1973

A radioautographic and vital staining study of formocresol on the dental pulp following pulpotemy

BOSTON UNIVERSITY

SCHOOL OF GRADUATE DENTISTRY

THESIS

A Radioautographic and Vital Staining Study of Formocresol on the Dental Pulp Following Pulpotomy

by

Gerald G. Udler, D.M.D.

University of Pennsylvania

School of Dental Medicine

Submitted in partial fulfillment of

the requirements for the degree of

Master of Science in Dentistry

(Pedodontics)

1973

CUSTOMER BOSTON UNIV. SCHOOL OF MEDICINE LIB.

BINDERY NO.

SCHED. NO. | ITEM NO. | VOLS. | NEW

BINDERY HAS PATTERN ☐ | SEE RUB

COLOR BLACK ☑

BIND AS IS ☐

NO TRIM ☐

COVERS IN ☐ OUT ☐

ADS IN ☐ OUT ☐

INDEX FRONT ☐ BACK ☐

FRONT COVERS ONLY ☐

BOOK ☑

LETTERING
GOLD ☑
WHITE ☐
BLACK ☐

UDLER

A RADIOAUTOGRAPHIC AND VITAL STAINING STUDY OF FORMOCRESOL ON THE DENTAL PULP FOLLOWING PULPOTOMY

SPECIAL INSTRUCTIONS

BIND BOTH COPIES BUT IN SEPARATE VOLUMES

PLEASE INSERT COPIES 1 & 4 IN BOOK

Approved by:

First Reader [redacted]

Richard E. Stallard, D.D.S., Ph.D.
Professor of Periodontology

5/11/73
Date

Second Reader [redacted]

Spencer N. Frankl, D.D.S
Professor of Pedodontics

5/11/73
Date

Acknowledgments

This thesis is the culmination of the efforts of many.

The original idea came from Dr. Spencer N. Frankl, Chairman of the Department of Pedodontics, Boston University School of Graduate Dentistry. His assistance, thoughtfulness, and guidance throughout the preparation of this paper were invaluable.

I am particularly indebted to Dr. Richard E. Stallard, Professor of Periodontology, and Director, Clinical Research, Boston University School of Graduate Dentistry. His accumulative years of knowledge and research experience were a constant source of immeasurable assistance and insight from the inception to the completion of this project.

All histological technics and preparations were due to the untiring efforts of Mr. Arthur Bloom, and his assistant, Mrs. Eileen Lahar. Their assistance, understanding, and kindness were well appreciated.

The photography preparations were made with the abled assistance of Dr. Victor Dietz, Assistant Professor of Orthodontics, Boston University School of Graduate Dentistry.

I am also indebted to Mrs. Kathi Ferland who worked so many hours at so difficult a task of typing and proofreading this thesis.

And last but not least I wish to express my sincerest appreciation to my fiancee, Miss Joyce Sargon. Her encouragement and support throughout this endeavor was a dominant motivating force.

TABLE OF CONTENTS

INTRODUCTION

A major problem in dentistry for children is the management of the cariously exposed dental pulp in primary teeth. In the dental literature numerous approaches have been advocated and used to preserve such teeth. These varied procedures include pulp capping, pulp curretage, pulpotomy, partial pulpectomy and complete pulpectomy.

In recent years two principle methods of treatment of exposed primary pulps have gained prominence in pulp management: the calcium hydroxide method of pulpotomy and the formocresol techniques of pulpotomy. The formocresol pulpotomy techniques for primary teeth, which have also been called "The Therapeutic Pulpotomy," has been accepted by many as the treatment of choice[1] based on the high rate of clinical success over other techniques advocated to preserve cariously exposed primary teeth.

REVIEW OF LITERATURE

Buckley,[2] in 1904, recommended the use of formalin and tricresol for the treatment of putrescent pulps. He contended that the equal parts of formalin and tricresol would react chemically with the intermediate and end products of pulp inflammation to form "new, odorless, and non-infective compounds of a harmless nature." He stated, however, that this form of treatment was based on empiricism and he also warned that the formaldehyde should not be forced through the root apices because of possible irritation to the periapical tissues.

Sweet,[3] in 1923, advocated a five-treatment modified pulpectomy procedure for cariously exposed deciduous teeth. The pulp was devitalized by arsenic and then treated with formocresol for three periods of forty-eight hours each. The pulp was filled on the fifth appointment with carbo-eugenol, followed by a cement base and amalgam.

In 1930,[4] Sweet modified his technique to a four-appointment treatment. He utilized zinc oxide and eugenol for final filling of the pulp chamber. Gardner,[5] in 1935, condemned the use of arsenic for devitalization and utilized local anesthetics when performing pulpotomies in deciduous teeth. After amputation, hemorrhage was controlled by application of formocresol on a cotton pellet for about two or three minutes. The chamber was then filled with zinc oxide mixed with beechwood creosote and a trace of formocresol. In 1937, Sweet[6] likewise discarded the use of devitalization agents and modifed his pulpotomy technique into a three-appointment procedure. Pulpal amputation was performed under local anesthesia.

In 1959, Emerson and his co-workers[7] studied the histologic change in pulp tissue following formocresol application on human and rat pulp tissues. The twenty human teeth were treated with formocresol for periods of application ranging from five minutes to three weeks. At the second visit either the pulp stumps were dusted with calcium hydroxide, the teeth filled with zinc oxide and eugenol, or the stumps were covered with a zinc oxide and eugenol-formocresol paste and a restoration was placed. The teeth were obtained for histologic evaluation two to eight weeks later.

In both the rat molar and the human pulps treated by Emerson et. al.[7] with formocresol, the pulpal reaction varied with the total time the formocresol was in contact with the amputated pulp. Tissue changes varied from surface fixation to complete calcific degeneration. A surface fixation of the pulp tissue occurred following a short period of application (five minutes to three days). Calcific degeneration resulted in pulps with application beyond three days. Histologic examination presented three distinct zones. Directly beneath the amputated area, the pulp showed a somewhat homogeneous yellow staining area--the immediate reaction of the formocresol with the blood clot. Subjacent to this area, a well defined and intact odontoblastic layer and normal appearing connective tissue elements were present. This was the zone of fixation. The pulp was considered in a status quo position and no metaplastic changes occurred. Below the "fixed" zone there was evidence of pulpal tissue degeneration and calcification. There was an absence of inflammatory cells throughout the treated pulps, as well as no evidence of internal resorption or metaplastic changes. The authors concluded

that this technique could be classified as either vital or non-vital, depending on the length of formocresol application.

Mansukhani,[8] in 1959, reported on the effects of formocresol on the pulps of rats and forty-three human deciduous and permanent teeth. The treatment intervals for the human teeth varied from one minute to three years. Histologic interpretation indicated that the surface of the pulp immediately under the formocresol became fibrous and acidophilic within a few minutes after the application of the formocresol. This was interpreted as a fixation of the living tissue. After seven to fourteen days of formocresol application, the pulp showed three distinct zones:

1. A broad acidophilic zone of fixation;
2. A broad pale-staining zone wherein the cells and the fibers were greatly diminished (atrophy); and
3. A zone of inflammatory cells concentrated at the junction with the pale-staining zone and diffusing deeply into the underlying pulp tissue of the apex.

At sixty days and thereafter, it was thought that the pulp was completely fixed and remained as only a strand of eosinophilic fibrous tissue. For maximal effectiveness as a germicide, and minimal irritation on pulp tissue, she suggested the formocresol be applied for two to three days and for no more than seven days prior to placement of a capping material.

In 1961, Dietz[9] described the histologic effects of formocresol on forty non-carious deciduous cuspids. After the pulpotomy and control of hemorrhage was obtained, a cotton pellet saturated with formocresol was placed over the amputated pulpal stumps and sealed in with zinc

oxide and eugenol. The experimental period varied from twenty-four hours to sixteen weeks. Dietz observed that there occurred a progressive non-inflammatory degeneration with an attempt to wall off the medication with a collagenous type of acellular band immediately below the amputation. This band, first seen as a thin zone in the twenty-four hour specimen, widened with each time interval until the sixteenth week, when it was still evident but seemed to be degenerating with the remainder of the original pulp tissue. Odontoblasts adjacent to the collagenous-like band maintained their vitality much longer than the odontoblastic layers in the remainder of the pulp. By the sixteenth week, no odontoblasts were noted. Dietz stated that the pulp tissue seemed to attempt to produce a new pulpal network of young proliferating fibroblasts. This appeared in the apical portion in the eight-week specimens and was present along the periphery of the pulp chamber at sixteen weeks. There was no evidence of inflammation in the pulp tissue until the eighth and sixteenth weeks and then to only a very mild degree. He concluded that, for any given tissue section, the greatest tissue breakdown was in the middle portion of the pulp tissue and the least was in the apical region.

Doyle,[10] also in 1961, compared the effects of the formocresol and the calcium hydroxide pulpotomy technique on sixty-five mechanically exposed human deciduous teeth and concluded from the clinical, radiographic and histologic results that the formocresol pulpotomy technique was superior. The time intervals for the histologic study group were four days to 380 days, and for the clinical study they were five to

nineteen months. Doyle stated that the effects of formocresol were almost as pronounced at four days as in later specimens. The histologic examination of the teeth treated with formocresol showed that the formocresol did not stimulate a healing response by the stump of the amputated pulp. At the amputation site were seen superficial debris from the blood clot, occasional dentin chips, and then a layer of well-preserved, compressed acidophillic tissue, which appeared somewhat fibrous. There was no evidence of calcification or organization for calcific bridging. Below the dark staining acidophilic layer, a large pale-staining area of slightly degenerated cells occurred, which extended nearly to the apex. Histologically, the formocresol pulpotomies were considered seventy-two per cent successful as opposed to a fifty per cent success rate for those teeth treated with calcium hydroxide. Clinically and radiographically, success rates were one hundred per cent and ninety-three per cent for the formocresol group as opposed to seventy-one per cent and sixty-four per cent for the calcium hydroxide group. Doyle felt that the success of the formocresol technique may be due to its "fixing" effect on the pulp tissue.

Spedding,[11] in 1963, attempted to duplicate in twenty monkeys the one-appointment formocresol pulpotomy technique and to observe the response of the pulp and the periapical tissues to the pulp dressing material. Formocresol was placed in contact with the amputated pulp stumps for approximately five minutes before a mixture of zinc oxide powder with one drop formocresol and one drop eugenol was placed as dressing over the amputated pulp stumps. All the teeth treated with formocresol pulp dressing material gave evidence of vital tissue in

either the apical one-half or one-third, or in the major portion of the canal. Fixation, as a result of the formocresol, was in many instances diffuse in action and was observed to penetrate to the apical one-half or one-third of the same tooth. No apparent damage or adverse effects to the periapical tissues were observed in the block sections of eight teeth.

In 1965, Berger[12] reported a study comparing the effects of pulp tissue to formocresol in a one-appointment formocresol pulpotomy technique to the effects of zinc oxide and eugenol cement on pulp tissue. Cariously exposed human deciduous molars were evaluated histologically, clinically, and radiographically three weeks to thirty-eight weeks postoperatively. Three weeks after formocresol treatment, the pulp tissue appeared compressed with good cellular detail. In the middle third of the canal, the cellular detail became less distinct and blended to complete absence of cellular detail in the apical third. These changes were amenable to repair by replacement with granulation tissue. The ingrowth of connective tissue through the apical foramen were seen after seven weeks, and appeared to be replacing the necrotic tissue coronal to it and, in the same region, to be resorbing the dentinal walls slightly. The teeth treated with formocresol in this study were vital on the basis of histologic and clinical criteria when examined thirty-one weeks following treatment. The pulps of all those teeth treated with zinc oxide and eugenol presented active inflammatory reactions, and internal resorption was found consistently.

In 1965, Spamer[13] studied a one-appointment formocresol technique in which the final pulpal covering was of zinc oxide and eugenol cement.

At the amputation site, the initial reaction was an eosinophilic staining zone of pulp tissue. Beneath this zone was a pale-staining, almost acellular zone characterized by the presence of intracellular edema, hyperemia, and acute inflammatory cells. The middle third of the pulp tissue presented a fairly normal appearance except for the presence of some acute inflammatory cells. The apical third of the pulp appeared normal and was free of any inflammatory reaction. Specimens of longer postoperative intervals showed similar results, except that the pale-staining zone became more acellular and the inflammatory reaction apical to this zone increased. There was proliferation of fibroblasts in the middle and apical thirds. Deposition of secondary dentin was noted, and by six months postoperatively vital tissue was seen throughout most of the radicular pulp.

Beaver,[14] Kopel and Sabes investigated the difference in pulp reaction after a five-minute formocresol application to subbases of either zinc oxide and eugenol with formocresol added or to zinc oxide and eugenol alone. Investigation periods were one, two, and three months. The pulpal responses seen in this study were not those various well-defined zones of degeneration, inflammation, calcific deposits, resorption and osteodentinization which had been reported by other investigators. Definite coagulation areas were predominant findings in this study, but "collagen-like" bends were not seen. There appeared to be no major difference in the histologic reaction, whether formocresol was used or not used in the subbase.

In 1968, Stratton and Han[15] examined the reaction to formocresol of connective tissue cells in sponge implants and femur wounds, by means

of quantitative radioautography following proline-H injection. Formocresol was diluted to 1/50 of normal concentration for the sponge implant study and the normal concentration was tested on the femur. Formocresol used in 1/50 concentration caused degeneration of cells in the immediate vicinity of the sponge implant as judged by histologic examination and radioautographic grain counts. However, in all animals treated with formocresol a definite reduction in the number of infiltrating inflammatory cells was observed. By the tenth day the experimental and control sponges showed a comparable recovery of connective tissue ingrowth. This was also true for the repair in the femur wound area. It was concluded that formocresol does not interfere with prolonged recovery of connective tissue and might suppress initial inflammatory response significantly.

Stratton and Han[15] felt that such a freedom from inflammation might be a beneficial aspect of formocresol treatment of the dental pulp because an inflammatory response subsequent to pulpotomy could create a serious clinical complication. However, the tooth would probably be able to withstand the temporary slowdown of the recovery process without manifesting any clinical signs.

Stratton and Han,[16] in 1970, by means of quantitative radioautography, studied the synthesis of RNA by connective tissue cells subjected to varying concentrations of formocresol. Formocresol was tested in full concentration and in dilutions of 1/5, 1/25, and 1/125. Tritiated uridine was the radioautographic marker used after implantation of polyvinyl sponges in the subcutis of hamsters. An evaluation of the labeling index indicated complete fixation of the tissue by the full concentration

of formocresol, whereas at one-fifth concentration, a similar obliteration of RNA synthesis was observed during the second week of the postoperative period. The relative number of functioning cells was high throughout the series and was clearly higher in sponges receiving lower concentrations of formocresol, suggesting a stimulated synthesis of RNA. They concluded that formocresol at one-fifth the usual concentration might be as effective as the full concentration, but much less irritating to tissue.

17

In 1971, Loos and Han assessed a histochemical profile of selected enzyme activities in connective tissue cells of polyvinyl sponge implants in the absence or presence of various dilutions of formocresol. The effects of formocresol were more severe among cells receiving the higher concentrations. Little differences were noted between the full concentration of formocresol and a one-fifth dilution in terms of initial effects of tissue fixation. However, an earlier recovery of enzyme activities was apparent after application of a one-fifth dilution. It was concluded that a one-fifth dilution of formocresol may be as effective as the full concentration and, at the same time, may provide a faster recovery from the cytotoxic effects of formocresol. Loss indicated that further studies on centration were necessary to arrive at the amount that will give the ideal tissue reaction.

18

In 1972, Berger reviewed the chemistry and pharmacologic actions of the constituents of formocresol: formaldehyde and tricresol. Formaldehyde is a colorless, strongly irritant, caustic pungent gas produced by the partial oxidation (incomplete combustion) of methanol

and has the chemical formulation $H-\overset{H}{\underset{|}{C}}=O$. It is readily soluble in water, with the aqueous solution (which is thirty-eight to forty per cent formaldehyde) being called formalin. Paraformaldehyde is the polymerized form of formaldehyde and precipitates from concentrated solutions. With dilution, paraformaldehyde is redissolved. It is a strong germicide and disinfectant. A solution of ten per cent formalin (four per cent formaldehyde) is utilized widely as a tissue fixative. Its action to prevent autolysis of tissue is believed to be caused by a complex chemical binding of formaldehyde with protein. This reaction of binding is reversible and the bond may be hydrolyzed in the human body by enzymatic action. The exact site of the chemical binding is thought to be the peptide groups of certain side chain amino acids, especially those amino acids that have dual peptide groups. Furthermore, formaldehyde is believed to link adjacent protein molecules by the formation of methylene bridges between peptide groups of adjacent amino acids. Such cross linkages connect protein molecules without changing their basic over-all structure and probably underlie some of the altered chemical reactivity and increased tissue hardness. Because of its chemical binding action, formalin is classified as an additive non-coagulation fixative. This is as opposed to some other fixatives or to heat, both of which drastically and irreversibly alter the chemical and physical properties of the protein molecules. The chemical binding with the proteins of microorganisms is thought to be the basis of formaldehyde's action as a bactericide.

The reaction between formaldehyde and protein is slow, with the diffusion through soft tissues approximately five times slower than through blood plasma. The pH of the environment has a complex effect on the binding of formaldehyde and protein, with the greatest interaction under slightly alkaline conditions (pH 7.5 to 8). Formaldehyde fixation does not cause a loss in volume of soft tissue, but subsequent dehydration in alcohol and paraffin embedding causes a shrinkage and distortion.

Cresol, commercially known as tricresol, is a mixture of three isomeric cresols obtained from coal tar, freed from phenol, hydrocarbons, and water.[19] It occurs as a colorless or straw-colored refractive liquid, having a phenol-like odor and a burning, caustic taste. It is soluble in fifty parts water at ordinary temperature (25 C.) and is miscible with alcohol, ether, glycerin, and alkali hydroxide solutions. Cresol is a strong antiseptic, resembling phenol in its local action. It is said to be 2.5 times as active as phenol, but less caustic. Cresol possesses marked analgesic properties and, like all phenols, cresol acts as a local obtundent. The specific action of cresol at the cellular level has not been determined.[15]

Cresol was empirically selected as an agent with which to dilute the formalin and modify the irritating action of the latter. According to Buckley,[20] the cresol was recommended for four principal reasons:

1. It is miscible with the liquor formaldehyde in all proportions, thus making, with the addition of alcohol, a good pharmacal product from which formaldehyde gas is constantly generated.

2. It is a good disinfectant--much more powerful than phenol.

3. It possesses an anodyne property which modifies the irritating action of formaldehyde.

4. It acts chemically upon fatty compounds, thereby disposing to advantage of these substances.

The irritational potential of formocresol has been studied by numerous researchers. Black,[21] in 1920, sealed three drops of cresol and formalin on the forearms of male volunteers. In all cases a deep inflammation was produced, with induration and necrosis existing after a period of two months. Grossman,[22] in 1944, repeated the same experiment. Necrosis from formocresol application lasted from two to three months. In 1958, Rubbo, Reich and Dixon[23] studied the tissue toxicity of formocresol following subcutaneous injection in the ears of rabbits. Tissue necrosis was found after twenty-four hours. In spite of the fact the formocresol was found to be an active bactericide and to cause instantaneous sterilization, it was concluded that "these substances....have no place in topical chemotherapy." Schilder and Amsterdam[24] used mammalian soft tissues (the abdominal integument and eyes of rabbits) to test the inflammatory potential of formocresol. Formocresol showed an extremely high irritant potential.

Radioautography

Radioautography has been defined as the production of a two or three dimensional image on a photographic film or plate by radioactive radiation.[25] The image may consist of a black area seen by the unaided eye or as a random distribution of grains or tracts seen under the microscope.

The first published biological application of radioautography was that of E. S. London,[26, 27, 28] in 1904. A live frog was exposed to radium emanation. After death, it was placed on a photographic plate producing an radioautogram of the specimen. In another experiment a portion of the skin was removed and again the entire animal was placed on the plate. The radioautogram showed less blackening in the area corresponding to the removed skin, indicating a deposition of the daughter products of emanation on or in the skin.

At the same time, Bouchard, Curie and Baithazard[29] used the microscopic radioautographic method in a similar experiment to study the distribution of inhaled radium emanation in tissue of a guinea pig. Their technique was not detailed, but they reported activity in varying amounts in the kidney, liver, spleen, heart, brain, lung, and adrenal gland.

Lacassagne[30] and Latles, in 1924 reported their studies of the distribution of polonium and other elements in histological specimens. Their method was to embed the tissues and place the flat surface of the paraffin block, from which sections had been cut for staining,

against the photographic plate. The radioautogram was compared with the stained sections, and the polonium located in the microstructures. Lacassagne called the results "autoradiographs" and "histoautoradiographs."

In 1930, Lomholt[31] apposed 20-micron tissue sections to a plate and later removed them for staining. In this manner the same section making the radioautogram could be studied histologically. With the help of radium-D, Lombolt studied the deposition of lead in the microstructure of the tissues of mice.

Belanger and Leblond[32] in 1946 developed the first successful method for simultaneously observing the radioautogram and the stained tissue section under the microscope. Their method consisted in covering the histologic tissue sections themselves with a photographic gelatin emulsion, which is developed after a sufficient exposure to the rays of the radio-element in the sections. Finally, the section is stained through the gelatin coating and mounted in the usual fashion.

In 1935, Chievetz and Hevesy[33] employed P^{32} in the first metabolism study utilizing an artificial radioactive isotope. Two years later, the same investigators[34] added sodium radiophosphate to the diets of rats and discovered that an appreciable portion of the radioelement quickly found its way into the bones, teeth, muscles, and other tissues of growing and adult animals. An exchange phenomenon in which individual phosphorous atoms in the teeth and bones were displaced by phosphorus atoms in the blood, and then were either eliminated or carried to other organs, was demonstrated.

In 1960, Sciaky and Pisanti[35] first employed a radioautographic technique in the study of pulp tissue. They used radioactive calcium ($Ca^{45}Cl_2$) contained within a paste of calcium hydroxide to trace whether a diffusion of calcium ions occurred from calcium hydroxide placed over amputated pulps into the dentin bridge or pulp of dogs' teeth. Radioautographs were obtained by pressing sections of the teeth tightly against the emulsion side of intraoral dental films for 36-72 hours. The radioautographs were then superimposed on tracings of the tooth outline. Their results showed that the Ca^{45} was confined to the calcium hydroxide dressing. Radioactivity could not be demonstrated in the area of newly formed dentin over the amputated pulp.

Pisanti and Sciaky[36] later demonstrated in dogs that the calcium in the newly formed dentin bridge might stem from the blood stream. Following the calcium hydroxide pulpotomies, for a three week period, the dogs were injected intravenously with 5 cc. of a solution of labeled calcium hydroxide. The sections were x-rayed and later radioautographs were made. The radiographs of each section were then superimposed on the radioautographic plates for orientation.

Stark, Myers, Morris and Gardner[37] also investigated the role of calcium hydoxide placed over an exposed pulp in the production of secondary dentin at the exposure site.

The pulpotomies were performed on Rhesus monkeys. Following extraction, the teeth were wrapped in aluminum foil and placed in a freezer to prevent dehydration. The teeth were later sectioned. The sections were then placed on the emulsion side of commercial x-ray film. Following eight weeks of exposure, the films and sections were

exposed for four seconds to outline the sections so as to permit orientation of any radioactive exposure. Their investigation presented evidence that the radioactive calcium migrated from its point of deposition in the cavity preparation and produced scattered areas of radioactivity in the pulp in twenty-five per cent of the sections studied.

Gatewood and Sorenson[38] studied the penetration of formalin into dentin and cementum. Freshly extracted human anterior teeth were totally immersed in a ten per cent neutral formalin solution to which C^{14} tagged formalin had been added. The teeth were removed at specified time intervals of 10 to 180 minutes and then cross-sectioned serially. X-rays were taken to help establish the patency of the root canal in each section. Radioautographs were then obtained. The degree of penetration was determined by comparison of the radiographs and the radioautographs. In no single time period was there complete penetration by the fixative.

Straffon and Han[15] studies the reaction of connective tissue to formocresol in sponge implants and femur wounds, by means of quantitative radioautography following proline-H^3 injection. They showed that the incorporation of proline-H^3 into secretory proteins of the connective tissue was severely suppressed in fibroblasts of polyvinyl sponge implants when a 1/50 dilution was applied. The radioautographic technique consisted of dipping selected histologically prepared slides which had been freed from paraplast and hydrated, into Kodak NTB - 3 nuclear track emulsion.* Following 4-8 weeks of exposure in an air tight slide

* Eastman Kodak Company, Rochester, New York

box, the slides were removed, developed, and stained. Radioautographic grain counts were then made to determine the fibroblast activity as correlated between the regressive changes occurring in the cytoplasmic ultrastructure and the amount of proline-H^3 taken up by the fibroblasts.

Straffon and Han[16] utilizing improved quantitative radioautographic techniques showed that formocresol, at 1/5 dilution, produced an effect equal to that of full concentration, in terms of suppression of RNA synthesis, but caused less of the side effects produced by full concentration.

Vital Staining

Vital staining is the method by which living cells are stained either after dissociation in the staining fluid (supra-vital staining) or by injection of the dye into the living organism (intra-vital staining).[39] Vital staining demonstrates cytoplasmic structures by the phagocytosis of particles of dye into the cytoplasm, or by the staining of pre-existing cellular components without any serious or obvious impairment of their vitality. The nuclear membrane of the living cell is impermeable to dyes, and it is not possible to stain the living nucleus. The surface membrane of the cell, however, under certain conditions, behaves as a permeable membrane towards some dyes. Vital dyes may be toxic if used in a sufficiently high concentration.[40]

Williams and Frantz[41] presented a study on the histologic technics of vital staining in both normal and damaged cells. They described four different circumstances or conditions of dyes occuring within cells and tissues through intraperitoneal injection:

1. storage and segregation of dyes by macrophages;
2. active excretion of the dye through renal proximal convoluted tubules, choriod plexus, and lactating mammary glands;
3. diffuse staining of certain elements such as small and multivacuolar fat cells and mural elements of blood vessels;
4. and staining or deposition in damaged cells.

A technique utilizing trypan blue to stain dense fibrous connective tissue was described by Swigert and Williams.[42] They showed that the rapid in vivo staining of dense connective tissue in mice resulted from attachment of the acid diazo dye to glycoprotein of the connective tissue ground substance. Williams and Frantz[43] found trypan blue to be one of the most satisfactory dyes for vital staining of normal, as well as damaged cells. An acid diazo dye, trypan blue is excreted by the kidney and stored as large granules in the macrophages of most tissues and organs.

Stallard and Schaffer[44] demonstrated in mice the reaction of the oral tissues to the injection of the vital dye trypan blue. The periodontal membrane, periosteum, subepithelial connective tissue, phagocytic cells and portions of the dental pulp were found to stain, while no staining was in evidence in normal epithelial cells, teeth and alveolar bone. Damaged epithelial cells, however, did stain with trypan blue.

STATEMENT OF PROBLEM

In recent years, the formocresol technique of pulpotomy has been advocated for the management of vital cariously exposed pulps of deciduous teeth. Its clinical use and broad evaluation have shown it to be a valuable treatment consideration. Extensive research, well controlled and critical, has covered the histologic response of the pulp tissue following treatment with formocresol. Periapical tissue involvement and reaction to the formocresol, however, has not been as well researched.

The major objective of this study was an attempt to duplicate the formocresol pulpotomy procedure in a dog, using C^{14} tagged formocresol and trypan blue bound formocresol, in order to study the depth of diffusion of the tagged formocresol into the pulp tissue and to determine if the medicament actually diffuses to and involves the periapical tissue.

METHODS AND MATERIALS

This investigation was based on two series of experiments, one using freshly extracted human teeth and the other using dog teeth. The in vivo study in the dog allowed for experimental analysis under carefully controlled conditions. Dog teeth resemble human teeth morphologically and developmentally.

Animal Series

A thirty-five pound male dog was selected as the experimental animal for this investigation. The dog was anesthetized with pentobarbital sodium five per cent veterinary solution, introduced intravenously, in an initial dosage of 28 mg./kg. of body weight (dosage determined by Turner et. al.).[45] Thereafter, additional increments of the agent were titrated against the animal's response during all procedures to assure a physiologically stable condition. Respiration, circulation and reflex activity were carefully monitored throughout the procedure and effectively maintained with proper anesthetic management.

This anesthetic technique was repeated when the dog was operated on the second time. The animal was positioned on a surgical table in a lateral position with the head extended for proper access to the oral cavity and dentition. The tongue was moved out of the field of work and positioned to insure an unobstructed airway. A cork stopper was used as a mouth prop and placed between the molar teeth on the opposite side of the field to be operated.

The working field was isolated with sterile 2" x 2" surgical gauze pads and cotton rolls. Segments of three teeth were isolated together. The working field and teeth were cleaned with tincture of Metaphen and then wiped with a 2" x 2" surgical gauze pad, moistened with seventy per cent isopropyl alcohol in order to enhance surgical cleanliness.

All instruments used in the operating room were previously sterilized in an autoclave. All procedures were done with the operator wearing rubber gloves.

The cavity preparations were prepared with a hi-speed dental engine, handpiece and sterile burs. Formocresol was the medicament used as pulp dressing. The posterior teeth were treated with Buckley's Formula* of formocresol to which trypan blue was added making a one per cent solution of the vital dye. Formocresol tagged with C^{14} ** was specially prepared for use as anterior pulp dressing.

The teeth were prepared for pulpotomy in the conventional manner. The pulps were exposed from the buccal and the coronal portion amputated by a sharp, long shank excavator. Hemorrhage was controlled with sterile cotton pellets moistened in sterile water. The pulp chamber was irrigated and cleaned of debris with sterile water. With hemostases, a pledget of cotton, immersed in formocresol and blotted dry of excess medicament, was

* Crosby Laboratories, Burbank, California.

** New England Nuclear, Boston, Massachusetts.
The solution contained 1 mc. of C_{14} formaldehyde with a specific activity of 56 mc.'s per millimole. The formaldehyde was diluted with non-radioactive formaldehyde to an end point of 315 µc/millimole, 1 µc/50 lambda and a total content of 35% cresol.

placed on the amputated stumps for a period of five minutes and then removed. A zinc oxide eugenol base, Cavitec*, was placed over the stumps, followed by a silver amalgam alloy restoration. (Fig. 1-8)

Twelve teeth were operated on. The maxillary right segment was first operated on with the C^{14} tagged formocresol used as pulp dressing on the amputated stumps of the three incisors. The three premolars were then treated with the trypan blue solution of formocresol. Six days later the maxillary left segment was similarly operated on. The incisors were treated with C^{14} tagged formocresol and the premolars were treated with the trypan blue solution of formocresol.

The dog was sacrificed twenty-four hours following the second operation. The maxilla was dissected from the soft tissues, and prepared in block sections. The unoperated mandibular premolar sections were also dissected in order to compare pulps and periapical structures with those of the treated teeth. The maxillary anterior segments were sectioned interproximally and then frozen. The maxillary posterior sections, following careful removal of the silver amalgam alloy restoration were placed in fixative, as were the mandibular premolar segments.

The frozen maxillary anterior sections were later cut in half in a bucco-lingual direction, parallel to the long axis. Each half was wrapped in clear food wrap** and placed with the

* Kerr Manufacturing Company, Romulus, Michigan

** "Stretch-N-Seal", Colgate-Palmolive Company, New York, New York.

flat surface of the exposed pulp and dentin against the emulsion side of unexposed industrial x-ray film* and held in position in specially constructed presses. The specimens were kept in light proof cartons. Seven days were allowed for exposure of the film. At the end of this period, the films, with the specimens in position, were exposed to x-ray radiation for three tenths seconds to outline the sections so as to permit orientation of the radioactive exposure. The films were then developed and fixed.

The maxillary and mandibular premolar sections were fixed, decalcified, dehydrated , and embedded in paraffin. Serial sections of each tooth were prepared. Ten micron thick sections were mounted and stained with hematoxylin and eosin. Fifteen micron thick sections were mounted and prepared for clear viewing unstained.

* Eastman Kodak Company, Rochester, New York

Human Series

Fifteen freshly extracted deciduous and permanent human teeth were used for this study. All but one of these teeth were non-carious and all were removed for orthodontic reasons.

The teeth were operated on within minutes following extraction. The teeth were kept in water prior to preparation. A hi-speed dental engine and handpiece, with a water spray coolant, was used to prepare the occlusal access to the pulp chamber. The coronal pulp tissue was excised with a long shank spoon excavator. The pulp chamber was then cleaned. A cotton pledget, immersed in formocresol and blotted dry of excess medicament, was placed in the chamber over the amputated stumps for a period of five minutes. The pledget was then removed. A base of zinc oxide eugenol was placed over the medicated stumps, followed by a temporary restoration of Cavit.*

C^{14} tagged formocresol was used as pulp dressing on seven of the extracted teeth. The remaining teeth were treated with the trypan blue solution of formocresol.

The teeth were placed upright on soft wax bases in sealed glass jars for four weeks. Gauze squares, 2" x 2", saturated with water were also sealed in the jars.

The specimens were then sectioned. The premolars and canine specimens were cut in a bucco-lingual direction and the molar specimen, in a mesio-distal direction. Those teeth treated with the radioactive tagged formocresol were prepared for radioautographic study. The remaining ground sections were studied microscopically.

* Premier Dental Products Company, Philadelphia, Pennsylvania

RESULTS

The radioautographs of both the seven day and twenty-four hour specimens of dog teeth demonstrated that the area of placement of the original radioactive formocresol material was confined to the coronal portion of the pulp chamber and to the cavity preparation. Some of the sections, however, did spread within the zinc oxide eugenol dressing, which was placed over the fixed pulp stumps. No difference was noted between the seven day and twenty-four hour preparations. (Fig. 14)

These findings were also duplicated in the specimens of the freshly extracted human teeth. The area of radioactivity was confined to the coronal pulp chamber and in the zinc oxide eugenol dressing. In no instance was radioactive material observed in pulp tissue below the point of amputation, in periapical tissues, in enamel, or in cementum. (Fig. 15)

On examination of the vitally stained human preparations prior to sectioning, a blue translucency was observed in the coronal portion. The color stopped near the cervical line, approximating the point of excision of the pulp. The sectioned specimens demonstrated blue stained dentin radiating laterally and occlusally from the amputated pulp tissue. The dye did not penetrate apically beyond the pulp tissue, nor did it penetrate into enamel or cementum. (Fig. 9-13)

Histologic analysis of the dog premolar teeth prepared for clear viewing demonstrated a zone of vitally stained pulp tissue cells, demonstrating damaged cells, in both the twenty-four hour and seven day specimens. These stained cells formed the superficial layers at the excision site where the formocresol was introduced. No vitally stained cells were observed beneath the surface necrosis in the twenty-four hour preparation. Periapical tissue was not vitally stained in either case.

Histologic examination of the twenty-four hour specimen of the dog premolar teeth which were stained with hematoxylin and eosin demonstrated a surface necrosis with the formation of a collagenous-like band below the necrosis. In the middle portion of the pulp tissue there was a beginning hyperemia, with slightly engorged blood vessels. The odontoblastic layer was normal and continuous. Signs of inflammation were absent. The periapical tissues were normal.

The seven day histologic sections exhibited a more highly organized and delineated collagenous-like band then was evident in the twenty-four hour section. There was surface necrosis, with fibrous tissue present. Cellular detail was poor. The pulp tissue in the middle portion of the pulp showed signs of advanced degeneration, necrosis, hemorrhage, extreme blood vessel engorgement and edema. The apical pulp tissue and periapical tissue showed a normal scattering of cells. No inflammatory cells were present. (Fig.16)

CONCLUSIONS

The radioautographic findings in the dog study strongly indicate that the penetration of the formaldehyde component of formocresol is limited to the superficial layer of necrotic pulp tissue which was fixed by the five minute application of formocresol. In both the twenty-four hour and the seven day specimens, the radioactive material was contained within the pulp chamber, and also observed in the zinc oxide eugenol base. In a radioautograph of a maxillary right central incisor, the radioactivity was especially well exposed and defined by the x-ray outline of the pulp chamber and the pulp tissue at the amputation site. The radioactivity did not diffuse through the dentin to the enamel. The other radioautographs were less exposed. The radioactivity, however, was observed in these specimens above the fixed pulp and localized in the zinc oxide eugenol base. The human extracted specimens likewise demonstrated a localized concentration of radioactivity in the zinc oxide eugenol base. In all the specimens no radioactivity was observed below the point of amputation, in pulp tissue or in periapical tissue.

The ground section specimens of freshly extracted human teeth treated with the trypan blue solution of formocresol were consistent with the radioautographic finding. The trypan blue partially diffused into dentin, although there was no penetration into the enamel. The path of diffusion was occlusally and laterally only. The excised pulp tissue, when fixed, acted as a barrier to further penetration and diffusion of the formocresol apically.

These findings are consistent with previous histologic [7,8,11,12,13] and radioautographic studies.[38] The surface fixation described by Emerson and his co-workers[7] which occurred following a short period of application of formocresol may act as a physical barrier limiting further penetration

of the fixative. The increased hardness of this surface tissue, according to Berger,[18] was related to the ability of the formaldehyde to unite with the tissue and to render it incapable of autolysis, yet amenable to replacement by granulation tissue between peptide groups of adjacent amino acids and connecting the protein molecules without changing their basic overall structure. Stratton and Han[15,16] and Loss and Han[17] found that formocresol affected cells primarily in the immediate vicinity of its application.

The absence of noticeable effects of the formocresol pulp dressing material on the periapical tissue, as observed by Spedding,[11] and Berger[18] is in agreement with the findings of this study. Diffusion of the formocresol is confined, above the excised pulp tissue and in the necrotic zone of tissue, and its penetration below this zone to periapical tissue does not occur. The periapical tissues remain unexposed to the pulp dressing and unaffected.

Likewise, the incomplete permeability of dentine to formocresol, as first described by Gatewood and Sorenson,[38] was confirmed by this study. The formocresol does not diffuse through dentine completely and therefore the pulp dressing remains confined within the pulp chamber. The enamel and dentine appears to be impermeable to formocresol.

The histologic observations of this study are in basic aggreement with the findings of Emerson,[7] Dietz,[9] and Spamer.[13] This project was only a seven day study, therefore, a more detailed comparison of the histology with these other investigations of longer duration is difficult.

It is suggested that this study be continued with refinements in the radioautographic technique utilizing more sensitive x-ray plates with better resolution. Also, in continuing with further radioautographic research, an invivo animal study, using the primary dentition is necessary to examine the pulpal and periapical response of primary molars when treated with a five minute formocresol application, following pulpotomy.

The radioautographic and vital staining findings of this study indicate that pulp tissue, when treated with formocresol for five minutes following a pulpotomy, forms a band of coagulated tissue immediately below the surface necrosis which acts as a physical and chemical barrier to further penetration of the fixative. The formocresol remains confined to the cavity preparation. The formocresol does not diffuse completely through dentine nor is enamel and cementum permeated by formocresol. Apical pulp tissue and the periapical tissue remains unaltered and unaffected by the formocresol.

Additional research is required on formaldehyde containing drugs whose use is still largely based on empiricism. Radioautography appears to be an excellent technique with which to study the diffusion and penetration of the formaldehyde component of formocresol, when used as pulp dressing for pulpotomies.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Sweet, C.A., Jr.: Formocresol Technique--Introduction, Method, and Procedure of Treatment. The 3rd International Conference on Endodontics. University of Pennsylvania, Philadelphia, October, 1963.

2. Buckley, J.O.: A Rational Treatment for Putrescent Pulps. Dent. Review, 18: 1193, 1904.

3. Sweet, C.A.: Root Canal Treatment in Deciduous Teeth Including Pulp Exposure, Pacific Dent. Gaz. 31: 718-721, 1923.

4. Sweet, C.A.: Procedure for Treatment of Exposed and Pulpless Deciduous Teeth, J. Am. Dent. Assoc. 17: 1150-1153, 1930.

5. Gardner, T.A.: The Treatment of Exposed Pulps in Deciduous Teeth and of Abscessed Deciduous Teeth, Int. J. Orthod. 21: 876, 1935.

6. Sweet, C.A.: Treatment for Deciduous Teeth with Exposed Pulps, Rev. of Dent. for Children 4: 11, 1937.

7. Emerson, C.C., Miyamoto, Osamu, Sweet, C.A., and Bhatia, H.L.: Pulpal Changes Following Formocresol Applications on Rat Molars and Human Primary Teeth, J. South Calif. Dent. Assoc. 27: 309-323, 1959.

8. Mansukhani, Nirmala: Effects of Formol-cresol on the Dental Pulp, Thesis, University Illinois, School Dentistry, Chicago, 1959.

9. Dietz, D.R.: A Histologic Study of the Effect of Formocresol on Normal Pulp Tissue, Thesis, University of Washington, School of Dentistry, Seattle, 1961.

10. Doyle, W.A.: Formocresol Versus Calcium Hydroxide in Pulpotomy, Thesis, Indiana University, School of Dentistry, Indianapolis, 1961.

11. Spedding, R.H.: The Effects of Formocresol and Calcium Hydroxide on the Dental Pulps of Rhesus Monkeys, Thesis, Indiana University, School Dentistry, Indianapolis, 1963.

12. Berger, J.E.: Pulp Tissue Reaction of Formocresol and Zinc Oxide-Eugenal, J. Dent. Child. 32: 13-28, 1965.

13. Spamer, Rolf: The Formocresol Pulpotomy: A Histologic Study of a Single Application of Formocresol on the Dental Pulp of Human Primary Teeth, Thesis, University of Washington, School of Dentistry, Seattle, 1965.

14. Beaver, H.A., Kopel, H.M., and Sabes, W.R.: The Effect of Zinc Oxide-Eugenol Cement on a Formocresolized Pulp, J. Dent. Child. 33: 381-396, 1966.

15. Straffon, L., and Hans, S.: The Effect of Formocresol on Hamster Connective Tissue Cells; a Histologic and Quantitative Radioautographic Study with Proline-H_3, Arch. Oral Biol. 13: 271-288, 1968.

16. Straffon, L., and Hans, S.: Effects of Varying Concentrations of Formocresol on RNA Synthesis of Connective Tissue in Sponge Implants, Oral Surg. 29: 915-925, 1970.

17. Loos, P.J., and Han, S.S.: An Enzyme Histochemical Study of Various Concentrations of Formocresol on Connective Tissues, Oral Surg. 31: 571, 1971.

18. Berger, J.E.: A Review of the Erroneously Labeled "Mummification" Techniques of Pulp Therapy, Oral Surg. 34: 131-144, 1972.

19. Dobbs, E.C.: Pharmacology and Oral Therapeutics, ed. 11, St. Louis, 1956, The C.V. Mosby Company, pp. 425-427.

20. Buckley, J.P.: Modern Dental Materia Medica, Pharmacology and Therapeutics, ed. 4, Philadelphia, 1917, P. Blakiston's Son and Company, pp. 338.

21. Black, G.V.: A Work on Special Dental Pathology, ed. 2, Chicago, 1920, Medico-Dental Publishing Company, p. 298.

22. Grossman, L.I.: Irritating Potentialities of Root Canal Medicaments, Am. J. Orthod. and Oral Surg. 30: 564, 1944.

23. Rubbo, S., Reich, J., and Dixson, S.: The Use of a Combination of Heomycin, Bacitracin, and Polynyxin in Endodontia, Oral Surg. 11: 878, 1958.

24. Schilder, H., and Amsterdam, M.: Inflammatory Potential of Root Canal Medicaments, Oral Surg. 12: 211, 1959.

25. Boyd, G.A.: Autoradiography in Biology and Medicine, Academic Press, New York, 1955, p. 10.

26. London, E.S.: Etudes sur la Valeur Physiologique et Pathologique de L'Emananation du Radium, Arch. Elec. Med., 12: 363-372, 1904.

27. London, E.S.: O Fiziologopatologicheskon Znachenii Emenatsii Radiya (Physiologo-Pathological Importance of Radium Emanation), Obshchestvie Okhraneniia Narodnogo Zdraviia, 15:111, 1904.

28. London, E.S.: O Fiziologopatologicheskom Znachenii Emanatsii Radiya, Ruskii Vrach (St. Petersburg) 3, 869-872, 1904.

29. Bouchard, C., Curie, P., and Balthazard, V.: Action Physiologique de L'Emanation du Radium, Compt. Rend., 138, 1384-1387, 1904.

30. Lacassagne, A., and Latles, J.: Repartition du Polonium dans L'Organisme de Rats Porteurs de Greffes Candereuses. Compt. Rend. Soc. Biol., 90: 352, 1924.

31. Lomholt, S.: Investigation into the distribution of lead in the organism of basis of a photographic method. J. Pharmacol. Exptl. Therap., 40: 235, 1930.

32. Belanger, L.F., and Leblong, C.P.: A Method for Locating Radioactive Elements in Tissues by Covering Histological Sections with a Photographic Emulsion. Endocrinology, 39: 8, 1946.

33. Chievetz, O., and Hevesy, G.: Radioactive Indicators in the study of Phosphorus Metabolism in Rats. Nature, 136, p. 754, 1936.

34. Chievetz, O., and Hevesy, G.: Metabolism of Phosphorus in Animals. Kgl. Danske Vidensk. Selsk. Biol. Medd., 13: No. 9, 1937.

35. Sciaky, I., and Pisanti, S.: Localization of Calcium Placed Over Amputated Pulps in Dogs' Teeth, J.D. Res., 39: 1128-1132, 1960.

36. Pisanti, S., and Sciaky, I.: Origin of Calcium in the Repair Wall After Pulp Exposure in the Dog, J.D. Res., 43: 641-644, 1964.

37. Stark, M.M., Meyers, M.M., Morris, M., and Gardner, R.: The Localization of Radioactive Calcium Hydroxide Ca^{45} Over Exposed Pulps in Rhesus Monkey Teeth: A Preliminary Report, J. Oral Therapeutic and Pharm., 1: 290-297, 1964.

38. Gatewood, D.C. and Sorenson, F.M.: Formalin Penetration of the Tooth Pulp Canal, Int. Assoc. Dent. Res., 41st Abstr., 1963.

39. Carleton, H.M., and Drury, R.A.: Histological Technique - for the Normal and Pathological Tissues and the Identification of Parasites, Ed. 31, Oxford University Press, London, England, 1957.

40. Carleton's Histological Technique, Ed. 4, Oxford University Press, London, England, 1967.

41. Williams, W.L. and Frantz, M.: Histological Technics in the Study of Vitally Stained Normal and Damaged Cells, Anat. Rec., 100: 547-557, 1948.

42. Swigard, R.H. and Williams, W.L.: The Relation of Histochemical Structure of the Vital Staining of Dense Fibrous Tissue of the Mouse. Anat. Rec., 112: 811-830, 1952.

43. Williams, W.L.: The Use of Dyes in the Study of Necrosis and Repair Following Acute Chemical Injury, Anat. Re., 107: 1-19, 1950.

44. Stallard, R.E., and Schaffer, E.M.,: Vital Staining of the Periodontium of White Mice, J. of Perio., 33: 183-85, 1962.

45. Turner, H., Ruben, M., Frankl, S., Sheff, M.: "Visualization of the Microcirculation in the Oral Cavity - A perfusion technic." Presented at the International Association for Dental Research, San Francisco, March 24, 1968.

BIBLIOGRAPHY

1. Beaver, H.A., Kopel, H.M., and Sabes, W.R.: The Effect of Zinc Oxide-Eugenol Cement on a Formocresolized Pulp, J. Dent. Child. 33: 381-396, 1966.

2. Belanger, L.F., and Leblong, C.P.: A Method for Locating Radioactive Elements in Tissues by Covering Histological Sections with a Photographic Emulsion. Endocrinology, 39: 8, 1946.

3. Berger, J.E.: Pulp Tissue Reaction of Formocresol and Zinc Oxide-Eugenal, J. Dent. Child. 32: 13-28, 1965.

4. Berger, J.E.: A Review of the Erroneously Labeled "Mummification" Techniques of Pulp Therapy, Oral Surg. 34: 131-144, 1972.

5. Black, G.V.: A Work on Special Dental Pathology, ed. 2, Chicago, 1920, Medico-Dental Publishing Company, p. 298.

6. Bouchard, C., Curie, P., and Balthazard, V.: Action Physiologique de L'Emanation du Radium, Compt. Rend., 138, 1384-1387, 1904.

7. Boyd, G.A.: Autoradiography in Biology and Medicine, Academic Press, New York, 1955, p. 10.

8. Buckley, J.O.: A Rational Treatment in Deciduous Teeth Including Pulp Exposure, Pacific Dent. Gaz 31: 718-721, 1923.

9. Buckley, J.P.: Modern Dental Materia Medica, Pharmacology and Therapeutics, ed. 4, Philadelphia, 1917, P. Blakiston's Son and Company, pp. 338.

10. Carleton's Histological Technique, Ed. 4, Oxford University Press, London, England, 1967.

11. Carleton, H.M., and Drury, R.A.: Histological Technique - for the Normal and Pathological Tissues and the Identification of Parasites, Ed. 31, Oxford University Press, London, England, 1957.

12. Chievetz, O, and Hevesy,G.: Radioactive Indicators in the study of Phosphorus Metabolism in Rats. Nature, 136, p. 754, 1936.

13. Chievetz, O., and Hevesy, G.: Metabolism of Phosphorus in Animals. Kgl. Danske Vidensk. Selsk. Biol. Medd., 13: No. 9, 1937.

14. Dietz, D.R.: A Histologic Study of the Effect of Formocresol on Normal Pulp Tissue, Thesis, University of Washington, School of Dentistry, Seattle, 1961.

15. Dobbs, E.C.: Pharmacology and Oral Therapeutics, ed. 11, St. Louis, 1956, The C.V. Mosby Company, pp. 425-427.

16. Doyle, W.A.: Formocresol Versus Calcium Hydroxide in Pulpotomy, Thesis, Indiana University, School of Dentistry, Indianapolis, 1961.

17. Emerson, C.C., Miyamoto. Osamu, Sweet, C.A., and Bhatia, H.L.: Pulpal Changes Following Formocresol Applications on Rat Molars and Human Primary Teeth, J. South Calif. Dent. Assoc. 27: 309-323, 1959.

18. Gardner, T.A.: The Treatment of Exposed Pulps in Deciduous Teeth and of Abscessed Deciduous Teeth, Int. J. Orthod. 21: 876, 1935.

19. Gatewood, D.C. and Sorenson, F.M.: Formalin Penetration of the Tooth Pulp Canal, Int. Assoc. Dent. Res., 41st Abstr., 1963.

20. Grossman, L.I.: Irritating Potentialities of Root Canal Medicaments, Am. J. Orthod. and Oral Surg. 30: 564, 1944.

21. Lacassagne, A., and Latles, J.: Repartition du Polonium dans L'Organisme de Rats Porteurs de Greffes Candereuses. Compt. Rend. Soc. Biol., 90: 352, 1924.

22. Lomholt, S.: Investigation into the distribution of lead in the organism of basis of a photographic method. J. Pharmacol. Expti. Therap., 40: 235, 1930.

23. London, E.S.: Etudes sur la Valeur Physiologique et Pathologique de L'Emananation du Radium, Arch. Elec. Med., 12: 363-372, 1904.

24. London, E.S.: O Fiziologopatologicheskon Znachenii Emenatsii Radiya (Physiologo-Pathological Importance of Radium Emanation), Obshchestvie Okhraneniia Narodnogo Zdraviia, 15: 111, 1904.

25. London. E.S.: O Fiziologopatologicheskom Znachenii Emanatsii Radiya, Ruskii Vrach (St. Petersburg) 3, 869-872, 1904.

26. Loos, P.J., and Han, S.S.: An Enzyme Histochemical Study of Various Concentrations of Formocresol on Connective Tissues, Oral Surg. 31: 571, 1971.

27. Mansukhani, Nirmala: Effects of Formol-cresol on the Dental Pulp, Thesis, University Illinois, School Dentistry, Chicago, 1959.

28. Pisanti, S., and Sciaky, I.: Origin of Calcium in the Repair Wall After Pulp Exposure in the Dog, J.D. Res., 43: 641-644, 1964.

29. Rubbo, S., Reich, J., and Dixson, S.: The Use of a Combination of Heomycin, Bacitracin, and Polynyxin in Endodontia, Oral Surg. 11: 878, 1958.

30. Schilder, H., and Amsterdam, M.: Inflammatory Potential of Root Canal Medicaments, Oral Surg., 12: 211, 1959.

31. Sciaky, I., and Pisanti, S.: Localization of Calcium Placed Over Amputated Pulps in Dogs' Teeth, J.D. Res., 39: 1128-1132, 1960.

32. Spamer, Rolf: The Formocresol Pulpotomy: A Histologic Study of a Single Application of Formocresol on the Dental Pulp of Human Primary Teeth, Thesis, University of Washington, School of Dentistry, Seattle, 1965.

33. Spedding, R.H.: The Effects of Formocresol and Calcium Hydroxide on the Dental Pulps of Rhesus Monkeys, Thesis, Indiana University, School of Dentistry, Indianapolis, 1963.

34. Stallard, R.E., and Schaffer, E.M.,: Vital Staining of the Periodontium of White Mice, J. of Perio., 33, 183-85, 1962.

35. Stark, M.M., Meyers, M.M., Morris, M., and Gardner, R.: The Localization of Radioactive Calcium Hydroxide Ca^{45} Over Exposed Pulps in Rhesus Monkey Teeth: A Preliminary Report, J. Oral Therapeutic and Pharm., 1: 290-297, 1964.

36. Straffon, L, and Hans, S.: The Effect of Formocresol on Hamster Connective Tissue Cells; a Histologic and Quantitative Radioautographic Study with Proline-H_3, Arch. Oral Biol. 13: 271-288, 1968.

37. Straffon, L., and Hans, S.: Effects of Varying Concentrations of Formocresol on RNA Synethesis of Connective Tissue in Sponge Implants, Oral Surg. 29: 915-925, 1970.

38. Sweet, C.A.: Root Canal Treatment in Deciduous Teeth Including Pulp Exposure, Pacific Dent. Gaz. 31: 718-721, 1923.

39. Sweet, C.A.; Procedure for Treatment of Exposed and Pulpless Deciduous Teeth, J. Am. Dent. Assoc. 17: 1150-1153, 1930.

40. Sweet, C.A.: Treatment for Deciduous Teeth with Exposed Pulps. Rev. of Dent. for Children 4: 11, 1937.

41. Sweet, C.A., Jr.: Formocresol Technique--Introduction Method, and Procedure of Treatment. The 3rd International Conference on Endodontics. University of Pennsylvania, Philadelphia, October, 1963.

42. Swigard, R.H. and Williams, W.L.; The Relation of Histochemical Structure of the Vital Staining of Dense Fibrous Tissue of the Mouse. Anat. Rec., 112: 811-830, 1952.

43. Turner, H., Ruben, M., Frankl, S., Sheff, M.: "Visualization of the Microcirculation in the Oral Cavity - A perfusion technic." Presented at the International Association for Dental Research, San Francisco, March 24, 1968.

44. Williams, W.L. and Frantz, M.: Histological Technics in the Study of Vitally Stained Normal and Damaged Cells, Anat. Rec., 100: 547-557, 1948.

45. Williams, W.L.: The Use of Dyes in the Study of Necrosis and Repair Following Acute Chemical Injury, Anat. Re., 107: 1-19, 1950.

APPENDIX

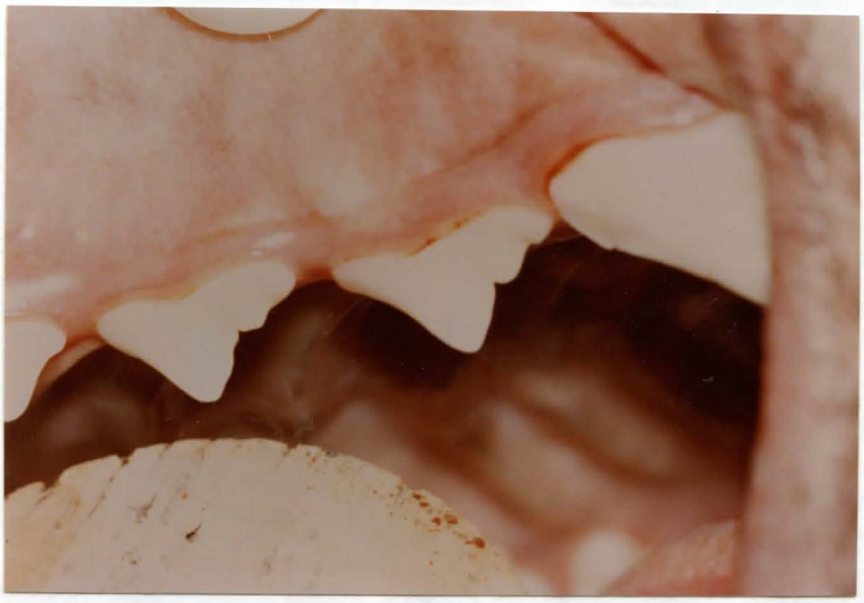

Figure 1 Preoperative view, maxillary left second premolar of dog.

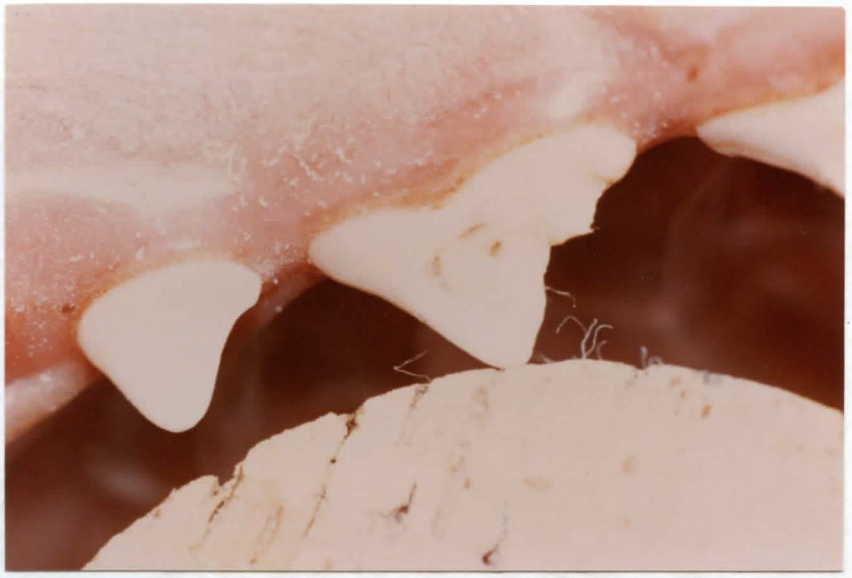

Figure 2 Buccal preparation of maxillary left second premolar of dog for access to coronal pulp.

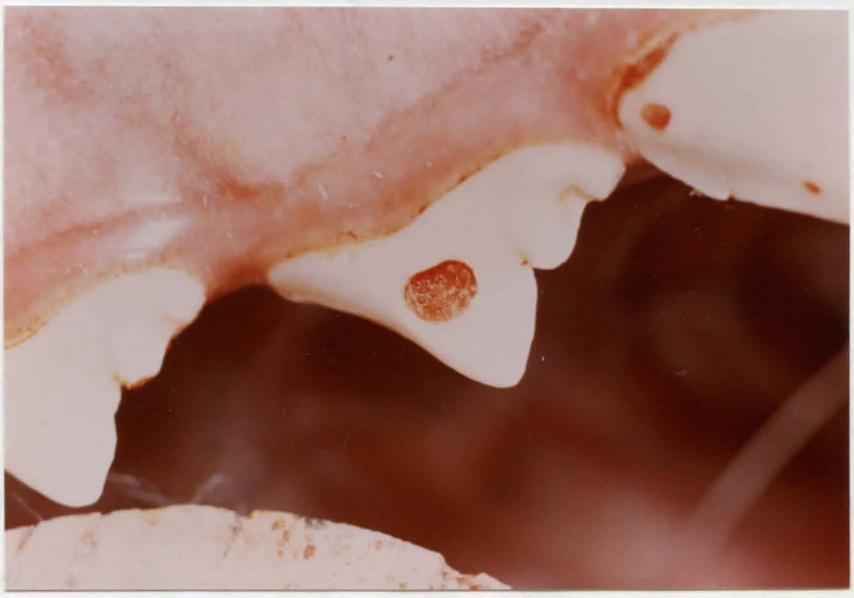

Figure 3 Moist sterile cotton pledget in contact with pulp tissue, to control hemorrhage, following amputation of coronal pulp tissue of maxillary left second premolar.

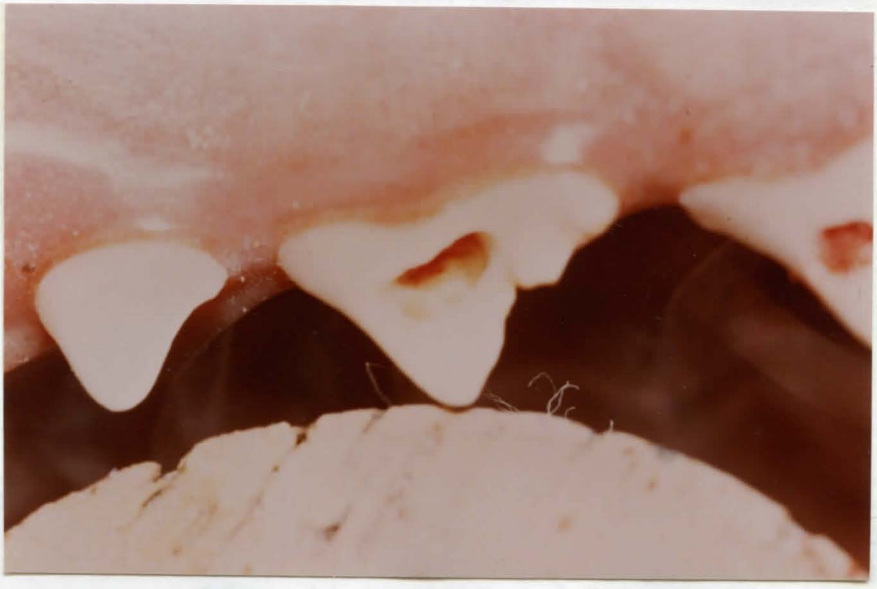

Figure 4 Pulp chamber and amputated pulp tissue stumps, of maxillary left second premolar, following hemostasis.

Figure 5 Pledget of cotton, moistened in 1% trypan blue solution of formocresol and blotted dry of excess medicament and placed on the amputated root stump of the maxillary left second premolar of the dog.

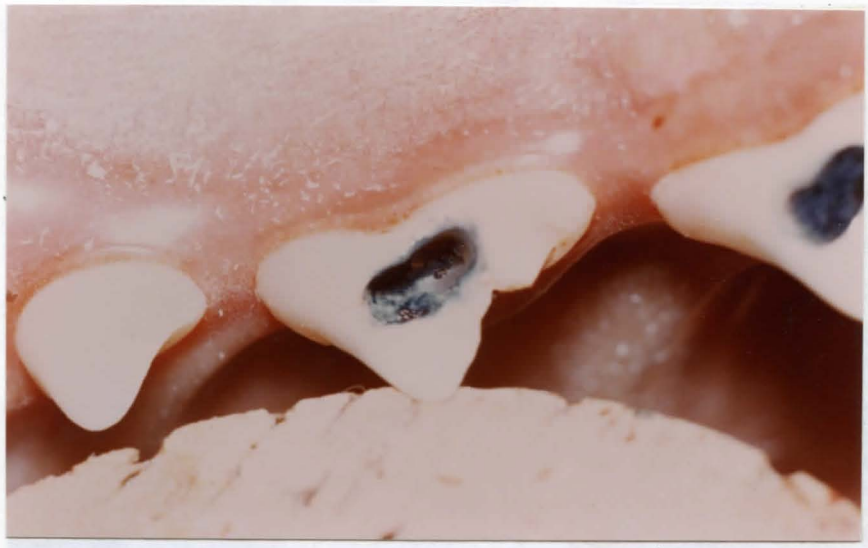

Figure 6 The pulp chamber and the amputated root stump of the maxillary right second premolar of the dog, fixed for five minutes with 1% trypan blue solution of formocresol.

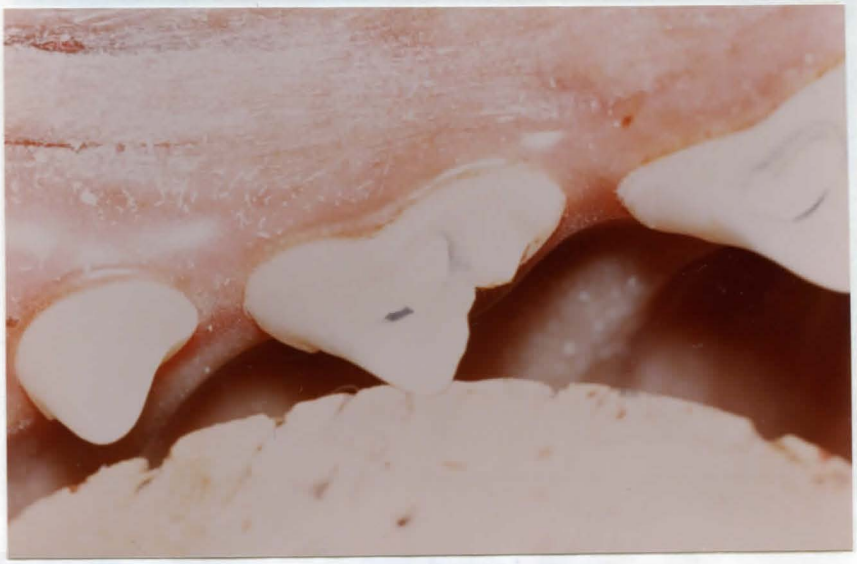

Figure 7 Cavitec on the pulp stumps of the maxillary left second premolar of the dog following amputation and fixation of the pulp.

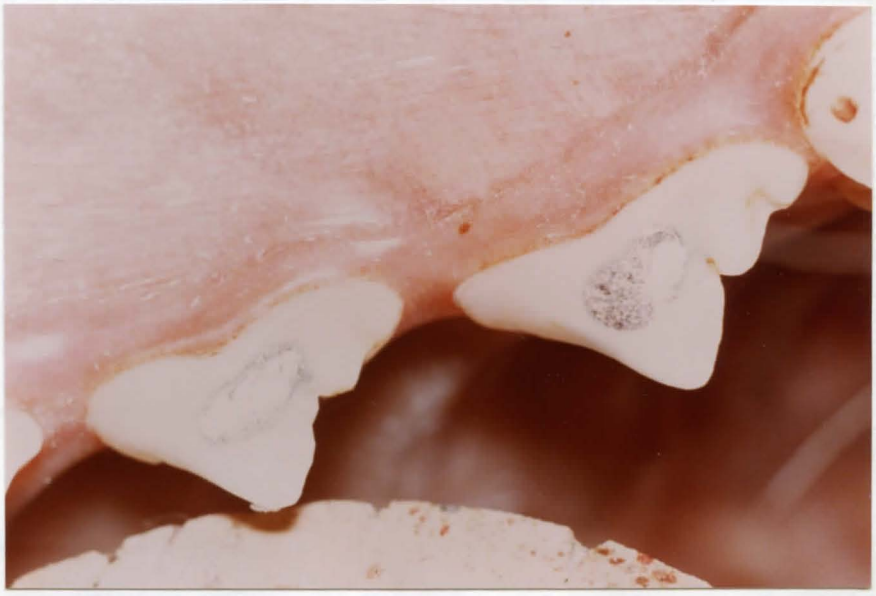

Figure 8 The maxillary left second premolar of the dog with the silver amalgam alloy restoration in position.

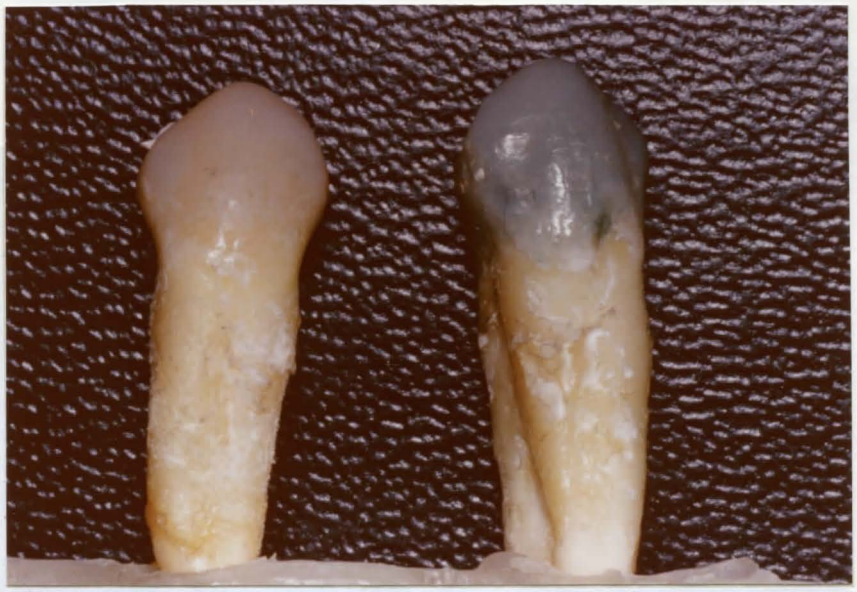

Figure 9a The buccal view.

Figure 9b The occlusal view.

Figure 9a and 9b Human premolars showing the diffusion of 1% trypan blue solution of formocresol following a five minute application of the pulp medicamen to an amputated pulp stump. The specimen on the right was the vitally stained.

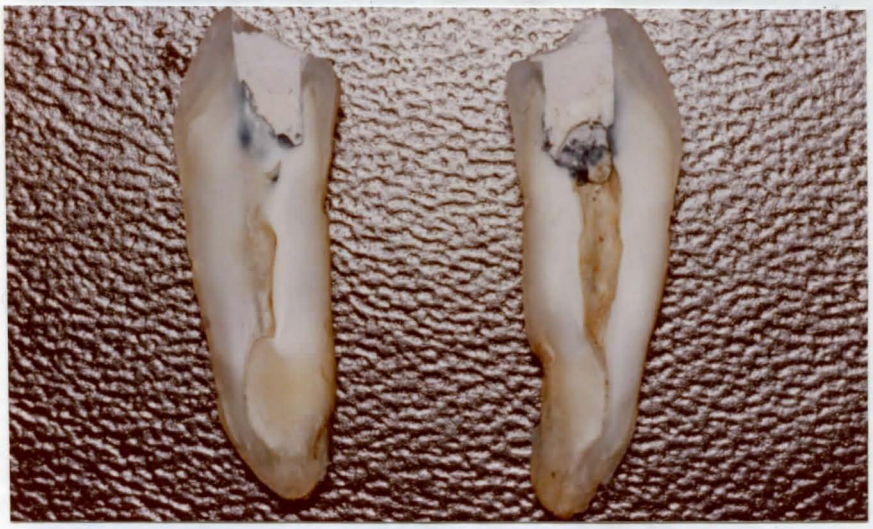

Figure 10 Bucco-lingual sections of a freshly extracted human premolar showing deposition and diffusion of the vital stain from its point of placement in the cavity preparation.

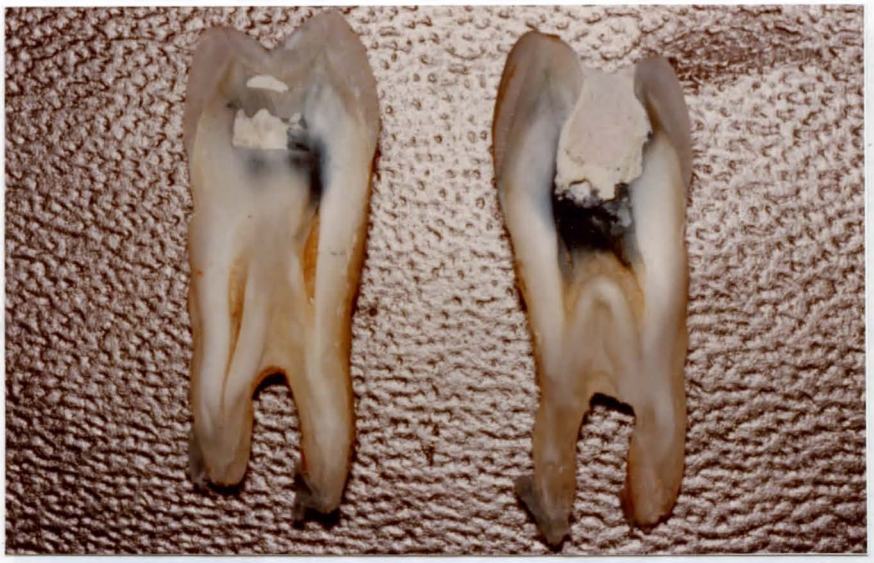

Figure 11 Bucco-lingual sections of a freshly extracted human premolar showing deposition and diffusion of the vital stain from its point of placement in the cavity preparation.

Figure 12 Bucco-lingual sections of a freshly extracted human premolar showing deposition and diffusion of the vital stain from its point of placement in the cavity preparation.

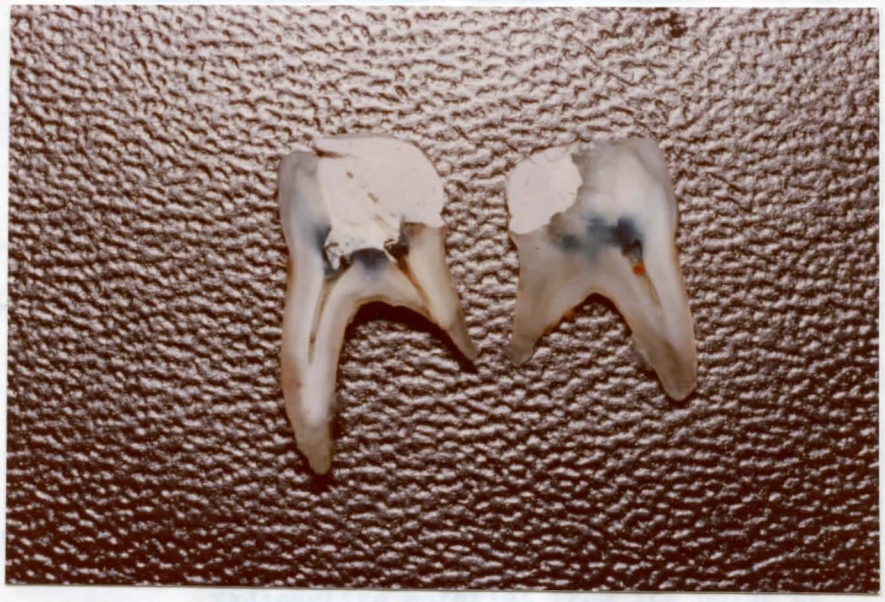

Figure 13 Mesio-distal sections of a freshly extracted human primary molar showing deposition and diffusion of the vital stain from its point of placement in the cavity preparation.

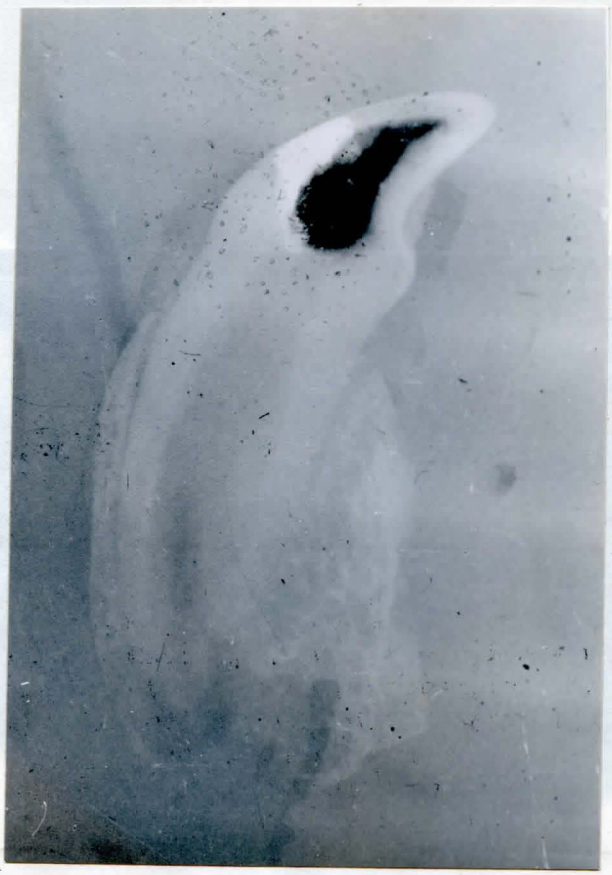

Figure 14 Radioautograph of maxillary right incisor of dog showing deposition and diffusion of radioactive material from its point of placement in the cavity preparation.

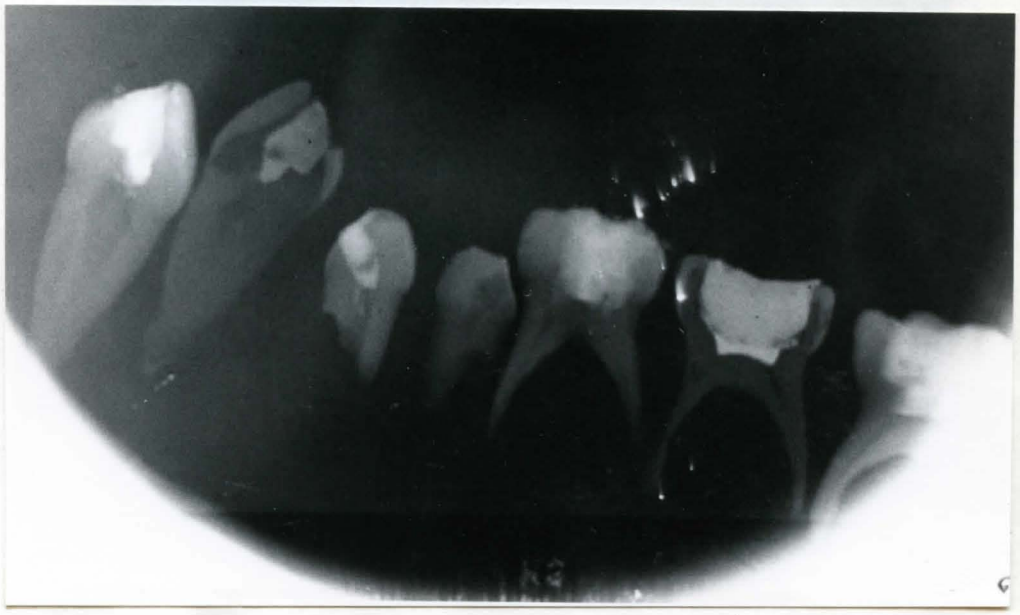

Figure 15 Radioautographs of freshly extracted primary and permanent human teeth showing deposition of radioactive material from its point of placement in the cavity preparation.

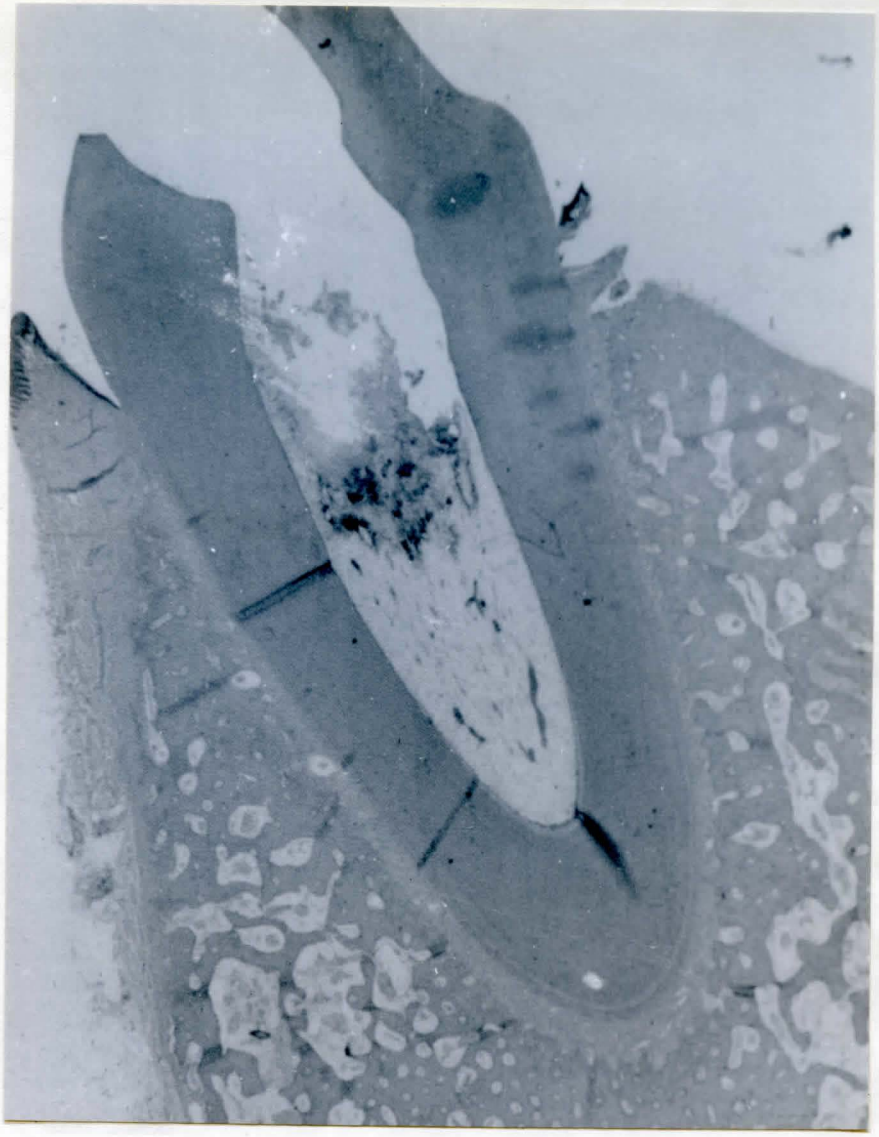

Figure 16 Photomicrograph of a decalcified section from a seven day specimen. The tissue was stained with hematoxylin and eosin and demonstrates the pulpal response to formocresol following coronal pulp amputation. (Mag. 100x)