinging on the pulp, or if full crown preparations had been substituted for the buccal amalgam fillings, increased pulpal inflammation might have occured. Kim (1982) reported a clinical stituation in which the operator noted an increased incidence of pulpal necrosis following full crown preparations after the operator began to use PDL injections. Although his study was not controlled, he recommended that the PDL injection technique be used only for exodontia or endodontia, and not for procedures requiring pulpal vitality. The preparations in this study extended only minimally into the dentin, but were in the buccal aspect near the pulp horn, where the dentin is thin. The pulpal responses noted were considered to be mild and within normal limits for recovery.

If the concomitant PDL injections on these teeth had an additive inflammatory effect on the pulp, then the pulps of Group 3 (preparation plus PDL anesthetic injections) should have exhibited a higher degree of inflammation than those in Group 4 (preparation alone). Such was not the case--in fact, the overall mean grade for Group 3 was slightly

lower than the grade for Group 4 (1.48 vs. 1.54). Both grades were well within "normal" limits, however, and the difference is not considered significant. It could be concluded, then, that neither the cavity preparation alone nor the combination of cavity preparation and PDL injections resulted in any significant level of pulpal inflammation or degeneration. Thus the report of Kim (1982) that was concerned with the adverse effect on pulps of the combination of the PDL injection and operative procedures was not confirmed.

The pulps of the 15-day and 2-day specimens were also graded well within the limits of "normal," and did not vary significantly from the control teeth. Since these pulps were basically as "healthy" as the 30-day specimens, it would appear that there were no short term inflammatory effects due to PDL injections which resolved in thirty days.

It can be concluded that the PDL injection technique, as used and studied by these techniques, causes little if any pulpal damage, and that any damage caused was reversible.

The pulps of the teeth injected just prior to sacrificing did not exhibit any inflammatory changes, but they did display, in some cases, a higher number of intravascular erythrocytes. The vessels in two samples were extremely engorged and packed with RBCs. (One of these, #2ULI1, was a Group 4 tooth--15 day pre-killing anesthetic injection only--which in other

cases did not act in this manner. However, the two adjacent incisors received ten minute pre-sacrifice anesthetic injections, and it is postulated that these injections produced stasis and the vessel packing.) Kim (1982) noted a similar result. If the PDL injection occluded the entering blood vessels to the pulp, then perfusion fixation would not be able to readily remove the RBCs. As the engorged vessels were only noted in teeth in the immediate vicinity of ten-minute pre-terminal injections, and as the other tissues appeared to be adequately fixed, the overall fixation would seem to be good. Perhaps the occlusion of the vessels was only partial and enough fixative entered the pulp space, but also retained many RBCs. It could also be suggested that additional fixative penetrated when the specimens were soaking in solution prior to decalcification.

Since only two of nine teeth considered here demonstrated a marked number of retained erythrocytes in the pulp, the PDL injection does not seem to routinely shut down the entering blood vessels. It could be, however, that sufficient time elapsed between injection and perfusion to allow vessels to re-open and permit passage of fixative. The study by Kim (1982) reported a marked immediate post-injection stasis of pulpal blood flow, but this was transient. Clinical studies have also noted that the PDL injection-induced anesthesia

may only last a few minutes. Further, it is not always effective with the first injection. An attempt was made to kill the dogs at about ten minutes post-anesthetic injection, but this was approximate. Two of nine specimens with engorged blood vessels may, then, be within expected ranges.

Are there any long-term harmful effects from this pulpal blood flow stasis? Two of nine (22.2%) ten-minute pre-terminal injected teeth showed intravascular non-cleared RBCs in the pulps, which was possibly due to the injections. Hypothetically, at least 22.2% of all the injected teeth had the same blood flow stasis immediately post-injection. If this lead to permanent pulpal damage, it should have been seen in 22.2% of the pulps of injected teeth. However, the fact that none of the 2, 15 or 30 day samples showed necrotic pulps suggest that no lasting harmful effects were due to cessation of blood flow.

As supported by the ANOVA, there was no significant statistical differences between the pulpal inflammation recorded for any of the groups. Further, the Scheffe' test showed that there was no statistical difference between each group and the controls. Since the mean scores for all the groups ranged between 1.28 and 1.59, with one representing no inflammation and two representing mild inflammation, it can also be concluded that, overall, the pulps in this

study were within the range of "normal," regardless of experimental procedure performed.

The mechanism of anesthetic action remains open to debate. Although Kim's study (1982) reported a reduced rate of clinically successful anesthesia when there was no vasoconstrictor in the solution, not everyone supports this conclusion. Malamed (1982) reported relatively equal percentages of successful anesthesia regardless of vasoconstrictor content of the anesthetic. Kaufman (1983) reported clinical trials that used Marcaine with 1:200,000 Epinephrine and showed unsatisfactory results. He stated that the epinephrine concentration was related to duration of anesthesia, but not necessarily to induction of anesthesia.

This study supports the work of Neidle and Liebman (1964), Edwall and Scott (1971), Olgart and Gazelius (1977) and Kim (1982), in that a PDL injected anesthetic solution containing epinephrine appears to have the capability of occluding the entering vessels of the pulps and thus reducing or interfering with pulpal blood flow. This may or may not be related to induction of anesthesia, and may or may not be related to duration of anesthesia. Further study is needed.

The PDL and Adjacent Structures

Group 7 specimens (ten-minute anesthetic injection) showed the immediate effects of the PDL injection in the periodontium. The findings reported in studies by Brannstrom et al. (1982) and Walton and Garnick (1982) were, for the most part, duplicated in this study. Needle penetration separated and tore the submucosal connective tissues. Broken strands of collagen fibers were scattered along the track, and cellular disruption was evident. Numerous extravasated RBCs were noted in and around the track. In some cases the needle point came to rest in the bone of the alveolar crest, chipping out a wedge-shaped portion and creating a fracture line below it. In other cases the needle point gouged into the root surface, cleaving away the cementum and leaving hard tissue remnants in the void. This tissue damage appeared to be limited to the immediate vicinity of the needle track. Tissue damage could not be related to the spread of the anesthetic solution. Although the solution is injected under high pressure, its force may be dissipated and reduced when it reaches compliant tissues.

In the two day specimens, the needle penetration produced an acute inflammatory reaction, characterized by a dense cellular infiltrate of PMNs, lymphocytes, plasma cells, and macrophages. The inflammatory reaction appeared

to spread beyond the actual needle track for a short distance. None of the specimens showed signs of root resorption or other hard tissue effects.

In the 15 day specimens, the needle damage was characterized by some reduction of inflammation in the connective tissue, but a marked resorption of hard tissue was noted in some cases. In the most severe example, specimen #2LRP2M, an extensive resorption of the alveolar crest and of the adjacent buccal root surface was seen. Osteoclasts and odontoclasts were noted along the resorptive fronts and there was no sign of resolution or repair of either lesion. This is in contrast to the findings of Brannstrom et al. (1982). They noted the same type of alveolar and dentinal resorption at two weeks, though to an apparently lesser extent. However, they reported that it was resolving and a "layer of cementoblasts was present." Their study did not extend beyond two weeks. Walton and Garnick (1982), in their 25 day research, detected bone resorption but no dentinal resorption. They noted bony repair as "the most prominent feature" at 25 days.

The 30 day specimens were noteworthy for an increased degree of root surface resorption present adjacent to probable needle penetration sites. Of the 32 roots injected, eight showed definite signs of tissue damage and six showed possible

damage thirty days after the injection. Hard tissue damage in the form of buccal root surface resorption was the most frequent sign. In some cases the resorption was large enough to be visualized without the aid of magnification. More important, it appeared to be an active process with numerous giant cells noted in resorption lacunnae, and showed no signs of reversal or repair. Granulation tissue filled the lumen of the scalloped resorptive areas, but little fibrosis was observed. A few cases appeared to have small areas of inactivity along the resorption front, with possible formation of a cementoid layer, but this was the exception, and abutting areas of active resorption could often be seen in the same specimen. These findings are in definite contrast to the findings of Brannstrom et al. (1982) and Walton and Garnick (1982), who reported that all tissue damage was either resolved or in a state of repair 14 to 25 days post-injection. Both studies used 30-gauge needles, though Brannstrom used the Peri-Press(TM) PDL syringe while the other study used a conventional syringe. Both studies were performed on monkeys. A species difference in the response to injection or in the rate of healing between monkeys and dogs may have influenced the results.

The resorption noted in this study appeared several days post-injection. None of the two-day specimens exhibited root resorption even though the inflammatory tissue response

was prominent, and the 15-day specimens showed only minimal effect. In the 30-day specimens, however, the resorption was frequent and marked. The lesions had a scalloped appearance, with frequent undermining of the cementum, and the defect was more widespreading than that seen initially. Some specimens showed additional areas of undermining resorption penetrating beneath layers of apparently intact cementum and undamaged PDL, and not apparently connected to larger adjacent areas. The cementum was only lost in a small superficial area where the resorption seemed to initiate its burrowing effect. Evidence of the tunneling of the resorptive process was graphically illustrated on two adjacent slides (#3ULP3-2 and #3ULP3-3). In one, perforation through the cementum was seen with spreading in the dentin (Figure 16a). In the other, the resorptive lesion was entirely encased in the root with an intact layer of cementum externally (Figure 16b). Although this could indicate repair or regeneration of the cemental layer, it is more likely that it represents a section taken beyond the area of cemental perforation. Undermining resorption here may have been enlarging the defect in all directions past the original entrant area of cemental disruption.

The evidence of an active resorptive process thirty days post-injection is a notable finding worthy of further investigation to answer the following questions: Did the

PDL injection initiate the process as a response to direct damage or indirectly due to inflammation? Will the process continue to erode the dentin? Will it just take a little longer for the process to either reverse and/or repair itself? Will the defect fill with fibrous tissue or continue to eventual tooth loss? Idiopathic external resorption in human teeth is not an uncommon finding, and is not usually known to be spontaneously reversible. However, it is also not usually severe and rarely causes loss of the tooth involved.

The preponderance of evidence in this study seems to indicate that the needle damage initiated the resorption. All the resorptive areas noted were on the buccal root surfaces, most of them toward the mesial or distal aspect of the root (as far as could be determined), and all were located approximately at the level of the needle point as it was placed for the injection. It is possible, however, that factors other than needle point trauma were involved. Perhaps the high pressure of the anesthetic in the PDL tissues following the injection led to a localized focus of pressure necrosis which, in turn, generated a resorptive response. An acute inflammatory response was noted at two days and root resorption at fifteen days. Was there tissue necrosis in the time interval between? This is possible but doubtful, given the complete repair of the PDL connective tissue at 30 days.

Could the anesthetic solution produce a vascular stasis and thus necrosis followed by resorption? Again, this is doubtful, given that no preponderance of intravascular RBCs were observed in the PDL specimens in the ten-minute specimens.

Why doesn't subgingival scaling and root planing, or root surface cavity preparation, induce the same resorption? In these procedures most of the trauma induced is above the epithelial attachment and only in superficial tissue contact. The PDL injection trauma is induced in, and mostly confined to, the narrow PDL space between cementum and alveolar bone, where the blood supply is not as abundant as in other areas which demonstrate more rapid healing. Endodontic periapical surgery also affects the root surface within the alveolar socket, but the surgical access opening is large, and the resultant blood clot and granulation tissue healing process is more vascularized and not subject to pressure. Periodontal surgery, too, often involves root manipulation in this vicinity--even including alveoloplasty--but, again, the healing process is on a much larger scale and unstressed state. Orthodontic movement can cause root surface resorption if the forces are too great. Perhaps, then, it is the confined nature of the traumatized area which contributes to the initiation of resorption instead of healing.

Further studies should be directed toward investigating the significance of this finding and should be extended over a longer time period. External root resorption is repairable, but not reversible, since dentin can only form from pulp tissue. Will this resorptive process cease after time and become lined with cementum? How extensive could it become? What would be expected in the presence of periodontitis, for example, or with a systemic condition such as diabetes that impairs healing, or with the addition of systemic steroids? Perhaps these test procedures can be used as an experimental model to study resorption.

Spread of Injectate

The tissues injected with India Ink and cleared in methyl salicylate provided an excellent three-dimensional visualization of the spread of injectate. The solution was heavily concentrated in the PDL space at the injection site, as could be expected, and spread laterally through the fenestrations of the lamina dura to partially envelope the adjacent teeth. The apical spread became more dispersed and tended to form in clumps, probably due to the density of the soft tissue in marrow spaces. It approached, but did not appear to enter the mandibular canal. This is in contrast to the work of Kim (1982) which reported that the

injectate readily entered the mandibular canal. Perhaps that study injected a greater volume of solution, or injected under greater pressure, or used dogs with younger, more porous bone, or perhaps the differences were due to a combination of these factors.

Smith and Walton (1983) stated that this widespread dispersion of injectate into the marrow spaces made the PDL injection synonomous with an intraosseous injection. It is doubtful, however, than an intraosseous injection would deposit much injectate into the actual PDL space, since that would be going against a pressure gradient, i.e., from a compliant tissue to a less compliant tissue. Rather, these results show that the PDL injection does, in fact, largely fill the PDL space, and also deposits a fairly large amount into the medullary bone. The spread of the injectate seems to differ from that of the intraosseous injection.

Does the pressure of the injection relate to the mechanism of anesthesia? Pashley et al. (1981) noted the relative noncompliance of PDL space tissues and theorized that the high pressure involved in the PDL injection might be involved with the anesthetic action. The lamina dura of the alveolar bone, however, is a cribriform plate. Even though the tissues are noncompliant, multiple fenestrations in the bone allowed notable spread of the India Ink solution beyond the PDL space, into the marrow spaces. This study

tends to support the work of Walker et al. (1978) who theorized that any PDL pressure increases would be rapidly attenuated by this low fluid resistance. It could be, however, that the initial injection-induced pressure may temporarily inhibit pulpal circulation, which would support the findings of Kim (1982), but for a different reason. The cause of the flow cessation would be pressure and not the vasoconstrictor of the anesthetic solution. Other studies have noted, however, that infiltration injections and even the inferior alveolar block injection can decrease pulpal blood flow when vasoconstrictors are used. These are quite remote from the PDL, so pressure-induced flow reduction would not be involved.

The question remains. Is it vasoconstriction, pressure induced and/or drug induced, or is it simply anesthetic action on the transmembranous ion exchange of the pulp nerves that produces anesthesia? Perhaps it is a combination of the above. Clearly, more research is required.

Dogs vs. Monkeys vs. Humans

It should be remembered that this study was conducted on dogs. The studies of both Brannstrom et al. (1982) and Walton and Garnick (1982) were conducted on monkeys. When extrapolating findings of all these studies, the differences between these experimental models must be kept in mind.

The apical canals of dog teeth are multiple and finer than the single canals of human or monkey teeth. The study by Kim (1982) noting the cessation of blood flow in pulps after PDL injections was also performed on dogs. Another study by Takahashi (1982), in which Kim was a co-author, noted the several small apical foramina of dogs in contrast to findings on monkey teeth, and suggests that variations "could be attributed to anatomical differences in the experimental animal." Perhaps the pulpal blood flow cessation after PDL injection is unique to dogs.

Furthermore, the dogs used were mature at five to six years of age. Age of the monkeys involved in the other studies is not known, although they were reported to have full or nearly full adult dentition. If this dentition was recently erupted, however, the apical foramina could still be not fully closed. Also, these older dogs demonstrated heavy supragingival calculus deposits around posterior teeth, with accompanying gingivitis. Did bacteria from this condition contribute to the external root resorption noted? This question was not answered.

Healing capabilities should also be considered. Perhaps Brannstrom et al. and Walton and Garnick observed tissue repair after 14-25 days because of their animal model, or because of a relatively young age of animal. Did the

monkeys heal faster than the dogs? How does this relate to humans?

Not all the variables examined in this and other related studies could be examined in humans, but more research is required with both lower animal and human models before all the ramifications of the PDL injection technique are resolved.

SUMMARY

The PDL injection technique was evaluated with 88 individual injections given to a total of 52 dog teeth, while 18 teeth were not injected and served as controls. The Ligmaject(TM) periodontal ligament injection syringe with a 30-gauge extra short needle was utilized to inject one of three solutions: 1) 2% Lidocaine with 1:100,000 Epinephrine, 2) India Ink, and 3) a pre-polymerized solution of methyl methacrylate. Nine experimental groups were generated: 1) the control teeth which received no injections, 2) 30 day injection only teeth, 3) 30 day injection plus buccal amalgam restoration teeth, 4) 30 day buccal amalgam restoration only teeth, 5) 15 day anesthetic injection teeth, 6) 2 day anesthetic injection teeth, 7) ten minute anesthetic injection teeth, 8) ten minute India Ink injection teeth, and 9) ten minute Methyl Methacrylate injection teeth.

The animals were killed by intrathecal perfusion at thirty days. Some specimens were block sectioned, decalcified, paraffin embedded, microtome sectioned, mounted, and stained with either hematoxylin and eosin, Masson's Trichrome, or Gomori's Stain. Histologic examination was made for the evidence of inflammation or relevant tissue alterations. Some block specimens were decalcified and cleared in methyl-salicylate as block tissues for analysis of injectate spread

in the tissues. Specimens injected with plastic were dissolved in potassium hydroxide to macerate the tissue and leave a positive plastic cast of solution spread.

Ten-minute pre-killing injection specimens showed PDL tissue separation and maceration, and alveolar bone and root cementum gouging and chipping in the vicinity of the needle penetration. Two-day specimens showed an acute inflammatory reaction in and around the needle track. In 15-day specimens, resorption of both alveolar bone and root surface dentin was noted. The inflammatory reaction was subdued and repair of the PDL connective tissues was progressing in some cases. At 30 days, significant active root resorption was noted in many specimens. Bone resorption was minimal and reversed in many areas, and connective tissue of the PDL was repaired.

Histological analysis of the pulps of the teeth in the first seven groups for the degree of inflammation was conducted by three qualified observers, independently, in a blind study. The mean pulp grades of all the groups ranged from 1.28 to 1.59 on a scale from 1 = no inflammation to 4 = pulpal necrosis. An analysis of variance revealed no statistical difference between the mean pulp scores of all the groups, and Scheffe' tests showed no statistical difference between the mean pulp score of each group in comparison to that of the control group.

The cleared specimens of the India Ink PDL injections graphically demonstrated in three dimensions the extensive spread of injectate in the tissues. The ink spread throughout the PDL, disseminated laterally to encompass adjacent teeth, and dispersed apically to congregate in the intrabony medullary spaces.

The polymerized methyl methacrylate injectate resulted in small clumps of yellow plastic after the tissues were dissolved, and did not demonstrate the injection path in the manner intended.

The PDL injection technique appears to be a safe procedure in relation to the pulp, PDL space tissues, and alveolar bone. It may, however, initiate foci of external resorption in the root surface. Further investigation is definitely warranted.

CONCLUSIONS

The injection of 2% Lidocaine with 1:100,000 Epinephrine into the PDL space of dog teeth utilizing the Ligmaject(TM) PDL syringe with 30-gauge extra short needles indicated the following:

1. If the procedure occludes entering pulpal blood vessels immediately after injection, the effect is transient.

2. The technique does not create significant permanent alterations of the pulpal tissues.

3. In the PDL, tissue damage is produced by the needle penetration. This damage includes soft tissue separation and chipping or gouging of both cementum and alveolar bone. The damage is localized and does not extend far beyond the actual needle track, at least initially. The above damages are only upon injection--further damage occurs with biologic changes.

4. In two days, localized acute inflammatory reactions occur along the path of needle penetration.

5. At two weeks, external root resorption of cementum and dentin and alveolar bone resorption is seen.

6. At thirty days post-injection, many areas of active surface resorption of the root are seen with only minimal signs of arrest. The alveolar bone resorption showed reversal lines and formation of new bone.

7. This study indicates that the PDL injection technique alone is not harmful to the pulp tissues, with or without a vasoconstrictor, or in combination with cavity preparation.

8. Damaged PDL tissues or alveolar bone appear to be healing at thirty days.

9. The external resorption of the root surface does not appear to be resolving at thirty days. Such root surface resorption may or may not be extensive, and may or may not be self-limiting.

10. The injected solution spreads down the PDL space to the apex, and laterally through the alveolar cribriform plate to encompass adjacent teeth. Apically, it concentrates in the intrabony medullary spaces. It does not appear to enter the mandibular canal.

11. The results of this study indicate that the PDL injection technique using the Ligmaject(TM) PDL syringe with 30-gauge extra short needles, and injecting 2% Lidocaine with 1:100,000 Epinephrine, is an acceptable procedure in terms of safety to the pulp in dogs. In relation to the periodontium, one should be aware that local foci of external root resorption may be initiated, the final ramifications of which are still unknown, and may be serious.

BIBLIOGRAPHY

Akinosi, J. A new approach to the mandibular nerve block. Brit. J. Oral Surg. 15:83-87, 1977-78.

Allison, M. L. Considerations in the approach to pain-free dentistry. J.A.D.A. 64:351-362, March 1962.

Bennett, C. R. Monheim's Local Anesthesia and Pain Control in Dental Practice. The C. V. Mosby Company, Saint Louis, Fifth Edition, 1974.

Birchfield, J. and Rosenberg, P. A. Role of the anesthetic solution in intrapulpal anesthesia. J. Endod. 1:26-27, January 1975.

Brannstrom, M., Nordenvall, K., and Hedstrom, K. Periodontal tissue changes after intraligamentary anesthesia. J. Dent. Child. 49:417-423, November-December 1982.

Carroll, M. J. Tissue necrosis following a buccal infiltration. Brit. Dent. J. 149:209-210, October 7, 1980.

Carter, R. B. and Keen, E. N. Journal of Anatomy (London) 108:433, 1971.

Chenaux, G., Castagnola, L., and Colombo, A. L'anesthesie itraligamentaire avec la seringue "Peripress." Revue mensuelle suisse d'Odont. 86:1165-1173, November 1976.

Cohen, S. and Burns, R. C. Pathways of the Pulp. Second Ed. The C. V. Mosby Company, St. Louis, 1980.

Cooley, R. L., and Robinson, S. F. Comparative evaluation of the 30-gauge dental needle. Oral Surg. 48:400-404, November, 1979.

Copenhauer, W., Bunge, R., and Bunge, M. Bailey's Textbook of Histology. The C. V. Mosby Co., St. Louis, 1971, 16th Edition.

Dobbs, E. C. L-arterenol as a vasoconstrictor in local anesthesia. J.A.D.A. 40:433-434, April 1950.

Dover, W.R. Dental anxiety. . .and other heavy stuff. Oral Health. 68:7-8, February 1978.

Edwall, L. and Scott, D. Influence of changes in microcirculation on the excitability of the sensory unit in the tooth of the cat. Acta. Physiol. Scand. 82:555-566, 1971.

Evans, H. E., and Christensen, G. C. Miller's Anatomy of the Dog. Second Ed., W. B. Saunders Co., Philadelphia, 1979.

Faulkner, R. K. The high-pressure periodontal ligament injection. Brit. Dent. J. 154:103-105, February, 1983.

Firestein, S. K. Patient anxiety and dental practice. J.A.D.A. 93:1180-1187, December 1976.

Fuhs, Q. M., Walker, W. A., Gough, R. W., Schinholer, W. G., and Hartman, K. S. The periodontal ligament injection: histological effects on the periodontium in dogs. J. Endod. 9:411-415, October 1983.

Galbreath, J. C. and Eklund, M. K. Tracing the course of the mandibular block injection. Oral Surg. 30:571-582, October 1970.

Gale, E. N. Fears of the dental situation. J. Dent. Res. 51:964-966, August 1972.

Giovannitti, J.A. and Nique, T. A. Status report: the periodontal ligament injection. J.A.D.A. 106:222-224, February 1983.

Gow-Gates, G. A. E. Mandibular conduction anesthesia: a new technique using extraoral landmarks. Oral Surg. 36:321-328, September 1973.

Gow-Gates, G. A. E. and Watson, J. E. The Gow-Gates mandibular block: further understanding. Anesth. Prog. 24:183-189, 1977.

Gustainis, J. R. and Peterson, L. J. An alternative method of mandibular nerve block. J.A.D.A. 103:33-36, July 1981.

Hammerstrom, L. Specific uptake of some drugs in ameloblasts and developing enamel. Acta Odont. Scand. 28:187-195, 1970.

Herzberg, F. Critical analysis of pain incidence during 1,508 operative procedures with rotating instruments. J.A.D.A. 55:515-521, October 1957.

Holroyd, S. Clinical pharmacology in dental practice. The C. V. Mosby Co., St. Louis, 1974.

Ingle, J. I. and Beveridge, E. E. Endodontics. Second Ed., Lea and Febiger, Philadelphia, 1976.

Jorgensen, N. B. and Hayden, J. Sedation, Local and General Anesthesia in Dentistry. Second Ed., Lea and Febiger, Philadelphia, 1973.

Kaufman, E. Intraligamentary anesthesia, Lecture presented at Oregon Health Sciences University, February 18, 1983.

Kaufman, E., Galili, D. and Garfunkel, A. Intraligamentary anesthesia: a clinical study. J. Pros. Dent. 49:337-339, March 1983.

Kim, S. and others. Interseptal or ligamental injection: clinical and physiological investigations. Proc. Am. Assoc. of Endodontists, Annual Session, Phoenix, April 1982.

Kraintz, L. and Conroy, C. Blood-volume measurements of dog teeth. J. Dent. Res. 39:1033-1036, 1960.

Kramer, H. S. and Mitton, V. A. Complications of local anesthesia. D.C.N.A. 17:443-460, July 1973.

Lafargue, R. Anesthesie intraligamentaire - possibilities d'une nouvelle technique. Acta Odont. Stomatol. 103:551-572, 1973.

Lane-Petter, W., Ed. Animals for Research. Academic Press, New York, 1963.

Langeland, K. Tissue changes in the dental pulp. Odont. Tidskrift, 65:4, 1957.

Laskin, D. M. Progress in pain control? J. Oral Surg. 31:423, June 1973.

Levy, T. P. An assessment of the Gow-Gates mandibular block for third molar surgery. J.A.D.A. 103:37-41, July 1981.

Lilienthal, B. A clinical appraisal of intraosseous dental anesthesia. Oral Surg. 39:692-697, May 1975.

Littner, M. M., Tamse, A., and Kaffe, I. A new technique of selective anesthesia for diagnosing acute pulpitis in the mandible. J. Endo. 9:116-119, March 1983.

Malamed, S. F. The periodontal ligament (PDL) injection: an alternative to inferior alveolar nerve block. Oral Surg. 53:117-121, February 1982.

Menke, R. A. and Gowgiel, J. M. Short-needle block anesthesia at the mandibular foramen. J.A.D.A. 99:27-30, July 1979.

Morse, D. R. Clinical Endodontology. C. C. Thomas, Illinois.

Najjar, T. A. Why can't you achieve adequate regional anesthesia in the presence of infection? Oral Surg. 44:7-13, July 1977.

Navia, J. M. Animal Models in Dental Research. University of Alabama Press.

Neidle, E. A. and Liebman, F. M. Effects of vasoactive drugs and nerve stimulation on blood flow in the tooth pulp and allied structures of the cat. J. Dent. Res. 43:412-422, June 1964.

Nelson, P. W. Injection System - Letters to the Ed. J.A.D.A. 103:692, November 1981.

Northrop, P. M. Practical technics in administration of local anesthetic agents. J.A.D.A. 38:444-448, April 1949.

Olgart, L. and Gazelius, B. Effects of adrenaline and felypressin (octapressin) on blood flow and sensory nerve activity in the tooth. Acta Odont. Scand. 35:69-75, 1977.

Pashley, E. L., Nelson, R., and Pashley, D. H. Pressures created by dental injections. J. Dent. Res. 60:1742-1748, October 1981.

Petersen, J. K. The mandibular foramen block: a radiographic study of the spread of the local analgesic solution. Brit. J. Oral Surg. 9:126-138, November 1971.

Phillips, W. H. Anatomic considerations in local anesthesia. J. Oral Surg. 1:112-121, April 1943.

Pohto, M. and Scheinin, A. Effect of local anesthetic solutions on the circulation of the pulp in rat incisors. Bibl. Anat. 1:46-49, 1960.

Poore, T. E. Maxillary nerve block: a useful technique. J. Oral Surg. 31:749-755, October 1973.

Rockert, H. O. E. Effects of local anesthetics on the respiratory activity in vitro of cells in the dental pulp. Scand. J. Dent. Res. 86:415-417, 1978.

Rood, J. P. The analgesia and innervation of mandibular teeth. Brit. Dent. J. 140:237-239, April 1976.

_____ Some anatomical and physiological causes of failure to achieve mandibular analgesia. Brit. J. Oral Surg. 15:75-82, 1977-78.

Seltzer, S. and Bender, I. B. The Dental Pulp. Second Ed., J. B. Lippencott Co., Philadelphia, 1975.

Shaw, J., Sweeney, E., Cappucino, C., and Meller, S. Textbook of Oral Biology. W. B. Saunders Co., Philadelphia, 1978.

deShazer, D. O. and Coffey, S. L. Is the mandibular block obsolete? Chronicle - Omaha Dent. Soc. 90-92, May 1981.

Sicher, H. and DeBrul, E. L. Oral Anatomy, Fifth Ed., C. V. Mosby Co., St. Louis, 1970.

Simon, D. E., Jacobs, T. L., Senia, E. S., and Walker, W. A. Intraligamentary anesthesia as an aid in endodontic diagnosis. Oral Surg. 54:77-78, July 1982.

Siskin, M., Ed. The Biology of the Human Dental Pulp. The C. V. Mosby Co., St. Louis, 1973.

Skoglund, A., Tronstad, L., and Wallenius, K. A microangiographic study of vascular changes in replanted and autotransplanted teeth of young dogs. Oral Surg. 45:17-28, January 1978.

Smith, F. N. and Walton, R. E. Periodontal ligament injection: distribution of injected solutions. Oral Surg. 55:232-238, March 1983.

Stanley, H. R. Design for a human pulp study, parts I and II. Oral Surg. 25:633-647, April 1968 and 756-764, May 1968.

Stanley, H. R. and Swerdlow, H. An approach to biologic variation in human pulpal studies. J. Pros. Dent. 14:365-371, April 1964.

Sticht, F., Woodbury, R. A., and Cox, R. A. The effects of certain drugs on the blood pressure within the tooth pulp. J. Oral Ther. and Pharm. 2:421-435, May 1966.

Takahashi, K., Kishi, Y., and Kim, S. A scanning electron microscope study of the blood vessels of dog pulp using corrosion resin casts. J. Endod. 8:131-135, March 1982.

Taub, J. A., McDade, G. O., Mitchell, J. N. and Stuver, F. E. Anesthesia for operative dentistry: time for a reevaluation. J.A.D.A. 100:181-183, February 1980.

Tonder, K. J. H. Blood flow and vascular pressure in the dental pulp. Acta Odont. Scand. 38:135-144, 1980.

Topazian, R. G. and Simon, G. T. Extraoral mandibular and maxillary block techniques. Oral Surg. 15:296-300, March 1962.

Traeger, K. A. Hematoma following inferior alveolar injection: a possible cause for anesthesia failure. Anes. Prog. 26:122-123, September-October 1979.

Travin, M. S. Ambulatory anesthesia then: sedation now. Oral Health, 68:11-14, February, 1978.

Walker, T. W., Ng, G. C. and Burke, P. S. Fluid pressures in the periodontal ligament of the mandibular canine tooth in dogs. Archs. Oral Biol. 23:753-765, 1978.

Walton, R. E. and Abbott, B. J. Periodontal ligament injection: a clinical evaluation. J.A.D.A. 103:571-575, October 1981.

Walton, R. E. and Garnick, J. J. The periodontal ligament injection: histologic effects on the periodontium in monkeys. J. Endod. 8:22-26, January 1982.

TABLES

Tables one through seven show the pulp scores recorded for each specimen, listed by group. The observers are numbered 1-3. The specimens are listed by dog and tooth and, in some cases, also by root. The dogs were numbered 0, 2, and 3. That is the first numeral in the specimen code. The next letter is either U for Upper or L for Lower. Next is either R for Right or L for Left. Next is tooth: I for Incisor, P for Premolar and M for Molar. Next is number of tooth, and finally, in some cases, M is for Mesial root or D for Distal root. Thus specimen #2URP2D is the distal root of the upper right second premolar of dog #2.

Table eight shows the percentage of times each observer agreed within one pulp score with each other observer in grading the pulps, listed by groups.

Specimen	Obs. #1	Obs. #2	Obs. #3
2LLI1	1	1	1
2LLP1	2	1-2	1
OURI2	1	1	1
3LRP1	4	4	3-4
3LLP1	2	1	1
2LRI2	2	1-2	1
2LRP1	1	1-2	1
2ULP1	2	1	1
OULP2	1	1	1
2LRM2	1-2	1-2	2
OULP1	1	1-2	1
3URI1	2	1-2	1

Table 1. Pulp grades given by each observer to the specimens of Group 1, the controls. Overall average was 1.50. Note the one tooth (3LRP1) which was evaluated disproportionally higher than all the others.

Specimen	Obs. #1	Obs. #2	Obs. #3
OULP2	1	1	1
OURP1	1	1-2	1
2ULI3	1-2	1-2	2
OURI1	1	1-2	1
2LRP4	1	2	1
3URI3	1	1-2	1
3ULP3	1-2	1	1
3LLP4	1	1-2	1
2URP3	1	1	1
OULI3	2	1	1
2LLI3	1-2	1	2
OULP3	2	1	1
2LRP3	1	1	2
3ULP2D	1	1	1
3LLP3	1-2	1-2	2
2URP2D	1	1	1

Table 2. Pulp grades given by each observer to the specimens of Group 2, the 30-day anesthetic injection only group. Overall mean pulp grade was 1.25.

Specimen	Obs. #1	Obs. #2	Obs. #3
3LRP4	2	1	2
3URP3	2	1	2
OURP3	1	1-2	1
OULP1	2	1	2
2LLP4	2-3	1-2	2
2LRI3	2	2-3	2
3ULI3	1	1-2	2
OURI3	1-2	1	2
2ULP3	2	1	1
2URI3	1	1	1
3LLI3	1	1-2	1
OULI1	2	1	1
2LLP3	2	2-3	2
3LRP3	1	1-2	1
2ULP2D	1	1-2	1

Table 3. Pulp grades given by each observer to the specimens of Group 3, the 30-day anesthetic injection plus buccal amalgam preparation teeth. Overall mean grade for this group was 1.49.

Specimen	Obs. #1	Obs. #2	Obs. #3
3LLP2D	1	1-2	1
0URP2	2	1-2	2
2LLI2	1	1	1
3LLP3	2	1-2	1
0ULI2	2	2	1
2URP2D	3	1-2	3
3URI2	1	1-2	2
2URP2M	1	1-2	1

Table 4. Pulp grades of the specimens of Group 4, the 30-day buccal amalgam preparation only teeth. Overall mean pulp score was 1.54 with one pulp (2URP2D) rated at an average of 2.50. Note, however, that the mesial root of the same tooth (2URP2M) averaged 1.17.

Specimen	Obs. #1	Obs. #2	Obs. #3
2LRP2D	1	1-2	2
2ULI1	3-4	1-2	2
2LLP3	2-3	1	1
3URP2D	1	1	1
2LRP2M	2-3	1-2	2
3ULP1	1-2	1	1
2URI2	1	2	1
2LLP2D	2	1	1-2
3URP2M	1	1	1
3LRI1	3	1	2

Table 5. Pulp grades of the specimens of Group 5, the 15-day anesthetic injection only teeth. Overall average of this group was 1.53.

Specimen	Obs. #1	Obs. #2	Obs. #3
3LRP3	2	1	2
2URP1	1	1	3
3LLP2D	1-2	2-3	2
2ULP2M	1-2	2	1
3LRP2D	1	1	1
3ULI1	2	2	2
3LLP2M	1-2	1	1
2ULP2D	1-2	1	1
2LRI1	2	1	2

Table 6. Pulp grades of the specimens of Group 6, the 2-day anesthetic injection only teeth. Overall mean pulp score was 1.57.

Specimen	Obs. #1	Obs. #2	Obs. #3
2LRP2D	1-2	1	1
2LLP2M	1	1	2
2URI1	2	1-2	2
2LRP3	1	1	1
3ULP2D	1	1-2	1
2ULI2	1	1-2	1
2LLP2D	1	1	1
3LRI2	2	1	1
3URP1	2	1-2	1

Table 7. Pulp grades of the specimens of Group 7, the ten-minute anesthetic injection only teeth. Overall mean pulp score was 1.28.

		OBSERVERS		
		1:2	1:3	2:3
	1	100%	100%	100%
	2	100%	100%	100%
	3	100%	100%	100%
GROUPS	4	88%	100%	88%
	5	70%	90%	100%
	6	100%	88%	88%
	7	100%	100%	100%

Table 8. Comparison of pulp grades given between individual observers. 1:2 is comparing observer one with two, and so on. Listed as a percentage of grades in agreement (plus or minus one grade) by groups. Overall agreement rate was 97%.

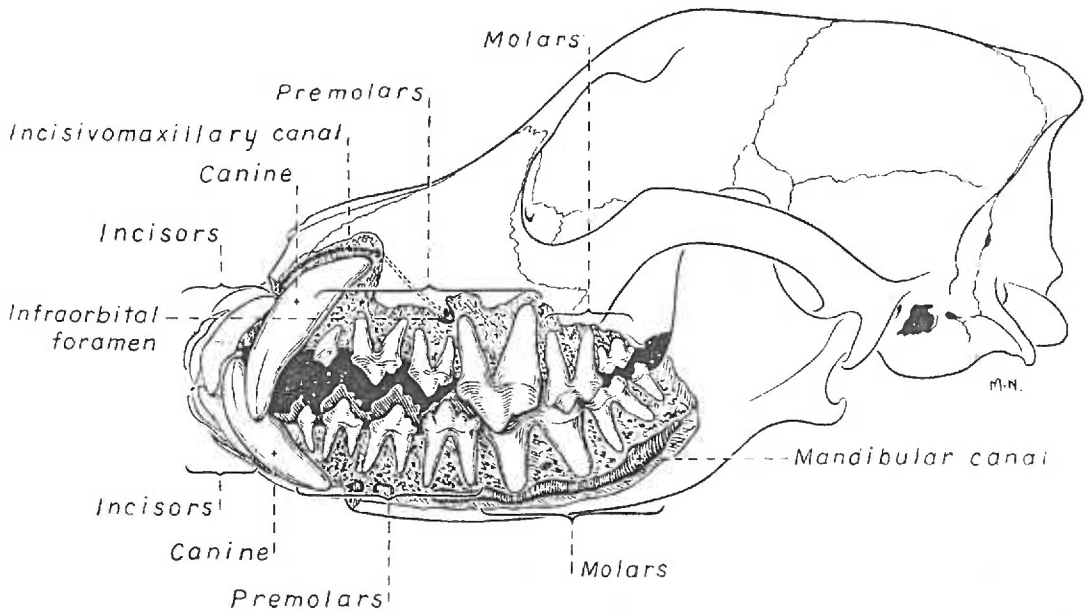


Figure 1. Schema of mandible, maxilla, and teeth of a mature dog. Note the relative crown size, root size, and position of each. Note tooth designations--cuspids, fourth upper premolars, and molars were not injected in this study, but some were used as controls. From Miller's Anatomy of the Dog, 2nd Edition.

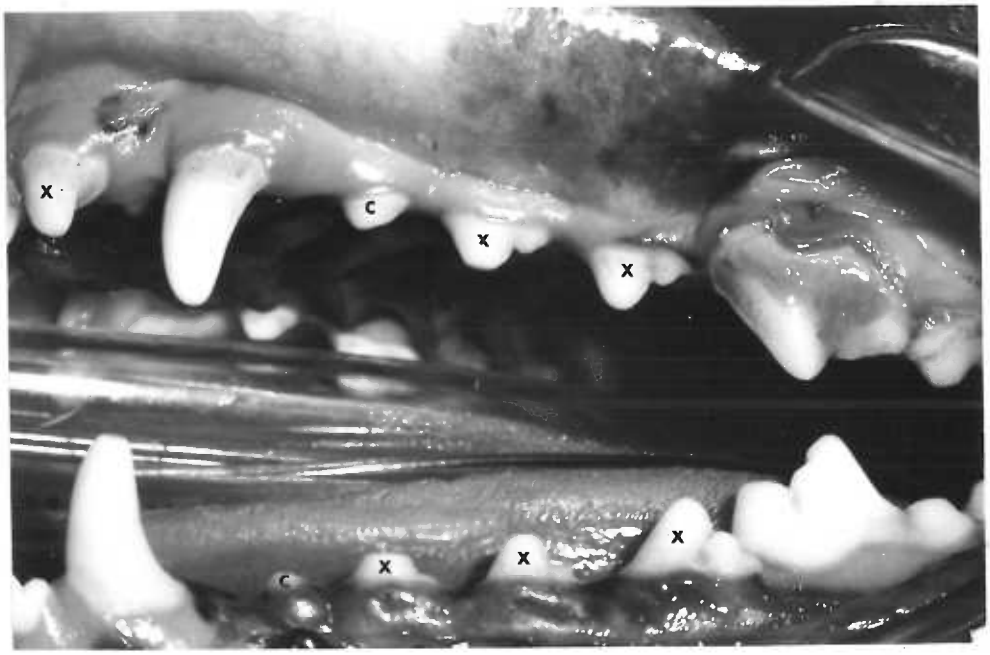


Figure 2. Photograph of left side view of dog #2 dentition. Teeth injected in this study are designated by "x"; those used as controls designated by "c".

Figure 3. Photograph of the Ligmaject(TM) periodontal ligament syringe, sterile 3-gauge extra short needle attached. A two inch advance of the "trigger" advances the plunger approximately 0.2 inch in the carpule.

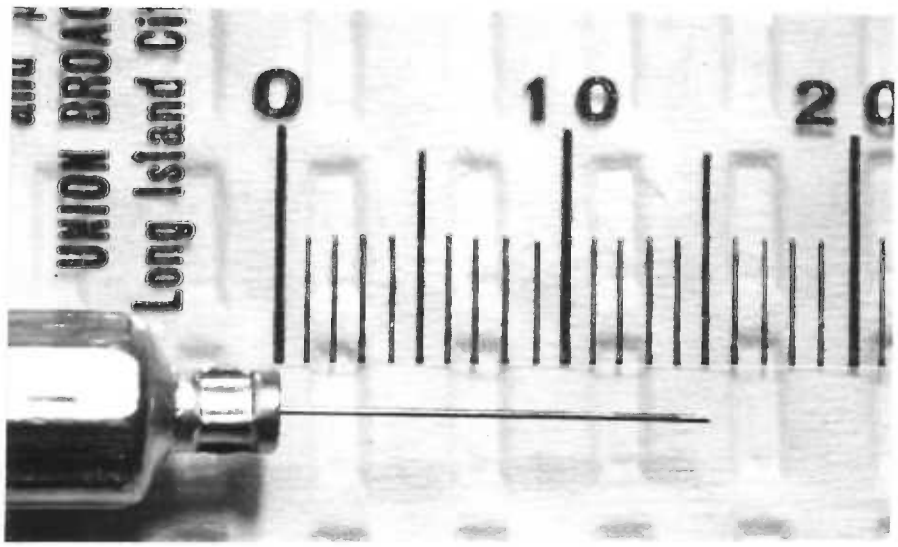


Figure 4. Photograph of the 30-gauge extra short (15 mm) disposable needle used with the PDL syringe. The length of a standard "short" needle is 19 mm and a "standard" long needle is 32 mm.

Figure 5. Photograph of the syringe and needle placed mesial to the upper right first premolar, ready for injection.

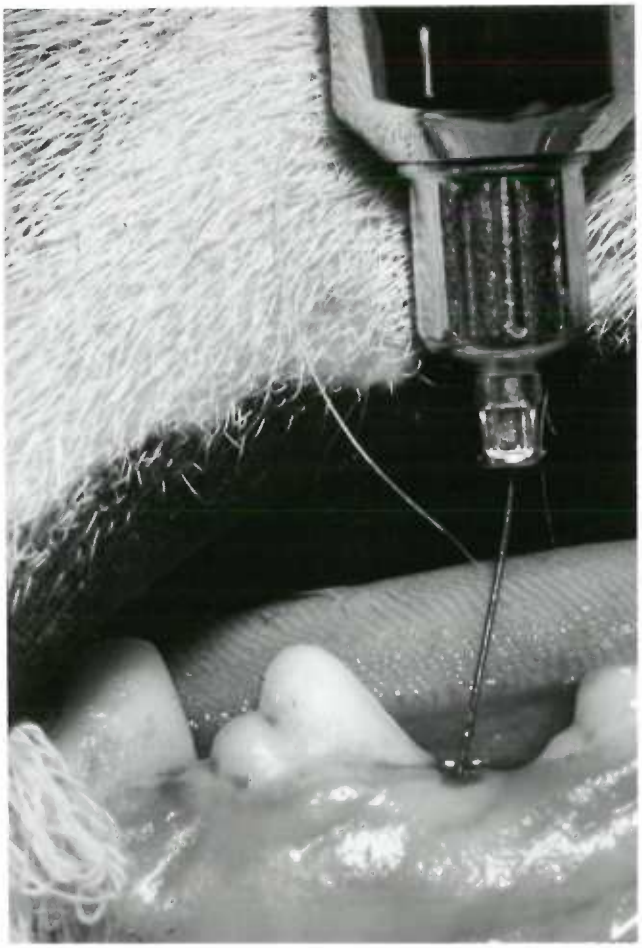


Figure 6. Photograph showing close-up of a PDL injection for the lower right fourth premolar with Ligmaject(TM). Note the angle of the needle as compared to the long axis of the tooth and note that the needle is beginning to bend at the hub.

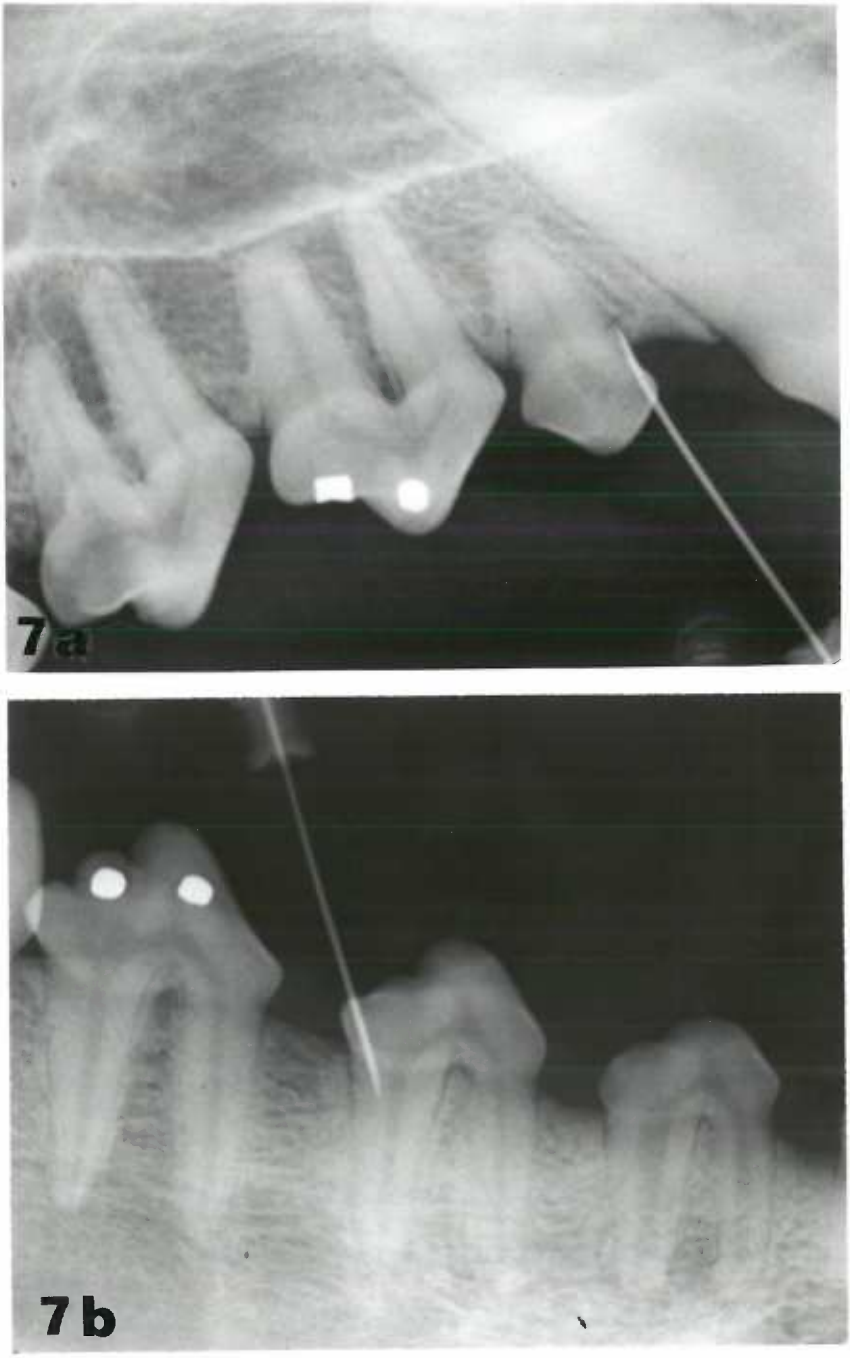
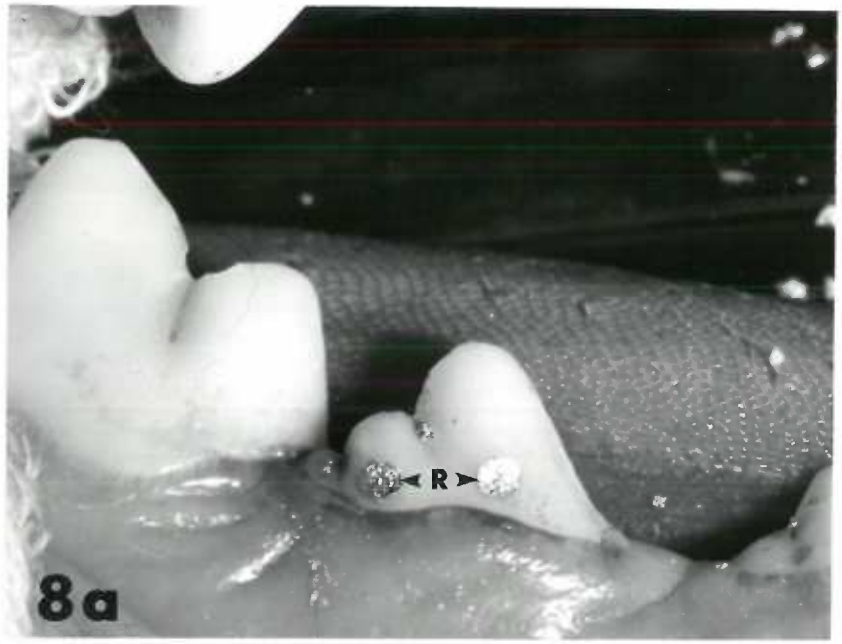


Figure 7. Radiographs showing needle placement for the PDL injection technique.

7a. Needle placement for injecting the upper right first premolar. Note the limited needle penetration.

7b. Needle placement for injection of the lower right third premolar. Note the angle of insertion required because of the cervical bulge of the crown.



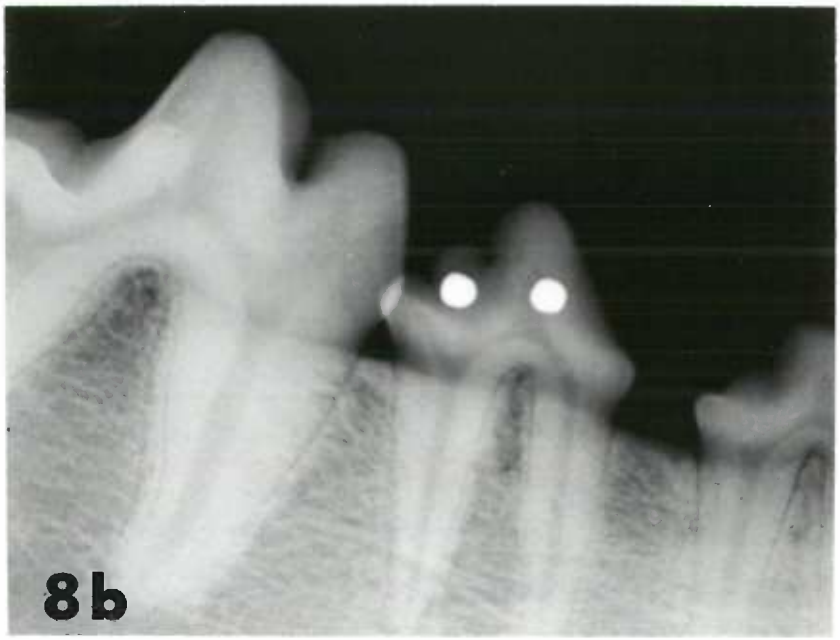


Figure 8a. Photograph showing the placement of two buccal amalgam restorations (R) in the crown of a lower right fourth premolar.

8b. Radiograph showing the two buccal amalgams in the same tooth. Note the close proximity of the restorations to the mesial and distal pulp horns.

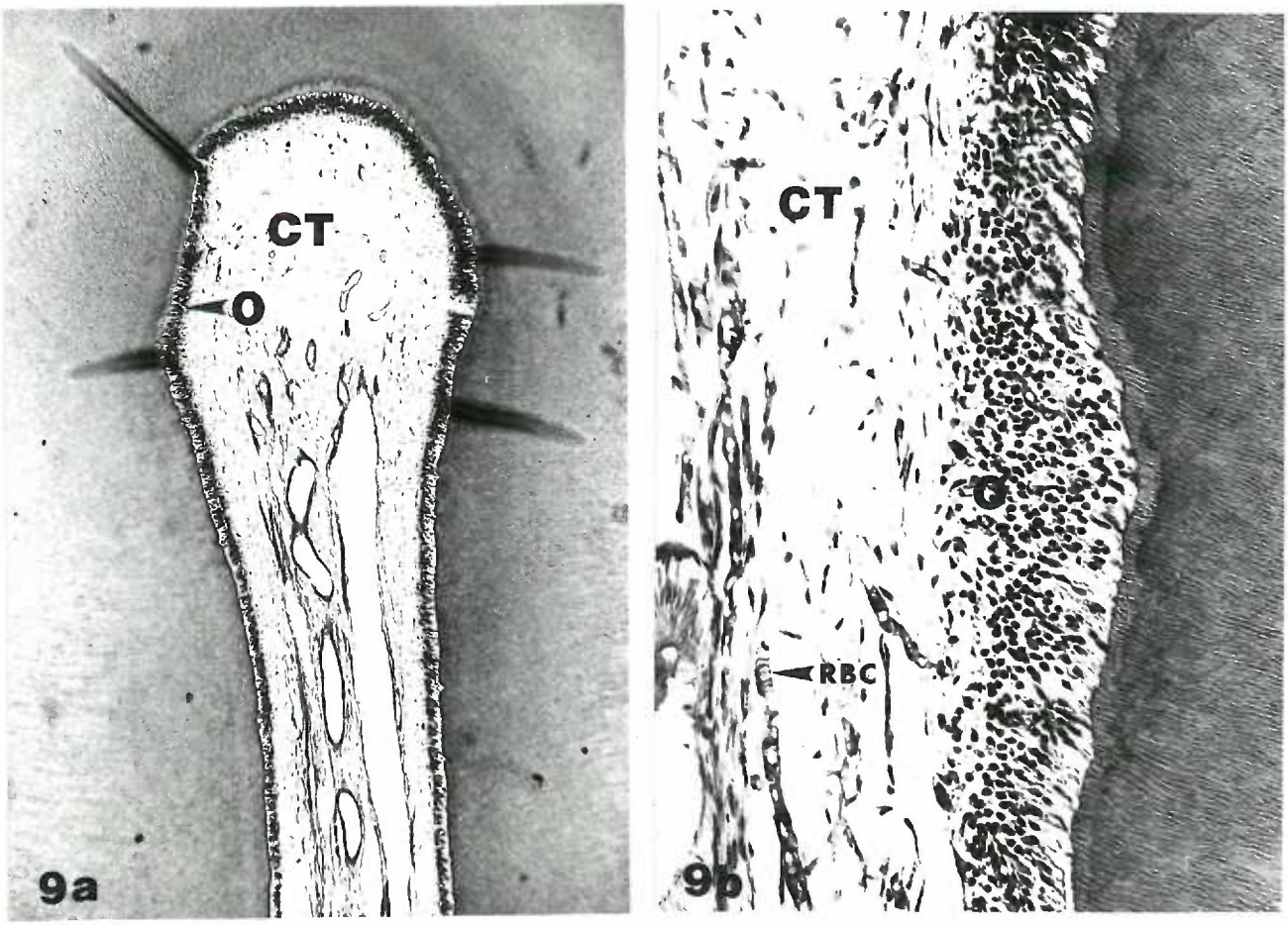


Figure 9. Photomicrographs showing longitudinal sections through the coronal aspect of pulps evaluated as Grade #1--no inflammation.

9a. Upper right third premolar. Note the pallisading odontoblastic layer (O) and the undisturbed loose connective tissue stroma (CT). H and E. x 10.

9b. Lower right third premolar. Note the thick odontoblastic layer (O) with few, if any, vacuoles, and the connective tissue stroma (CT) containing very few RBCs (RBC), indicating good fixation perfusion. H and E. x 64.

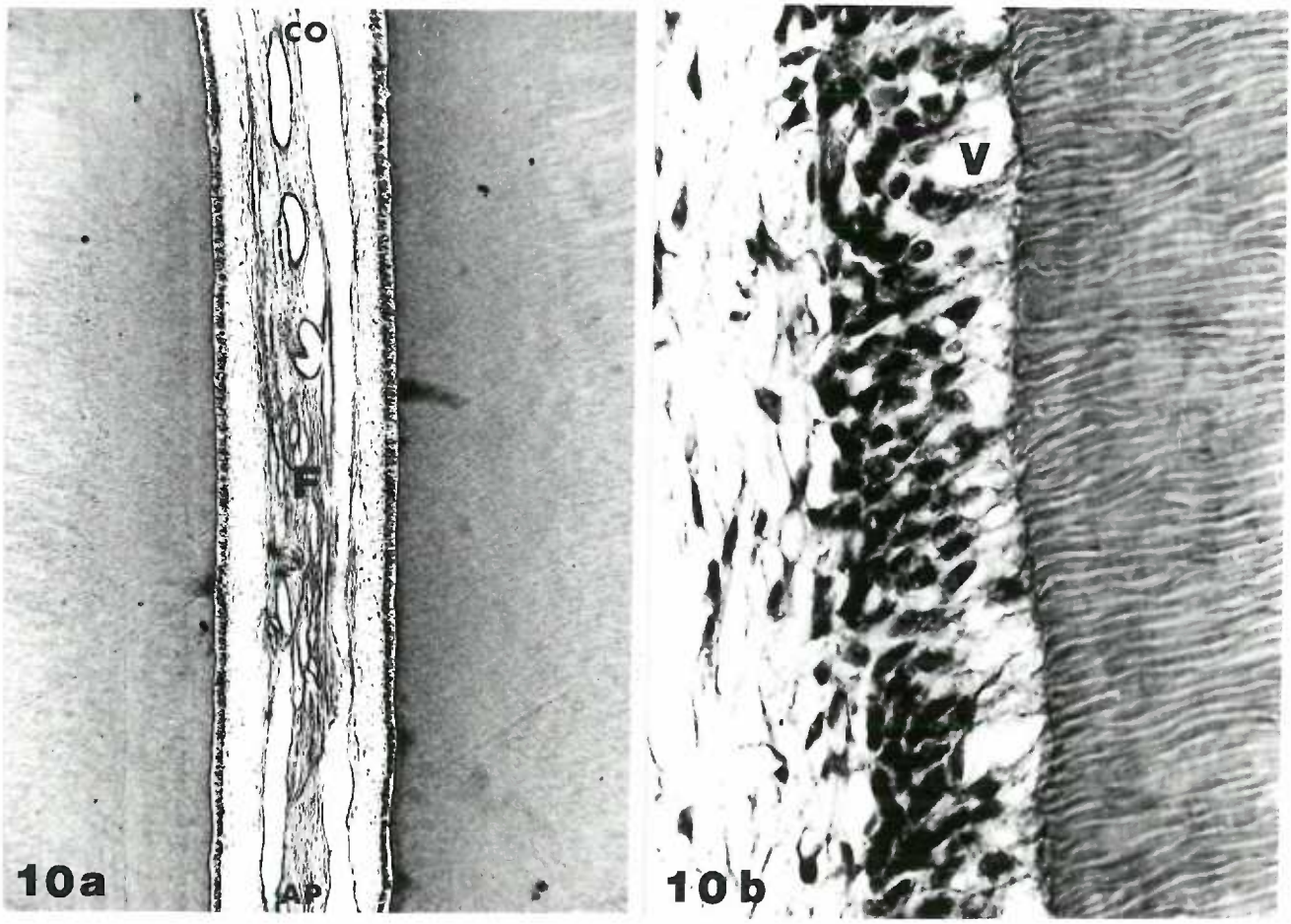


Figure 10. Photomicrographs showing longitudinal sections of Grade #1 pulps at the mid-root level.

10a. Note the thinning of the odontoblastic layer progressing from the coronal (CO) to the apical (AP) aspect of the pulp. Note also the increase in fibers (F) compared with Fig. 9. H and E. x 10.

10b. Higher power of the dentin-pulp interface. Note vacuoles (V) evident in this section. H and E. x 64.

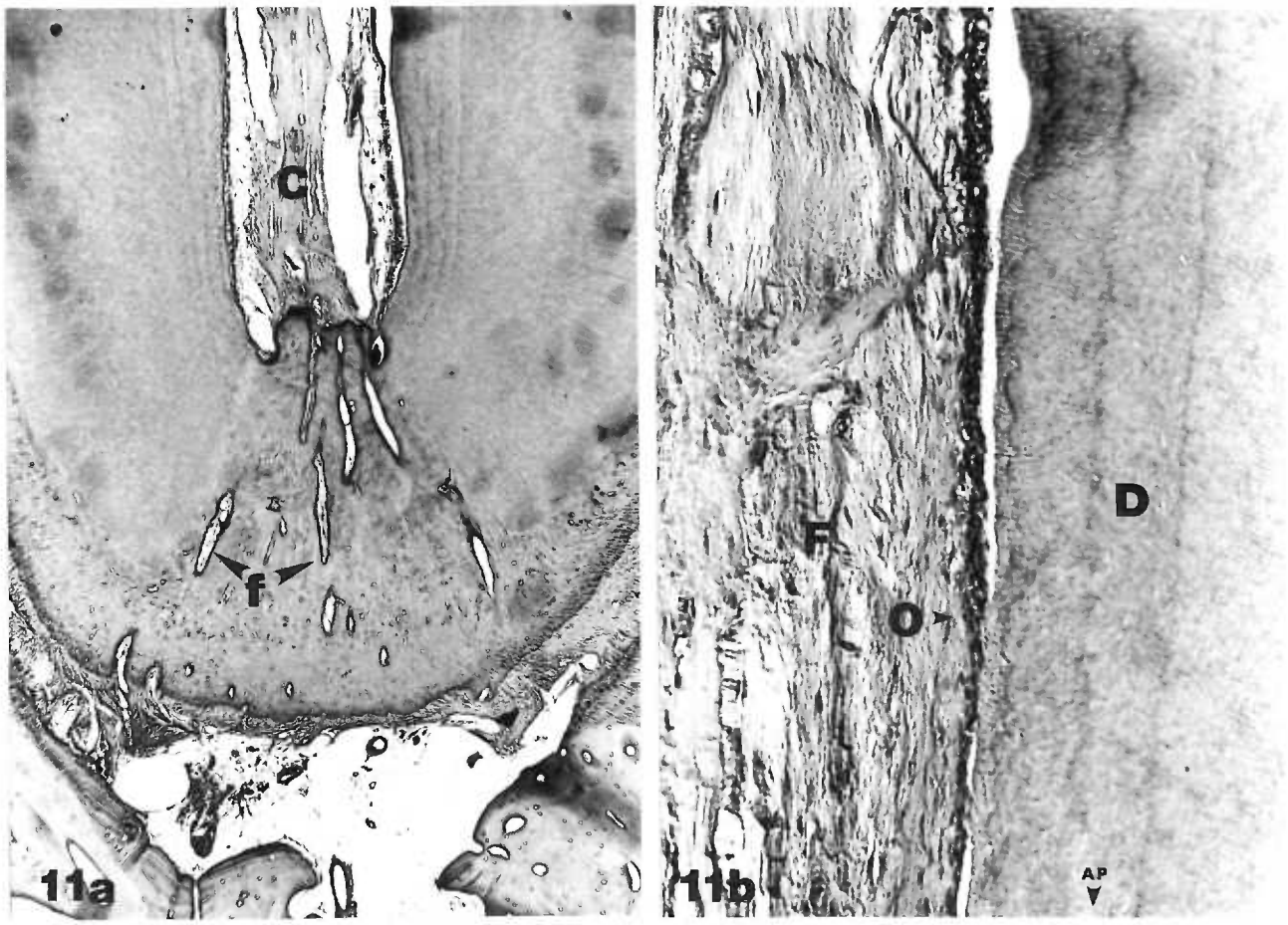


Figure 11. Photomicrographs showing the apical portion of teeth with Grade #1 pulps.

11a. Upper right third premolar. Note the abrupt cessation of the canal (C) and the wide apical layer of hard tissue perforated with multiple small foramina (f). H and E. x 10.

11b. High power of apical pulp, lower right third premolar. Note the density of fibers (F) and the thin odontoblastic layer (O) becoming nearly indistinct toward the apical aspect (AP). The separation between the odontoblastic layer and the dentin (D) is artifact. H and E x 64.

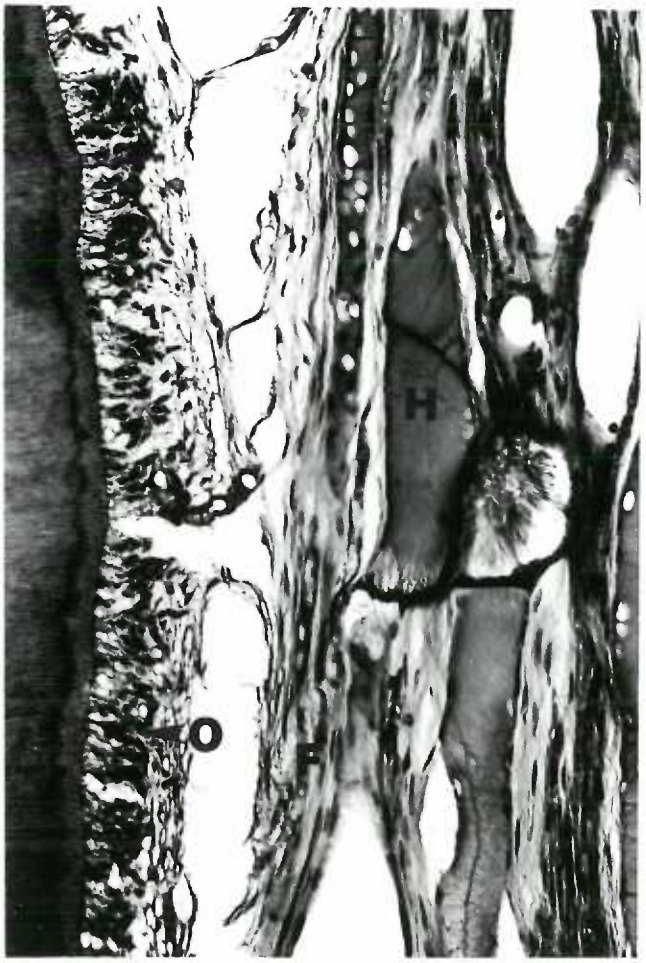


Figure 12. Photomicrograph of the pulp at the coronal level of the tooth #2LLP3, graded #2--moderate inflammation. Note the increase in vacuolization and disorganization of the odontoblastic layer (O), substantial fiber content (F), and apparent hard tissue deposits (H). H and E. x 64.

Figure 13. Photomicrograph of the pulp in tooth #2ULI1, evaluated by one observer as severely inflamed (Grade 3) and by the other two as mild to moderately inflamed. Large numbers of inflammatory cells are not evident, but note the thin, flattened odontoblastic layer (O). H and E x 64.

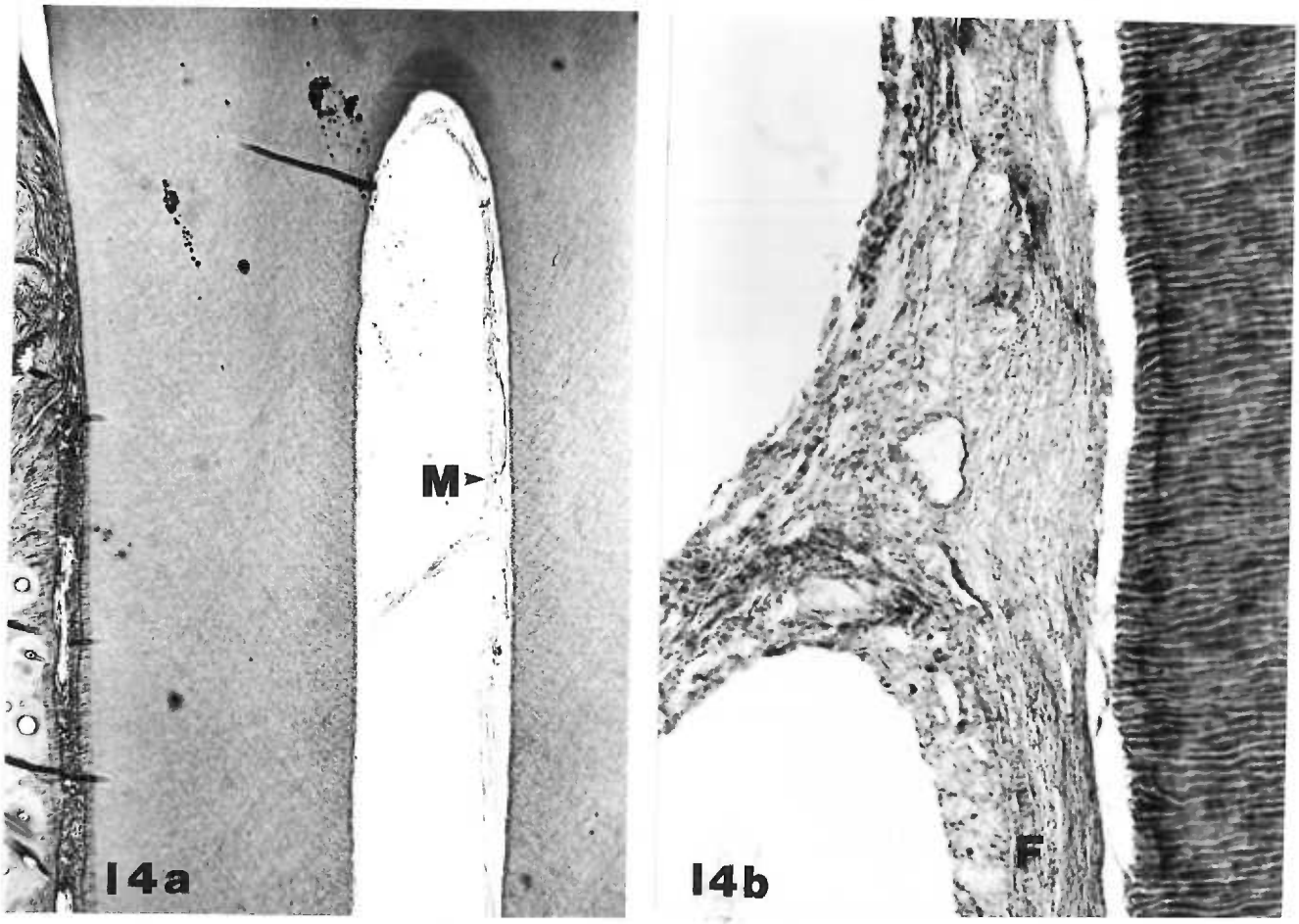


Figure 14. Photomicrographs of the coronal pulp in tooth #3LRP1, evaluated as Grade #4--necrotic.

14a. Note the nearly empty pulp chamber with a thin, amorphous material (M) along one wall. H and E. x 10.

14b. Higher power of the tissue remnants in 14a. Note the fibers evident (F), but few, if any cells. Note also the complete absence of an odontoblastic layer. H and E. x 100.

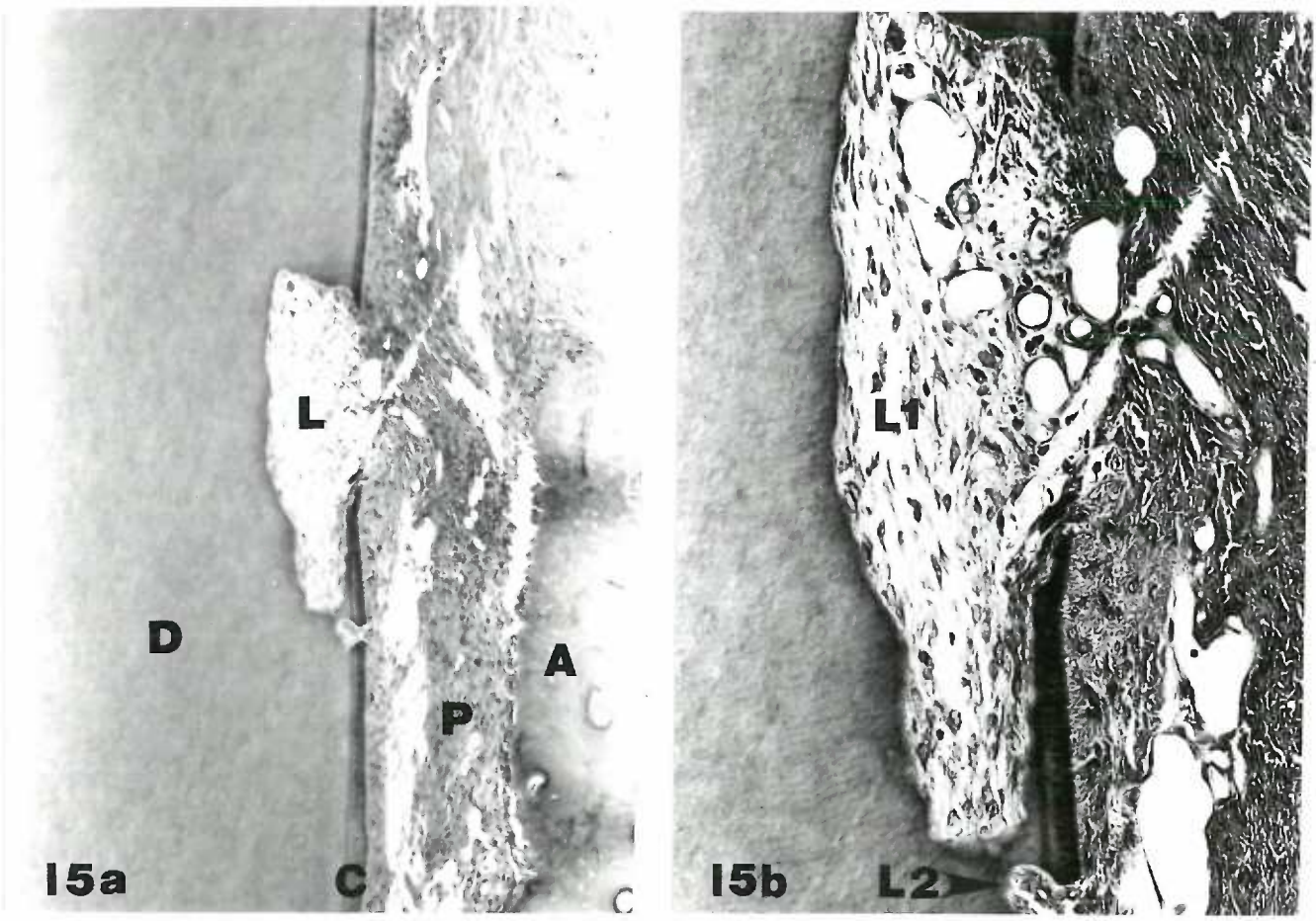


Figure 15. Photomicrograph of external resorption in the root of tooth #OULP3--a Group #2 (30-day anesthetic injection only) specimen.

15a. Note the intact alveolar crest (A), PDL tissue (P), dentin (D), and cementum (C). The resorptive lesion (L) is just apical to the CEJ and extends beyond the area devoid of cementum. H and E. x 26.

15b. Higher power of the resorptive lesion (L1). Note a second, smaller lesion (L2) penetrating the cementum into the dentin apical to the primary lesion. H and E. x 64.

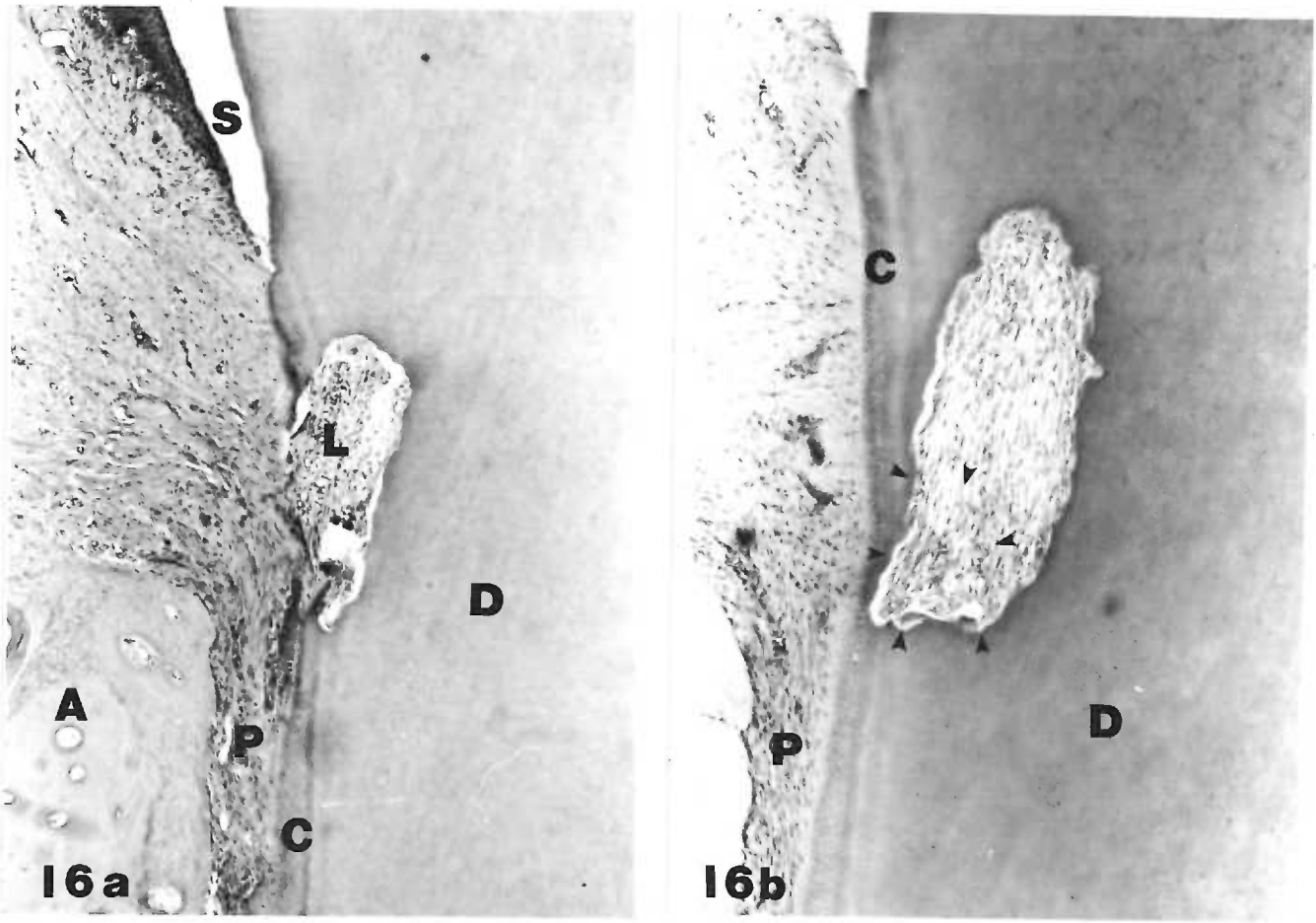


Figure 16. Photomicrographs of two adjacent serial sections of tooth #3ULP3--a Group #2 (30-day anesthetic injection only) specimen.

16a. Note the alveolar crest (A), dentin (D), and enamel space (S). The resorptive lesion (L) penetrates the cemental layer (C). Note also the intact PDL tissues (P). H and E. x 16.

16b. The adjacent section shows the lesion apparently totally encased in dentin (D). Note the intact outer layer of cementum (C) and "normal" PDL tissues (P). This "burrowing" type of resorption has not been previously reported. The area delineated by arrows is shown in higher power in Fig. 17. H and E. x 26.

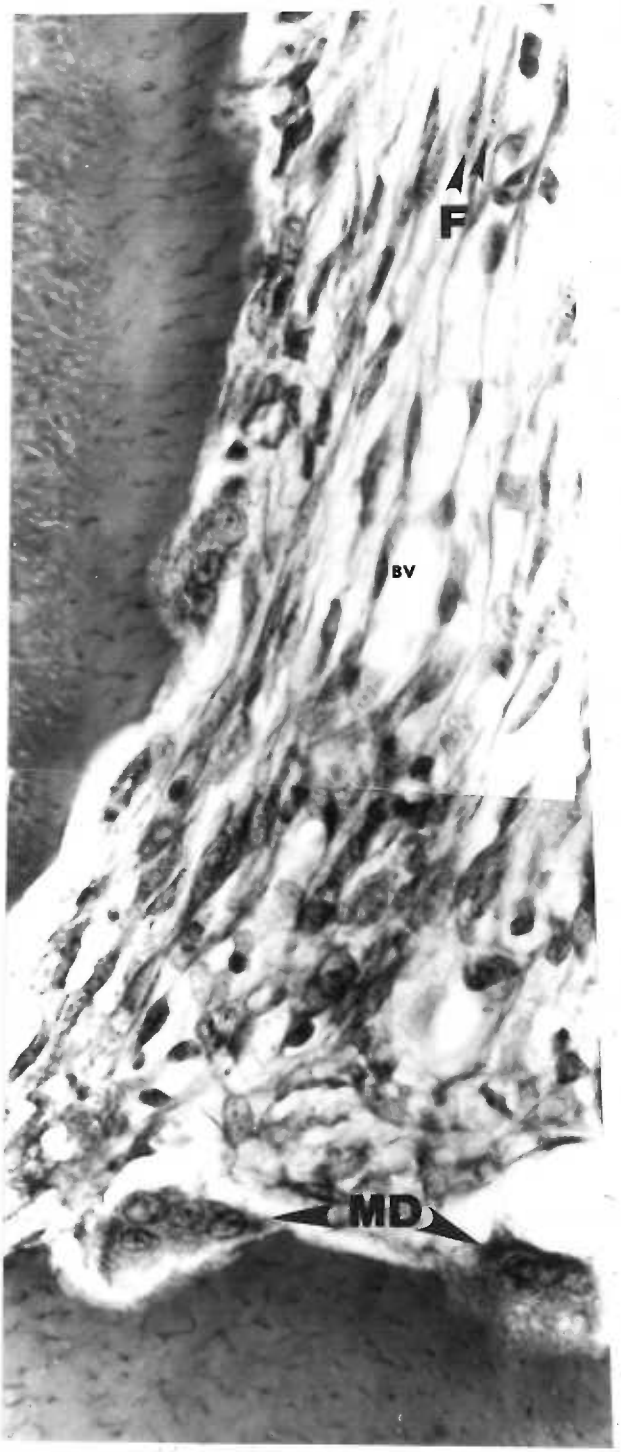


Figure 17. Composite photomicrograph of the area outlined by arrows in Figure 16b. Tooth #3ULP3, a Group 2 (30-day anesthetic injection only) specimen. Note the multi-nucleated dentinoclasts (MD) in areas of active resorption. Note also the fibroblasts (F) and empty blood vessels (BV) in resorptive tissue. H and E. x 160.

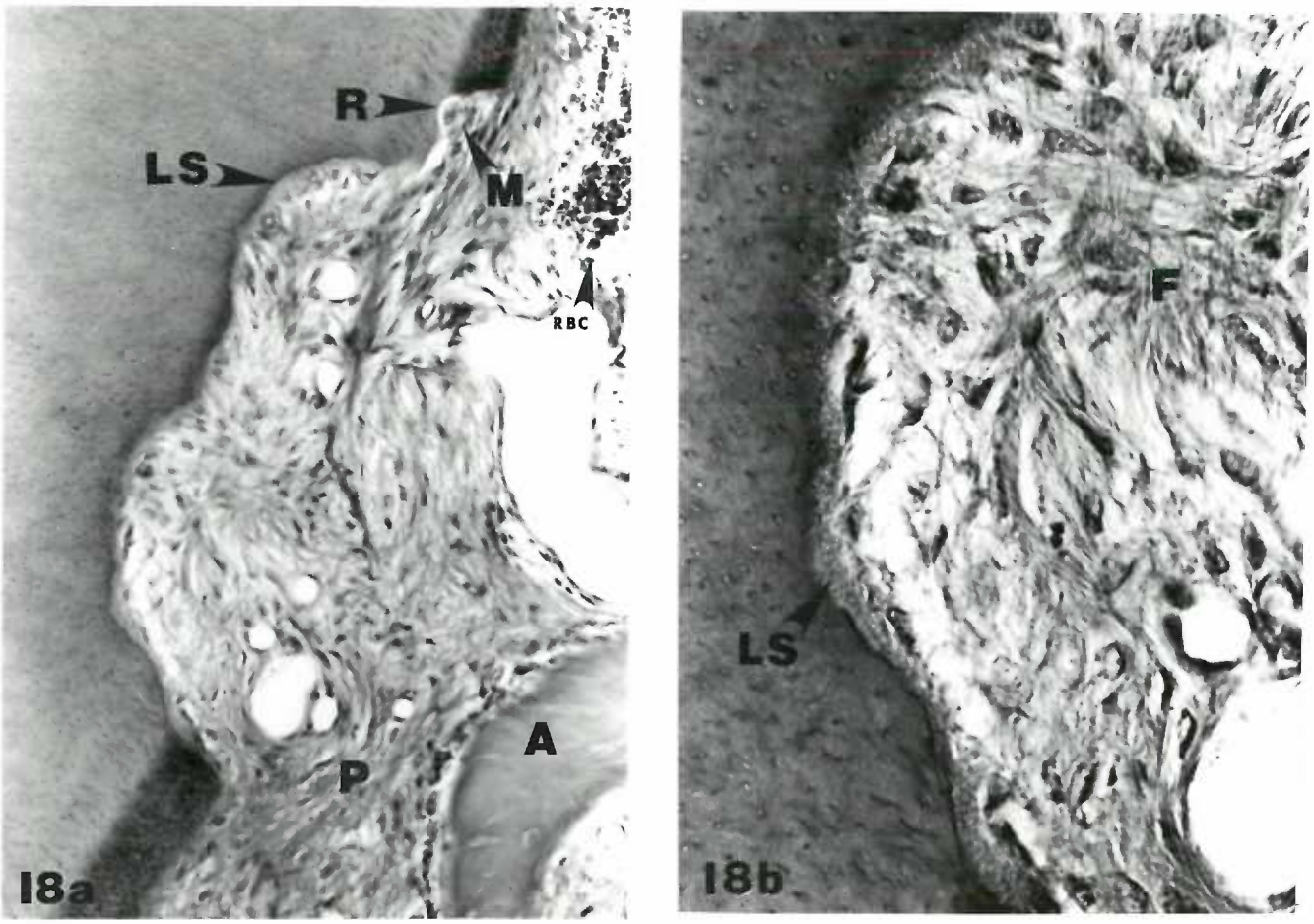


Figure 18. Photomicrographs of a resorptive lesion on tooth #3ULP2D, a Group 2 (30-day anesthetic injection only) specimen.

18a. Note the areas of extravasated RBCs (RBC) in the PDL tissues (P). These are not related to the resorptive area. Most of the lesion demonstrates a thin layer of lighter-staining tissue (LS) at the resorptive front. One area shows a multinucleated cell (M) and apparent active resorption (R). A = alveolar bone. H and E. x 64.

18b. Higher power of the dentin-resorption interface. Lighter staining tissue (LS) at the interface may be cementoid, indicating repair of the lesion. Note the numerous collagen fibers (F) in the lumen. H and E. x 160.

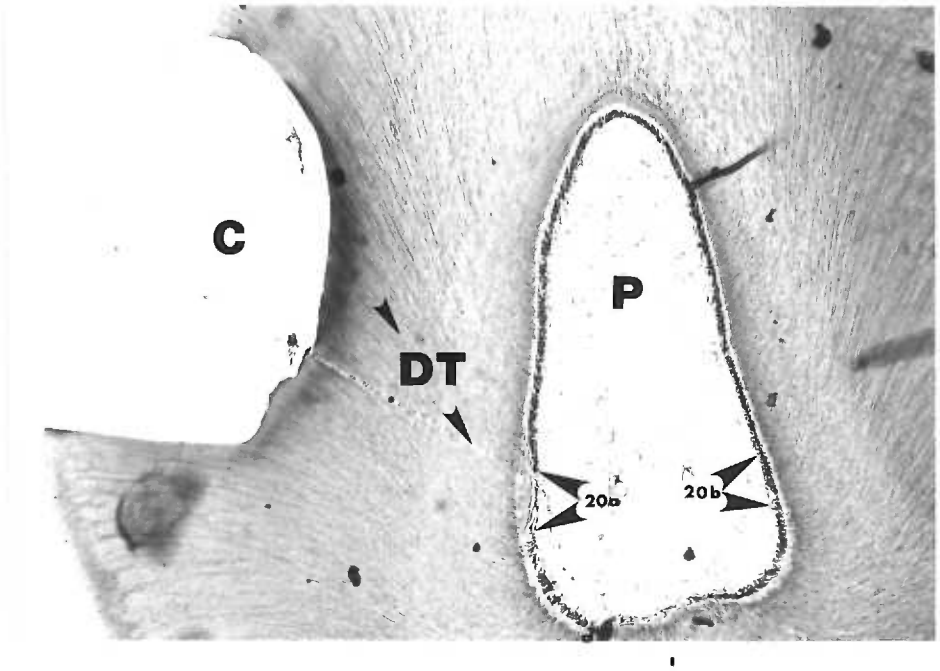


Figure 19. Photomicrograph of tooth #OURP2 (30-day--cavity preparation only). Note the pulp (P), cavity preparations (C) and direction of dentinal tubules (DT). The odontoblastic layer subjacent to the tubules tracking from the cavity preparation is thin and disorganized, with a thickened predentin layer, indicating initiation of a layer of reparative dentin. This pulp had a mean pulp grade of 1.83 (1 = no inflamamtion, 2 = mild inflammation). The areas delineated by arrows in pulp are expanded in Figures 20 a and b. H and E. x 10.

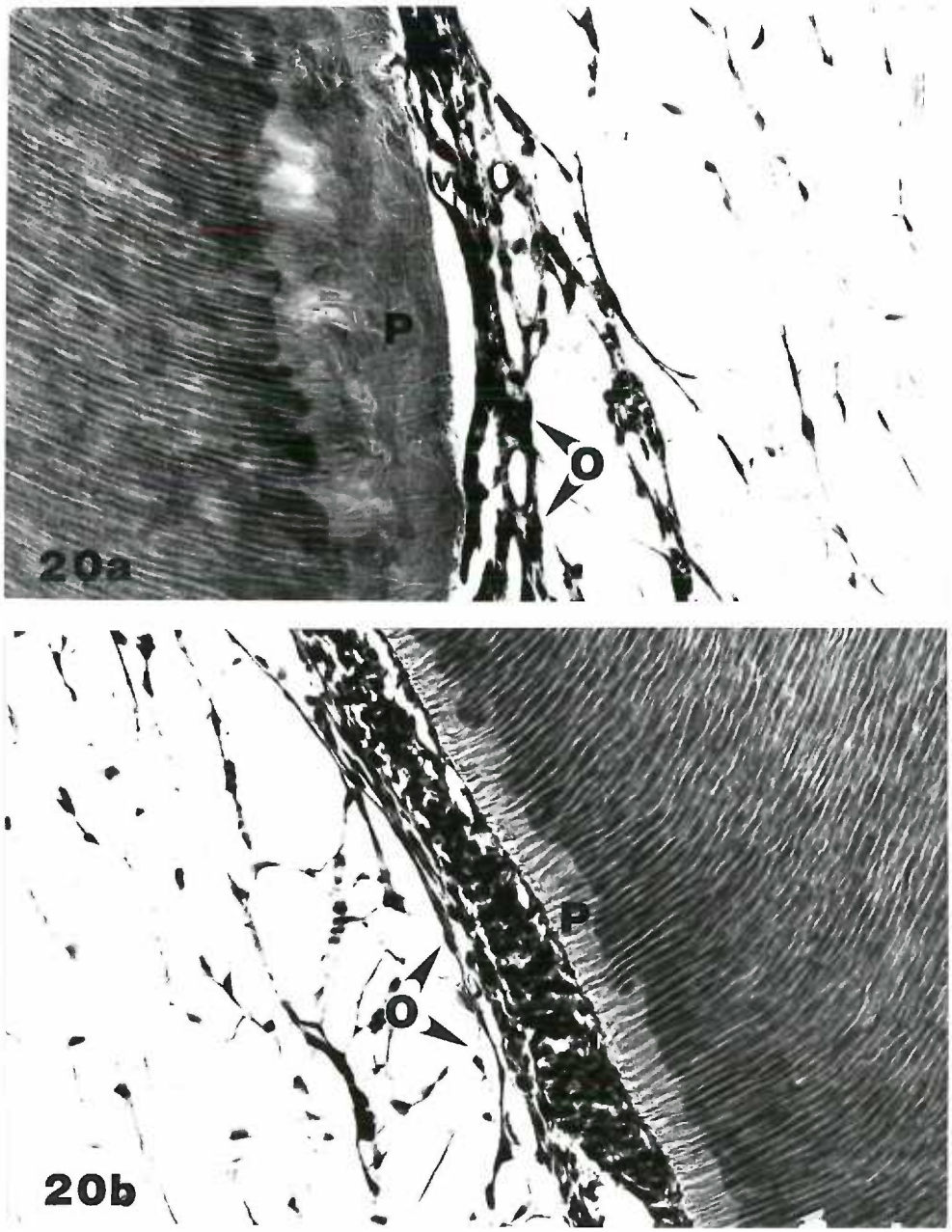


Figure 20. Photomicrographs at higher power of the portions of the pulp-dentin interface delineated by arrows in Figure 19.

20a. The area subjacent to the dentinal tubules tracking from the cavity preparation. Note the thinned odontoblastic layer (O), vacuolization (V), and thickened predentin (P) with disorganization of the dentinal tubules. H and E. x 100.

20b. The area on the opposite side of the pulp chamber from 20a. Note the thicker, more organized odontoblastic layer (O), the thin predentin (P) with continuous dentinal tubules, and less vacuolization. H and E. x 100.

Figure 21. Photomicrograph of the mesial root and periodontium of tooth #2URP2, a Group 4 (15-day anesthetic injection only) specimen. Note the large areas of resorption (R) in both the root dentin (D) and the alveolar crest (A). The area of resorption is in the buccal aspect of the root in the vicinity of the needle point termination with the PDL injection. The areas of resorption were entirely separate. S = enamel space. H and E. x 10.

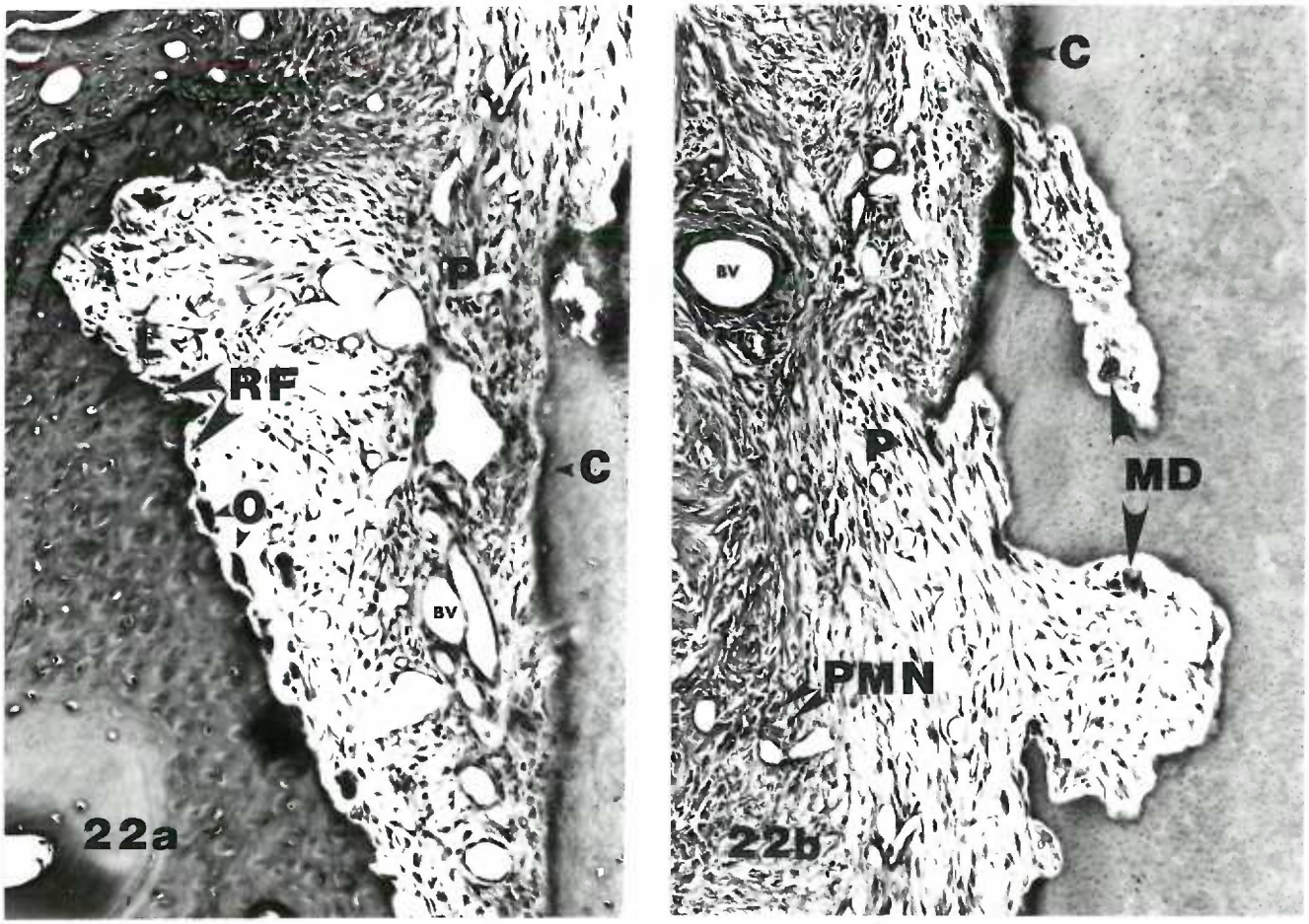


Figure 22. Photomicrographs at higher magnification of the resorption areas in the alveolar crest and the root surface in specimen #2LRP2.

22a. Alveolar crest. Note the resorption front (RF) with large osteoclasts (O), and the lacunnae (L) in the bone subjacent to the resorption front either empty or with pyknotic nuclei. The tooth cementum (C) is thick in this section and the PDL tissues (P) have numerous blood vessels (BV). H and E. x 40.

22b. Root surface. Note the upper area of resorption with an apparent narrow entry through the cementum (C) and a "burrowing" effect into the dentin. Note the multi-nucleated dentinoclasts (MD). The PDL tissues (P) are inflamed with PMNs (PMN) and numerous blood vessels (BV). H and E. x 40.

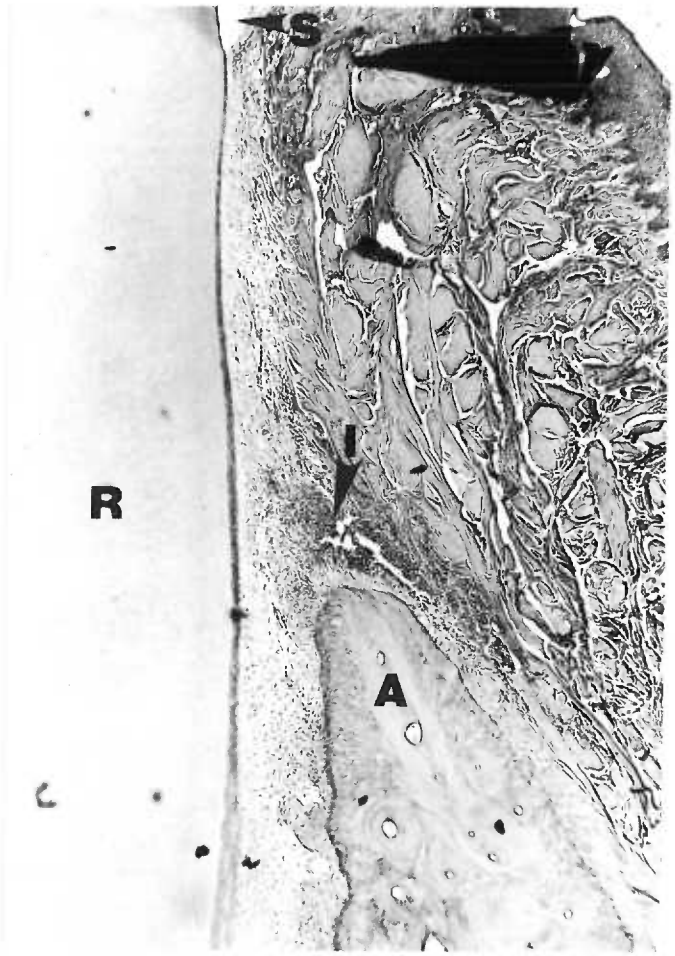


Figure 23. Photomicrograph of the root surface and periodontium of tooth #3ULI1, a Group #5 (2-day anesthetic injection only) specimen. Note the acute inflammatory reaction (I) in the vicinity of the alveolar crest (A), external to the buccal aspect of the root (R). There is no evidence of root surface or bone damage or resorption. S = enamel space. H and E. x 10.

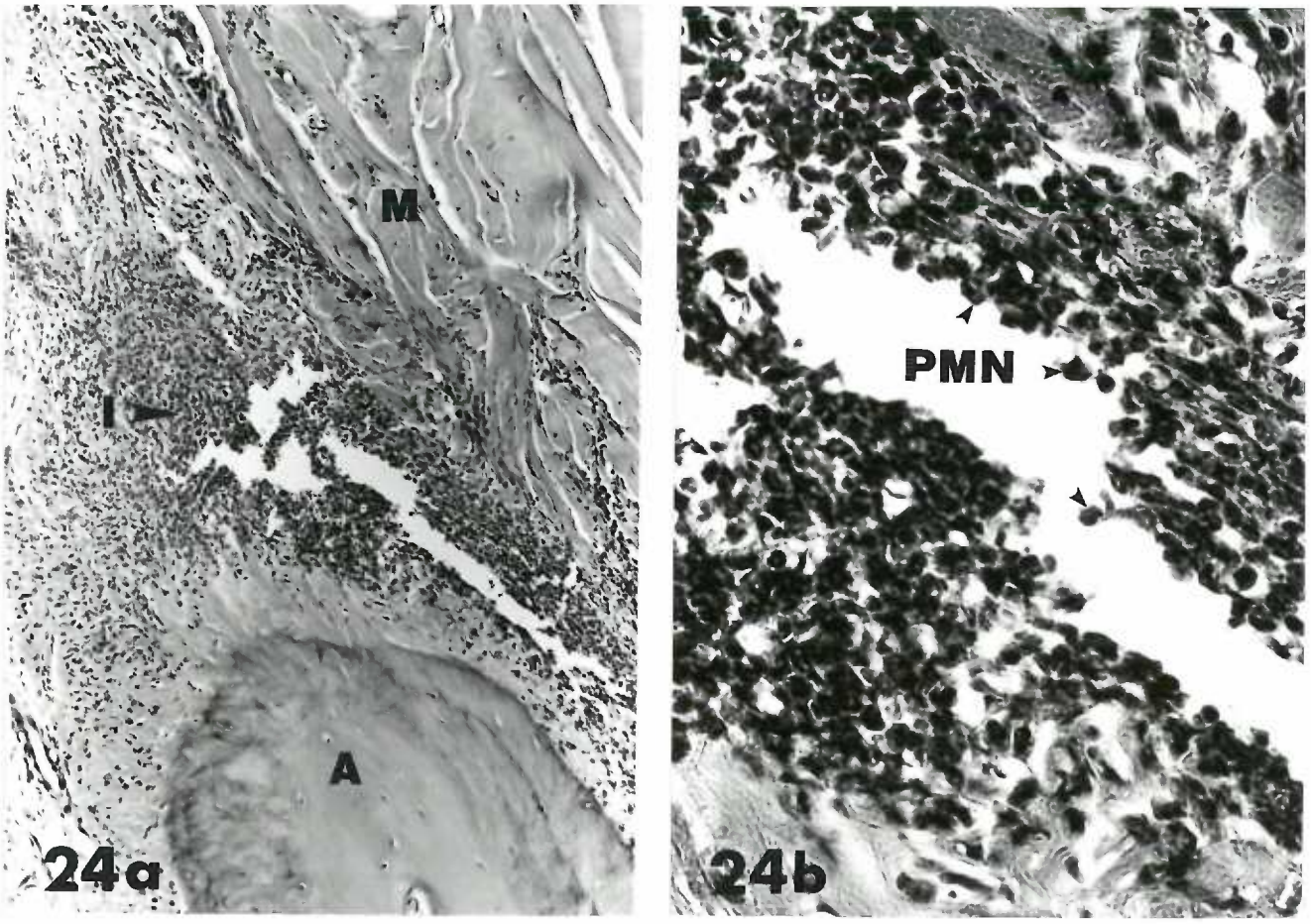


Figure 24. Higher power photomicrographs of the inflammation seen in Figure 23.

24a. Note the intact alveolar crest (A), and the spread of inflammatory cells (I) interstitially between muscle fibers (M). H and E. x 40.

24b. Note the abundance of PMNs (PMN), indicating an acute inflammatory process. H and E. x 160.

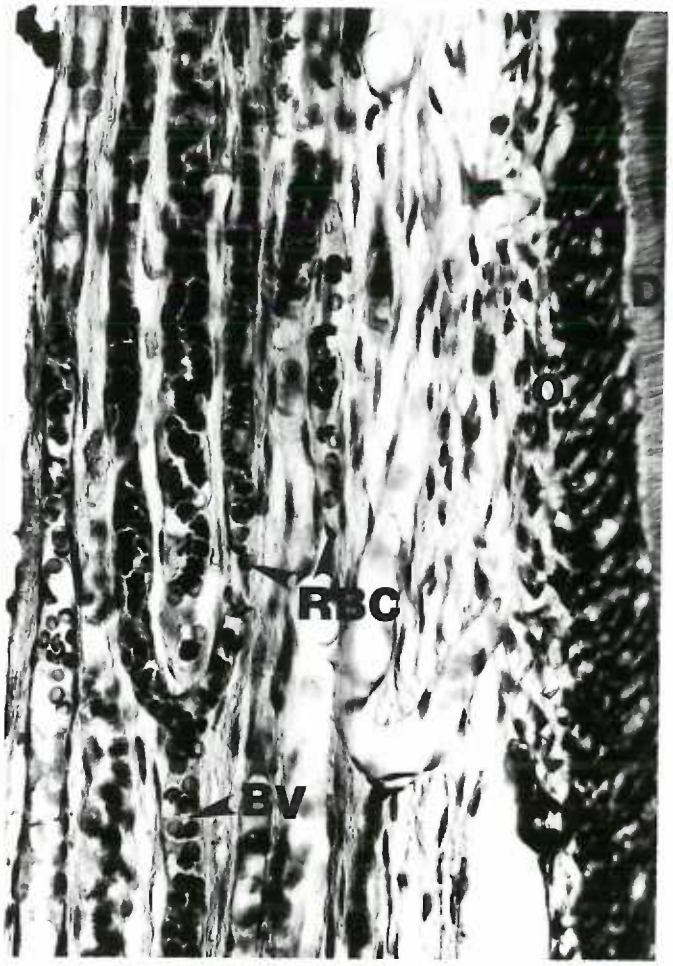


Figure 25. Photomicrograph of the pulp of tooth #2URI1, a Group 7 (ten minute pre-sacrificing anesthetic injection) specimen. Numerous intravascular erythrocytes (RBC) can be seen in most of the blood vessels (BV). This possibly indicates that the fixative did not enter these vessels during the perfusion fixation. Since this tooth received a PDL injection of Lidocaine with 1:100,000 Epinephrine ten minutes prior to perfusion, it is possible that the entering blood vessels were occluded, preventing fixative flow. D = dentin. O = odontoblastic layer. H and E. x 100.

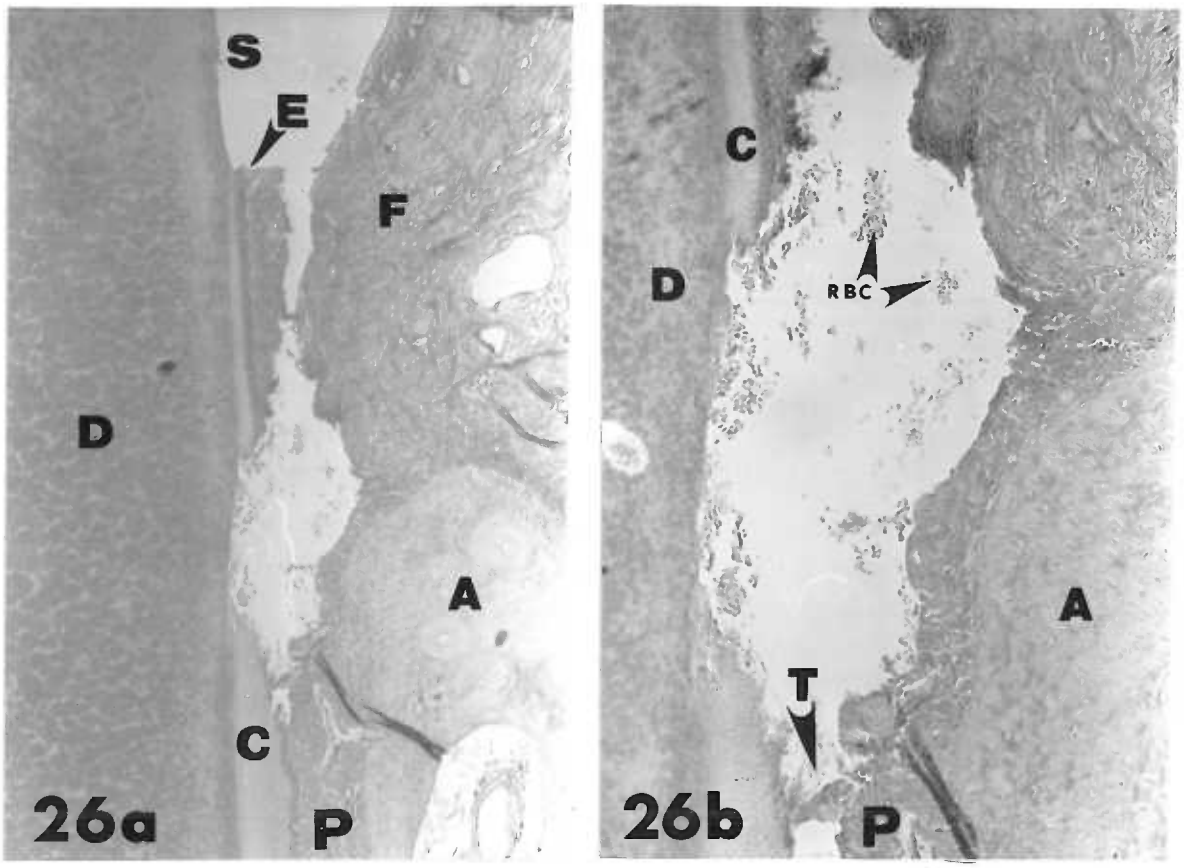


Figure 26. Photomicrographs of the periodontium and root of tooth #2LRP2D, a Group #7 (ten minute pre-sacrifice anesthetic injection) specimen.

26a. Note the path of needle penetration through the enamel space (S), the attachement epithelium (E), and the connective tissue fiber groups (F). The needle gouged away the cementum (C) covering the root dentin (D) and came to rest in the PDL (P) between the root and alveolar bone (A). Masson's x 16.

26b. Higher power of the needle track in the PDL (P). Note the complete stripping of cementum (C) from root dentin (D) in some areas, the intact alveolar bone (A), and the numerous tissue remnants (T) and extravasated RBCs (RBC) in the track area. Masson's x 40.

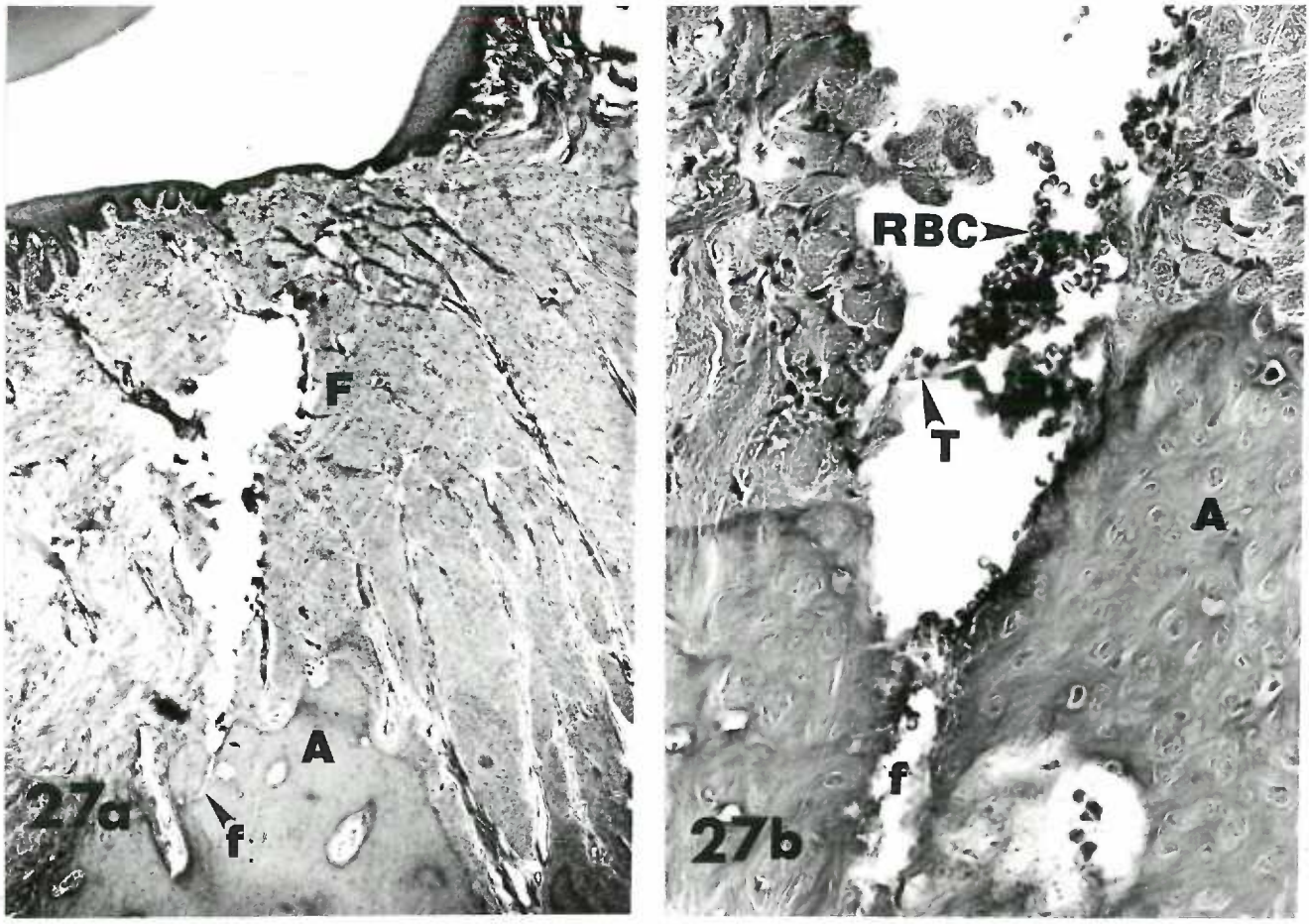


Figure 27. Photomicrograph of the periodontium adjacent to tooth #2ULI1, a Group #7 (ten minute pre-sacrifice anesthetic injection) specimen.

27a. Note the needle track through the gingival connective tissue fibers (F) and into the bone of the alveolar crest (A), and the fracture line in the bone below the needle path (f). The needle may have stopped at this point or may have glanced off the bone and continued into the PDL. H and E. x 10.

27b. Higher power of needle path into the alveolar bone (A). Note the bone fracture line (f) and numerous extravasated RBCs (RBC) and tissue remnants (T). H and E. x 100.

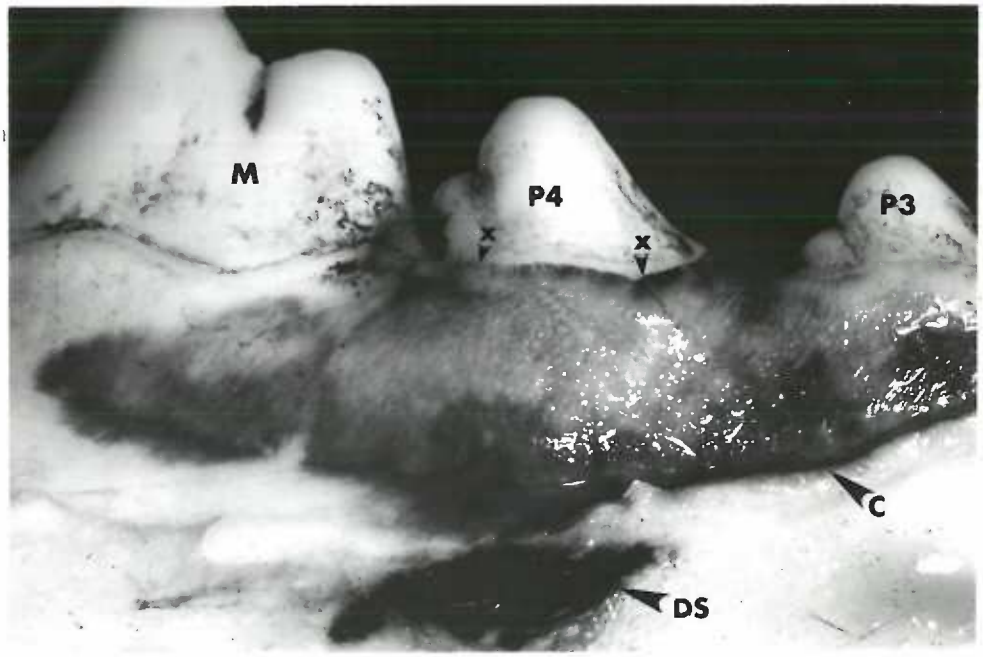


Figure 28. Photograph taken prior to clearing of the posterior teeth in the lower right quadrant injected with India Ink. The injections were made at the points designated "X". In disarticulating the mandible, the gingival tissues were cut at the line marked "C". Note the dark isolated stain (DS) in the submucosa, indicating that the injectate may have penetrated through the buccal plate from the PDL to the submucosa. M = molar. P4 = fourth premolar. P3 = third premolar.

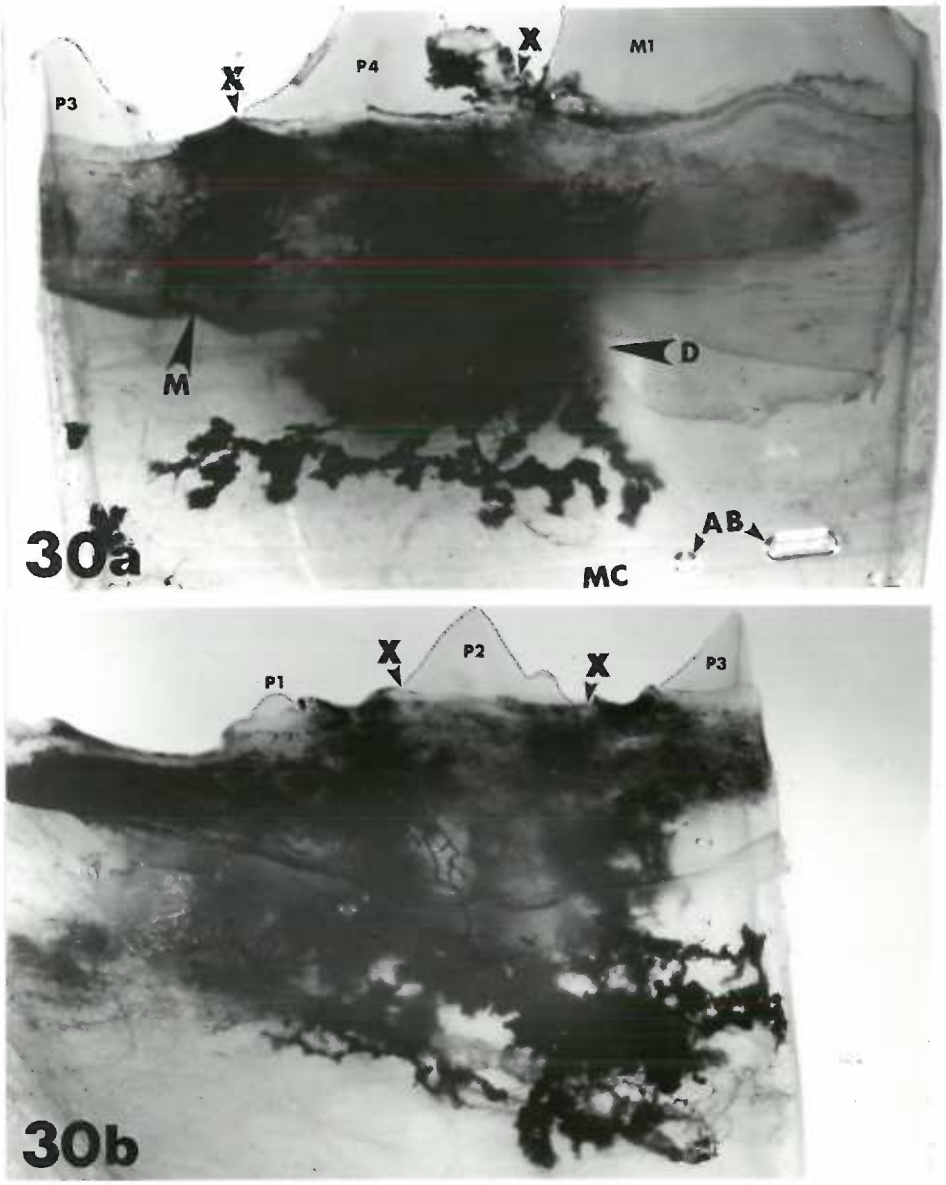


Figure 30. Photographs of India Ink injected specimens after clearing in methyl salicylate. Tooth crowns are outlined for clarity.

30a. Tooth #P-4 was injected with India Ink both mesially and distally at points marked "X". Note the wide spread of the distal injectate (D) and lesser spread of the mesial injectate (M), extending to the root of #M1 and apically into the intramedullary spaces. Note the mandibular canal (MC) with air bubbles (AB), but no injectate.

30b. The middle tooth (P2) was injected with India Ink both mesially and distally at points marked "X". Note the wide spread of both injections, engulfing the roots of both P1 and P3.