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The Histopathology of Ti-6A1-4V and Silver Endodontic Fillings in Monkeys

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THE HISTOPATHOLOGY OF Ti-6Al-4V AND SILVER ENDODONTIC FILLINGS IN MONKEYS

BY

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

MAY 1974
Gene R. Palmer was born in South Bend, Indiana, on April 23, 1945, to Richard J. and Grace M. Palmer. He attended a township grade school in northern Indiana and attended high school at Culver Military Academy, Culver, Indiana, from which he received his diploma in 1963. He attended college at Indiana University, Bloomington, Indiana, and received a Bachelor of Science degree in 1967. He received the degree of Doctor of Dental Surgery from Indiana University School of Dentistry in 1970.

After serving two years in the United States Navy, he matriculated to Loyola University School of Dentistry in 1972 where he began graduate study toward the degree of Master of Science in Oral Biology and clinical specialty training in Endodontics under Dr. Franklin S. Weine.

Gene is married to Mary Ann Johnson Palmer, daughter of Donald L. and Margaret M. Johnson. Mary was also graduated with the degree of Doctor of Dental Surgery from Indiana University School of Dentistry in 1970 and has served with various organizations and is currently a Lt. Commander in the United States Public Health Service.
DEDICATION

To Mary, my wife, friend, and colleague.
ACKNOWLEDGEMENTS

To my advisor, Dr. Franklin S. Weine, my long-time friend, I wish to express gratitude for the guidance he has given me for these many years.

To Dr. James L. Sandrik, Dr. Louis J. Blanchet, and Dr. Patrick D. Toto who supplied the analytic criticism necessary to direct the presentation of this experimental endeavor in a logical and scientific manner, I wish to offer my sincere thanks.

In addition, I wish to express my thanks to the Department of Endodontics for my graduate education.
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CHAPTER I

INTRODUCTION

A recent investigation by Palmer, Weine, and Healey indicates that at least 50% of teeth instrumented to the radiographic apex are actually overinstrumented by "1 mm or more". Other studies indicate similar results if using the radiographic apex as the landmark to end an endodontic filling procedure. Since many dental schools teach and many practitioners advocate the use of the radiographic apex as the terminus of endodontic procedures, one can conclude that many roots, in fact, have overextended fillings.

Throughout the history of dentistry, endodontic filling procedures have become more refined. Methods of filling roots have ranged from "driving a well-fitted hickory plug dipped in creosote" to condensing warmed gutta percha into a tooth.

The soft core filling materials in greatest use today are the gutta percha compounds. According to Friedman the constituents do not vary significantly from manufacturer to manufacturer, but proportions of the constituents do vary.

In 1933, Jasper reported using silver cones to fill canals that had been instrumented with a corresponding size file, thus the emergence of the reverse inlay technique of root canal filling.

The most common solid-core filling material in use today is still silver. A recent investigation by Seltzer indicates that silver when
in contact with tissue fluids corrodes badly and that the corrosion products are cytotoxic.

The reverse-inlay technique in which corresponding sizes of instruments and silver cones are used to prepare and fill canals is simple and has application in many cases. Therefore, a material which does not corrode or yield cytotoxic effects on the periapical tissues would be ideal for use with this technique.

It is the purpose of this study to histologically compare tissue reaction to silver and an experimental metal (Ti-6Al-4V) when placed into the periapical tissues of monkeys by means of purposely over-extending root canal fillings.
CHAPTER II

REVIEW OF RELATED LITERATURE

Any material which is likely to be in contact with the body tissues should be considered an implant and possess the desirable characteristics of an implantable material. According to Ferguson, Laing, and others, the most desirable qualities of an implant are: 1) corrosion resistance, 2) ability to endure stresses in a corrosive environment, 3) general inertness, and 4) ease of fabrication.

SILVER

Noble metals were used in the body very early in time. In 1565, Petronius used gold in cleft palate repair. Dornkreilius described a method of filling root canals with silver amalgam. Other non-noble metals also have been used on a trial and error basis. In 1883, Lister used silver wire to fix fractures, and in 1894, Doisy used aluminum for the same purposes.

In 1911 Algrave experimented with the use of silver wires in bone and found that the resulting corrosion produced a chloride of silver which he determined was harmful to bone.

A. A. Zierold compared tissue reaction to metals used in orthopedics in 1924. Gold, aluminum, and cobalt chrome alloys appeared to be well tolerated although he advocated the use of the cobalt chromes due to superior strength and inertness.
At about this point in time, suppuration was a major finding associated with the failure of orthopedic implants. Using implants purposely contaminated with *Staphylococcus aureus* and *Bacillus pyocyaneus*, Rugh found that silver, gold, and tin were well tolerated by the body. He was surprised to find that his uncontaminated control implants of iron, steel, copper, zinc, and nickel produced an aseptic suppuration.

In 1933, Rickert and Dixon implanted silver both subcutaneously and intramuscularly in rabbits and found that after 3-5 months, the implants were surrounded by a band of fibrous connective tissue. During the same year, Jasper described the use of silver cones and instruments of the same size for endodontic use.

Use of silver cones for the reverse inlay technique of filling roots gained much popularity through the 1940's and in the early fifties. Auerbach recognized that teeth with overfilled silver points were not as successful as those teeth with fillings which appeared slightly short. In 1953, he published a histologic study which indicated that the overfills were most harmful to the periapical tissues when the tooth was in function.

Hunter in a study on guinea pigs in 1957 reported a fibrous capsule around silver implants placed in the tibia for up to six months. He further stated that no infection was seen around the silver.

In 1964, Feldman and Nyborg in a study comparing histologic reaction to implants of silver and a set sample of AH26 root canal sealer noted that the thickness of the fibrous capsule surrounding implants might be of significance.
Spangberg studied the effects of endodontic materials on cultures of HeLa cells in 1969. He found that silver points unhandled and sterilized did not significantly inhibit the growth of the tissue culture.

In 1972, a study by Seltzer, Green, Weiner, and DeRenzis actually put many aspects of previous studies in perspective by combining tissue culture and electron microscopic investigation. They removed twenty-five silver cones from a total of thirteen teeth which had been treated endodontically from three months to thirteen years beforehand and were considered to be failing cases. These cones were compared with five cones removed from cases considered to be successful, one of which was treated 25 years previously. The control cones numbered 13 and were produced by two different manufacturers. Five of these had been in a vial, unhandled for five years.

Electron microscopy showed that all of the used cones whether from failing or successful cases showed corrosion when compared to unused cones, although those from the failing cases showed much greater corrosion. Microprobe analysis indicated that the corrosion products were predominately silver amine sulfate amide hydrate.

Upon exposure to tissue cultures of mouse fibroblasts the control cones showed no inhibition of cell growth whereas the used cones caused inhibition of cell growth due to rupture of cell membranes.

The authors concluded that even highly polished metals appear rough under ultra-high power magnification and any rough areas may be a nidus for the corrosion process. Transfer of dissimilar metals to dentin and the silver cone itself during instrumentation can and may
set up anodic and cathodic areas. Additionally, stresses caused during manipulation and under function facilitate cracking and re-arrangement of the crystals of metal. Perhaps most significantly, frank overfill or poor seal allowing tissue fluids to contact the silver cone will set up electrochemical corrosion due to ionic gradients. Combinations of these factors will cause corrosion to occur at varying rates. Since the authors reported that the corrosion products of silver points are cytoxic, they urge avoidance of overfills wherever possible and discontinuation of the use of silver points.

It is upon the basis of these studies that a different metal than silver for the reverse inlay technique of filling root canals is indicated:

OTHER METALS CONSIDERED

In 1929, Austenal Laboratories developed a cobalt-chrome molybdenum alloy called Vitallium for use in dental implantology. It was widely accepted and is still in use today for implants in many parts of the body.

The first significant study on electrolytic effects on metallic implants came in 1937 by Venable, Stuck, and Beach. Using various metals for bone pins in dogs, they found Vitallium to be electropassive. However, any transfer of metals even from instruments to Vitallium could cause corrosion due to the effects of dissimilar metals immersed in an electrolyte. The corrosion products were found to be metallic salts which were concentrated in the livers of the experimental animals.

a) Howmet Corporation, Austenal Dental Division, 5101 South Keeler Avenue, Chicago, Illinois
Additionally, the authors found local electrolytic forces caused cellular proliferation and inhibition of bony growth.

Use of Vitallium in humans was reported by Venable and Stuck and Tuell to have short-term success. Other clinical studies followed, and successful use in the head and mandible for trauma cases was reported in the early 1940's.

In 1941, Fink and Smarko showed that under function, the Vitalium crystals may become realigned making the material subject to stress corrosion.

Although Strock had advocated the use of Vitallium as an implant in alveolar bone in 1939, Bernier and Clancy in 1943 were the first to do histologic studies of Vitallium as an intraosseous implant in alveolar bone. The results were favorable.

Also in the 1940's work with the stainless steels types 316 and 317 was underway. Uhlig indicated that the stainless steels exhibited pitting corrosion in electrolytic fluids at pH's similar to those found in the human body.

In 1948, Newman and Van Huysen reported a layer of fibrous connective tissue surrounding Vitallium implants in dogs. Other authors were able to reproduce similar histologic results.

That only metals with extreme resistance to corrosion should be implanted in the body was concluded in the 1950's by Bowden, Williamson, and Laing. In 1959, Ferguson published a book on implantable metals and reiterated the perils of corrosion.

Subperiosteal implants of Vitallium in dogs showed "no significant microscopic changes" after three months duration according to a study by Cobb in 1960. These results did not agree with those of
Herschfus' in 1958. The discrepancy appears to be involved with the
type of implant. Subperiosteal implants apparently showed less inflam-
matory response than endosseous implants of the same material.

In 1954, Herschfus noted plasma and lymphocytic cells around
Vitallium implants of three-months duration. Fitzpatrick reported
that chronic inflammatory cells should not be seen more than four weeks
after implantation of a metal. However, Frank and Abrams noted a
small number of inflammatory cells around endodontic endosseous im-
plants after 24 months and this was considered acceptable. Other
authors report different amounts of inflammation for different time
periods and each appears to have his own standard of histological
success.

Laing and Cohen considered the thickness of the connective
tissue capsule around implants and agreed that a thinner fibrous cap-
sule indicated better tissue response. Laing went on to say that
the better tissue responses were due to less corrosion.

In 1965, Vincent included mechanical irritation, galvanism and
corrosion in the list of factors which can influence host acceptance
of implanted materials.

The products of corrosion from metals have been traced by Ferguson,
Akahoshi, Laing, and Hodge to the surrounding connective tissue and
to distant organs in rabbits. Lung, spleen, liver, and kidney concen-
trated the corrosion products although no toxic effects could be attri-
buted to the stainless steel and chrome-cobalt alloys they had implanted.

Stress corrosion had been referred to by Fink and Smarko as
being responsible for corrosion of some Vitallium implants as early as
1948.
Toginow in 1966 refined this concept noting that only tensile forces and not compressive forces would cause corrosion. Additionally, he indicated that stress alone would not cause the type of corrosion seen in his implant studies but that the corrosive environment of the body was necessary. He further indicated that corrosive environment alone also would not cause the observed effects.

Takahashi re-emphasized the importance of dissimilar metals in the corrosion process. In 1968, he introduced an intriguing answer to a difficult problem. Corrosion had been seen around some implants which could not be attributed to stress or dissimilar metals by way of transfer during manipulation. He stated, "Even with the same metal, a circuit can also be produced by differences in concentration of the fluid surrounding the metal forming a concentration cell." This phenomenon is known as galvanic action. Therefore, simply an oxygen gradient as is seen in the endodontic endosseous implant could be responsible for corrosion.

Seltzer, Green, de la Guardia, Maggio, and Barnett reported corrosion of Vitallium endodontic endosseous implants in dogs. The authors speculated that ionic gradients were the cause of such corrosion from this quite inert material.

Ti-6Al-4V

As early as 1951, Leventhal suggested the use of titanium for surgical implants. After considering this proposal, Laing reported in 1966 that Ti-6Al-4V should be used due to its superior resistance to fatigue corrosion and crevice corrosion in a chloride environment.
Additionally, he found the small amounts of titanium salts released to be non-toxic.

In 1967, Laing, Ferguson, and Hodge studied the histologic reaction of rabbit muscle to implanted materials. Various metals including several types of stainless steel alloys, titanium, and several titanium alloys were implanted and the thickness of the resulting fibrous capsule measured. The authors found titanium and its alloys "remarkable" for thinness and consistency of the surrounding fibrous capsule. Titanium-aluminum-vanadium alloys demonstrated the thinnest fibrous capsules.

Morse, Barnett, and Maggio reported in 1972 a scanning electron microscopic study of Plantanium, a titanium-aluminum-vanadium alloy. Examination of a used implant revealed titanium dioxide to be the main corrosion product. Titanium dioxide was considered inert and non-toxic by the authors. Additionally, they reported closely adherent opaque protruberances on a used implant. These nodules appeared bone-like, and electron probe analysis revealed the presence of calcium and phosphorous.

In 1972, an in vitro study comparing corrosion of type 316-L stainless steel and Ti-6Al-4V by the passive current-time technique, was reported by Aragon and Hulbert. They reported that after 100 hours in isotonic solutions at body temperature, Ti-6Al-4V corroded 176 times slower than type 316-L stainless steel.

Therefore, due to its high strength, high resistance to corrosion, and low inflammatory potential, Ti-6Al-4V was chosen for this study.
SEALERS

In the course of routine endodontics and in the placement of endodontic endosseous implants, a sealing cement is necessary. Failure to seal the prepared tooth is the primary cause of unsuccessful results of treatment according to Ingle and Morse. With the use of sealers comes the extrusion of these materials into the tissues in varying amounts. Although Strindberg discourages the introduction of sealers into the periapical tissues, it is not always possible to avoid this clinically. Since sealers are likely to be introduced into the body tissues, these agents should also be considered implanted materials even though they are, for the most part, resorbable.

Grossman lists eight requirements of a root canal sealer:

1. tackiness when mixed to provide adhesion
2. slow setting time
3. radiopaque
4. fine powder in order to be readily mixed
5. non-irritating
6. non-staining
7. minimal shrinkage upon setting
8. soluble in a common solvent

Perhaps one further quality could be added; inertness.

In 1957, Hunter compared the histologic reaction of guinea pigs to implants of silver and Rickert's sealer (available as Kerr's sealer) in the tibia. After six months, a sustained foreign body reaction was seen even though a fibrous capsule had formed around the implants.
Stewart in 1958 studied three sealers in conjunction with both silver points and gutta percha points. He found Kerr's, new Grossman's (available as Proco-Sol non-staining) and Diaket sealers to be tolerated equally well when implanted subdermally into a rabbit. The new Grossman's formula and Diaket had better anti-microbial action than Kerr's sealer. Diaket possessed the greatest tensile strength and resistance to permeability.

Another experiment involving the implantation of cements sub-dermally in animals was done by Mitchell in 1959 with rats. Histologic reaction to zinc oxide-eugenol cement was mild in comparison with zinc phosphate cement.

Over a decade later, Guttuso was the first to actually explain the importance of bio-assay of endodontic materials by subdermal implantation in animals. He used samples of N₂, N₂ Medical, Proco-Sol, Tubli-Seal, Riebler resin, and Diaket root canal sealers. Kerr's and Diaket sealers gave the mildest tissue response after 32 days implantation in the rat.

Feldman and Nyborg compared pure silver to set samples of AH-26 sealer when implanted into rabbit mandibles. Bone totally healed over the silver implant but not the AH-26 implant. The fibrous capsule around the AH-26 was three times thicker than that seen around the silver implants. Also, the inflammatory cell response was greater around the AH-26.

In 1964, Rappaport et. al. distilled the requirements of the ideal root canal cement to a list of four:

1. compatible with periapical tissues
2. non-toxic
3. non-inflammatory
4. good adherence to tooth structure

The authors did a four-part study including Kerr's (Rickert's formula), Mynol, AH-26, Diaket, Kloropercha, Proco-Sol (Grossman's original formula), N, N Medical, and zinc oxide eugenol cements. The results of subdermal implantation in the rat revealed that zinc oxide eugenol gave a moderate response, Proco-Sol gave a severe response as did Kloropercha, whereas N gave a severe response with edema and necrosis. In tissue culture, all the cements appeared extremely toxic until large dilutions were made. Tests for anti-microbial activity placed zinc oxide eugenol first with Diaket, N, N Medical, Mynol, and Proco-Sol in a mass tie for a close second place followed by Proco-Sol non-staining and AH-26 with Kloropercha last. Irritational effects on rabbit cornea depended upon the powder and liquid proportions used to mix each cement. Proco-Sol non-staining powder was the least irritating along with zinc oxide.

In a bacteriologic study performed in 1965, Maurice, Kroger, and Krieger found zinc oxide eugenol to be the most inhibitory to bacteria often found in cultures of root canals. Proco-Sol non-staining and Kerr's sealer were nearly as good as zinc oxide eugenol, whereas cloropercha was deemed to be almost uniformly compatible with bacterial growth. After subcultures were made, Proco-Sol non-staining was found to have a bacteriocidal effect.

Erasquin and Maruzabal compared Diaket and AH-26 in a 1966 experiment. Sixty and ninety day specimens of rat molars filled with the cements purposely extruding were compared histologically. Diaket had a better short-term appearance, but after ninety days both sealers were being phagocytized although AH-26 appeared to give a slightly
greater inflammatory response.

The physical properties of five sealers were compared by Higginbotham in 1967. Kerr's, Kerr's Tubli-Seal, Diaket, Proco-Sol, and Kloropercha root canal sealers were tested for sealant properties. Kerr's sealer and Proco-Sol sealed best and Kloroperka sealed least.

In 1968, Curson and Kirk tested several root canal cements for setting time, sealing properties, tensile strength, and tissue reaction in rat muscle. The authors reasoned that the results of Stewart's tissue studies were invalid since cements are introduced into the tooth in the unset rather than the set state. They found that most cements set faster in the presence of moisture. Diaket, AH-26, and Kerr's Tubli-Seal gave the least amount of leakage. Zinc phosphate and zinc oxide eugenol cements were the strongest while Proco-Sol non-staining, Kerr's, Diaket, Kerr's Tubli-Seal, and AH-26 cements were approximately equal in strength. Tissue reaction to zinc oxide eugenol, Proco-Sol non-staining, Kerr's, and AH-26 sealers was moderate while Diaket and Tubli-Seal sealers gave a moderately severe response. Zinc phosphate and Bioxol cements produced a severe inflammatory response.

In a comparison of tissue responses to cements by Erasquin in 1968, zinc oxide eugenol, Kerr's, Proco-Sol, and N cements were introduced into rat molars. After 20 days, all roots with overfillings had necrosis of the surrounding periodontal ligament due to pressure necrosis. After 90 days, all extruded cements were encapsulated although they showed a tendency to be resorbed. The most favorable tissue reaction was found around roots which had been filled short of the foramen and had had "minimum" damage to the remaining pulp stumps.
Spangberg compared the cytotoxicity of several materials using HeLa cell cultures as the test system in 1969. He diluted samples until he found the level at which cell growth would not be inhibited. In decreasing order, relative cytotoxicity was as follows: Riebler's Paste, N, Diaket, calcium hydroxide paste, AH-26, pure silver, zinc phosphate cement, gutta percha, Gutta Percha-Germicidal, and Kerr's Tubli-Seal. Additionally, N and Diaket were found to increase in cytotoxicity with time due to the leaching out of water-soluble products.

Since no authors actually agree to what sealer is the one best product, which should be used in this study? Since silver points are to be tested, immediately any sealer containing silver powder must be ruled out. Proco-Sol (Grossman's original formula), AH-26, Kerr's (Rickert's formula), Kerr's Tubli-Seal, all contain silver particles for radiopacity. The resins appear to have great toxicity as seen by tissue response to Diaket and Riebler's Resin. N Medical and N are normally used as a complete filling material, not in conjunction with cones of gutta percha or silver. These materials also have unwanted inflammatory properties. Chloropercha and Kloroperka NØ are generally used with gutta percha and not usually in conjunction with metallic hard-core filling materials. Zinc oxide eugenol and Proco-Sol non-staining cements are the two outstanding in bacteriocidal non-inflammatory and non-toxic properties. They both resorb, however, Proco-Sol non-staining cement somewhat slower than zinc oxide eugenol cement. Zinc oxide eugenol is cheapest but is not in general use today as a sealer-cement for use with metal cones. Therefore, Proco-Sol non-staining cement was chosen for this experiment.
CHAPTER III

METHODS AND MATERIALS

Two monkeys, one male and one female Maculata Rhesus were chosen as experimental animals since the anatomic and histologic characteristics of these animals are similar to that of the human.

The male monkey, referred to as Alpha, weighed 9.5 Kg while the female, Gamma, weighed 8 Kg. The oral status of each monkey was that of a young adult, all teeth having erupted. The ages of the two animals were estimated at approximately four years.

The animals were housed in restraining cages at the Animal Research Facility of Loyola Hospital and were under the care of Charles Larson D.V.M., M.S. and his staff.

To prepare the animals for intraoral procedures, an IM injection a) of phencyclidine hydrochloride 20 mg/ml was given in dosages of .1 ml/kg. Approximately ten minutes after injection, the animals were removed from their cages and placed in the operating room. Any additional IM doses of phencyclidine hydrochloride were then given in 0.1 ml increments although this procedure was not usually necessary.

Preoperative radiographs of the jaws using ultraspeed type two b) periapical film were taken.

A general purpose portable hospital x-ray unit was used and by trial and error 90 kvp at 15mA at 0.6 seconds for molar and premolar teeth.

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a) Sernylan (for veterinary use only), Bio-Centric Laboratories, Inc., St. Joseph, Missouri
b) Eastman Kodak Company, Rochester, New York
areas and 0.4 seconds for anterior shots were found to be satisfactory. The films were developed and fixed in the usual manner. Mouthprops were used on the side of the mouth not to be operated and a rubber dam was placed on the tooth.

Both tooth and rubber dam were swabbed with Bactine on sterile cotton pellets.

Access openings into the operated teeth were made with a slow-speed portable belt-driven electric engine equipped with contra-angle handpiece and a #557 carbide bur. To obtain the most advantageous access to the apical areas of the incisors, some of the incisal portions of the tooth were removed.

Working measurements were determined by routine radiographic techniques after the insertion of #8 reamers. The canals were irrigated with 5% NaOCl solution frequently during instrumentation with the reamers. The preparations were made through both dentin and bone to a length approximately 3 to 5 mm. longer than the root of the tooth. The tooth was then dried with paper points, a cotton pellet placed in the chamber and the access sealed with zinc oxide eugenol cement.

During the second operative procedure approximately 48 hours later, each tooth was again opened under the rubber dam and the canals reinstrumented to assure patency. Either a silver cone or a Ti-6Al-4V cone was then chosen and fitted after 1 mm. was removed from the tip to allow for a tight fit in the prepared portion of the tooth and to avoid

a) Bactine; Miles Laboratories, Elkhart, Indiana
hitting the "stop" prepared into the bone. The cone was then removed, notched, and replaced in the salt sterilizer. Meanwhile, the canal was dried and the sealer was mixed.

a) The sealer was then placed only on the calculated intradental portion of the cone. The cone was grasped in a hemostat, seated forcefully to place, and severed by bending the cone back and forth until fracture occurred at the previously notched area.

A layer of zinc oxide eugenol cement with zinc acetate crystals mixed in to reduce setting time was then placed over the implant and the occlusal opening was filled with silver amalgam. The occlusion of the tooth was then reduced and the occlusal table narrowed buccolingually to minimize forces on the operated teeth.

Sixteen teeth totalling twenty-four roots were prepared and filled with the metal cones. Eight teeth or a total of twelve roots were filled with each material. Four teeth from the right side of the mouth of one animal and four from the left side of the other animal were filled with Ti-6Al-4V; therefore, both animals had equal numbers of roots filled with both materials. Tooth location was selected so that each operated tooth was separated by at least one tooth which was left undisturbed. This was possible in all except the maxillary and mandibular anterior teeth. In the maxillary anterior, the wide nasal cavity divided the roots of the central incisors. Only two millimeters of bone separated the mandibular central incisors.

After a period of 155 days, the animals were sacrificed using a

lethal dose of sodium pentobarbital. The mandible was removed in three sections and the anterior maxilla in one section using a Striker bone saw. The specimens were immediately placed in 10% buffered formalin solution for ten days.

After decalcification in 5% formic acid for 60 days, the sections containing the teeth were trimmed with a scalpel and the metal cones removed. The specimens were then embedded in paraffin.

a) Barb-Euthol, Haver-Lockhart Laboratories, Shawnee, Kansas
*Semi-serial sections eight microns in thickness were made in a mesio-distal direction and stained with hematoxylin and eosin.
CHAPTER IV

RESULTS

HISTOLOGIC OBSERVATIONS

Microscopic examination was made of the tissue specimens after the 155-day experimental period. Inflammatory and reparative reactions were classified as follows:

I - Minimal reaction: Minimal inflammation present around area surrounding metallic point. Thin fibrous capsule intact and boney remodelling seen around implant space.

II - Mild reaction: Fibrous capsule around metallic point with active bone repair as evidenced by reversal lines. Some residual granulomatous reaction still seen in adjacent bone marrow.

III - Moderate reaction: Predominance of lymphocytes, plasma cells, macrophages, and foreign body giant cells containing darkly pigmented granules adjacent to metallic point. Presence of root and bone resorption with some evidence of repair seen in this group.

IV - Severe reaction: A more dense infiltration of chronic inflammatory cells than seen in moder-
ate reaction with the addition of eosinophils suggesting an immunologic component. Frank granulomatous or abscess reaction with foci of polymorphonuclear leukocytes.

SILVER GROUP

Twelve roots were included in this group. Of the twelve periapical areas studied, only one showed a grade II response while six had a grade III and five demonstrated grade IV responses. The average grade of response was 3.33.

Six grade III responses were recorded. A darkly pigmented material was found to be lining the channel occupied by the implant. Attempt at fibrous encapsulation was evident. Howship's lacunae were frequently found in the adjacent bone as were reversal lines. Inflammatory infiltrate was localized to the immediately surrounding bone marrow. The predominate cells were lymphocytes and plasma cells. Also in evidence were many macrophages and a few foreign body cells containing brown pigmented granules in their cytoplasms.

The five periapical areas with grade IV responses revealed more severe findings. The darkly pigmented lining of the fibrous capsule was observed along with the chronic inflammatory infiltrate of lymphocytes and plasma cells. The surrounding bone appeared to have less reparative activity and the inflammation was more widespread into the marrow spaces. Eosinophils were also in evidence. In three instances, frank abscess formation was observed.
One root-end showed a grade II response demonstrating little inflammation and a rather thin fibrous capsule. However, the periodontal ligament appeared atrophied and the root was ankylosed on at least one aspect.

**Ti-6Al-4V GROUP**

Twelve roots were examined in this group. Seven of the periapical areas showed a grade II result while one exhibited grade III and four revealed grade I responses. The average grade of response was 1.75.

Four periapical areas revealed a grade I response. This was typified by a thin, non-pigmented, lightly staining fibrous capsule continuous with the periodontal ligament. The immediately adjacent bone was arranged in an organized fashion along the channel occupied by the metallic point. Minimal inflammation was observed in the adjacent bone marrow.

One root produced a grade III response. This root was one root of a double-rooted tooth. The response of the other root was grade I. This particular grade III response was remarkable for the great number of macrophages and giant cells seen. The cytoplasm of these cells were heavily loaded with brown pigmented granules. The fibrous capsule was well organized but considerably thicker than that seen on the other root specimen of the same tooth. Although some evidence of bone repair was present, lingering inflammation was observed in the adjacent bone marrow spaces.

Seven of the periapical areas resulted in grade II reactions. The fibrous encapsulation of the metal appeared to be as good as that seen on the four roots with grade I responses. However, inflammatory cells
consisting mostly of lymphocytes, were seen in the fibrous capsule. At times, these were observed to reach one to two millimeters into the periodontal ligament. The bone surrounding the fibrous capsule showed reversal lines and evidence of excellent remodelling by its organized arrangement. Minimal inflammation was observed in the adjacent bone marrow.
CHAPTER V

DISCUSSION

According to Maruzabal and Erasquin, an evaluation of results requires comparison of histologic findings of an experimental group with a previously standardized scale. No exact scale of expected results exists for either short or long term evaluation of this experimental procedure. Consequently, the responses observed were segregated into graded levels.

Naturally, any observation which cannot be directly measured or weighed must be subjectively evaluated. In order to eliminate bias from the grading of the histologic responses, an oral pathologist was handed slides in random order and asked to record his observations. The slides were labeled only with a letter and number. No indication of which metal had been present was given before the slide was scrutinized.

The four different grades of response are subjective divisions, also. Perhaps ten or three gradations could have been used. However, four main categories of response appeared to both include all responses while dividing all responses in a logical manner. The normal state does not include a metallic cone extending through the root end. Therefore, no grade 0 or normal was awarded to any of the specimens. A brief description of the normal anatomic and histologic findings in the Maculata Rhesus will, however, help bring the experimental results into focus.
As in the human, the cortical plates of bone are much thinner in the maxilla than in the mandible. Mandibular cortical bone is thickest buccally in the molar region and thinnest lingually in the molar region. In the anterior region of the maxilla the cortical bone is quite thin, and spongy bone is not in abundance. These findings are generally consistent with human anatomy but can be compared by adding that the relationships are more exaggerated in the monkey.

Histologically, the cortical plates consist of circumferential lamellae and these are buttressed by longitudinal lamellae. The interdental and inter-radicular spongy bone contain mostly fatty marrow between the trabeculae. Inferior to the teeth in the mandible, hematopoietic marrow is a frequent finding. Marrow in this area in the human would be a normal finding in children. The animals used in this experiment were approximately four years old and were considered young adults.

The cribriform plate of the alveolus is perforated in many places by blood vessels and nerves which supply the periodontal ligament and dental pulp. Venous sinuses in the periodontal ligament are a regular finding. Bundle bone and Sharpey's fibers are found in the same relationships as in the human.

It is important to understand that the trabeculae are arranged in a nearly horizontal plane connecting alveolar bone to cortical plate. Masticatory stresses are transmitted through the tooth and the periodontal ligament to these trabeculae. Adding a metal rod which extends through the tooth changes the relationships in this system. The tooth is now longer and forces are distributed differently. Coordination of destructive and formative activity adapts bone to changes in mechanical stresses. Osteoclasts and cementoclasts remove tissue which is no
longer suited to existing mechanical forces. Osteoblasts and cementoblasts then redeposit bone, osteoid, and cementoid. Intermittant resorption and reapposition of bone leaving so-called reversal lines is an anticipated histologic finding if effective remodelling of bone is taking place. While evaluating the tissue responses, expected physiologic bone and soft tissue remodelling was considered.

Classification of individual inflammatory cells was at times difficult since the decalcification process tends to affect the clarity of cellular structure as seen under oil immersion with the light microscope. The extension of the inflammatory process was noted. The presence of abundant hematopoetic tissue in the mandible was rather misleading at times since this tissue resembles chronic inflammatory tissue but contains megakaryocytes.

Thickness of the fibrous capsule was heavily weighted on the overall grade of the tissue response as advocated by Cohen and Laing. A fibrous capsule confluent with the periodontal ligament was considered a good response. Evidence of pressure necrosis due to mechanical trauma of the overfilled root as described by Maruzabal and Erasquin was not a universal finding. Evidence of root resorption, however, was observed and weighted in the overall grade.

Grade III and Grade IV tissue reactions totalled twelve out of twenty-four. Lymphocytes and plasma cells were characteristically present. At times, eosinophils were seen and under close examination with oil immersion lenses, brown pigmented granules were noted in the nuclei. This is suggestive of an immune response. The brown pigmented granules seen here and in the cytoplasm of macrophages are speculated to have been particles of sealer.
Interestingly, of the twelve grade III and grade IV reactions, eleven were from the silver group. Upon gross inspection, the eleven silver points removed from these eleven roots showed the dark discoloration which Seltzer, et. al. has associated with corrosion. The twelfth silver point looked comparatively shiny and came from the grade II silver specimen. Dark pigmentation was found to line the channel left by the silver cones when the histologic specimens were viewed.

The last of the twelve specimens in the group III and IV responses was a Ti-6Al-4V specimen and was one root of a double-rooted tooth. The other root contained Ti-6Al-4V and demonstrated a grade I response. This seemingly strange result is easily explained. The implant channel contained no dark pigmentation as in the silver group. However, brown granules were in evidence in many of the adjacent macrophage cells. Many more brown pigmented granules were seen around this periapical area than around most of the others viewed. Consequently, the reaction was attributed to the presence of sealer.

The method of placing cement only on the intradental portion of the cone before insertion into the preparation as described by Scopp et. al. was employed during the operative procedure. The large amount of sealer in the tissues around this particular specimen must be attributed to operative error since in most instances very little sealer was noticed in the tissues around the other specimens.

As has been mentioned, eleven of the silver group fell into the grade III and grade IV categories. The last specimen of this group showed a grade II response. This root was the second of a two-rooted tooth. The other root exhibited a grade III response. The grade II
specimen exhibited little inflammation and a well-organized fibrous capsule. The periodontal ligament appeared quite thin or atrophic and the root was found to be ankylosed. This was the only specimen which exhibited ankylosis, so a separate classification was not created since it was unknown whether or not the ankylosis was present before the experimental procedure was performed.

Of the twelve roots included in the Ti-6Al-4V group, all fell within the grade I and grade II response categories except the one grade III response which has been previously discussed. An interesting grade I response was recorded from one specimen which may be considered significant. Upon readying the tissue blocks for introduction into formalin, one Ti-6Al-4V cone was found to have perforated the boney floor of the nose. The soft tissue overlying was intact and the histologic picture was that of grade I response. Whether or not this is indicative of low inflammatory potential of the metal can only be speculated. This result can be compared directly with another perforation of cortical bone on the lingual aspect of the mandible in one of the silver specimens. A sinus tract through the soft tissues existed and histologic observation revealed a grade IV response with an acute inflammatory component evidenced by frank abscess formation.

A clinical usage study was chosen rather than subcutaneous or intraboney implantation studies in order to maintain the effects of function on periapical tissues during mastication. Cell toxicity studies on HeLa and mouse fibroblast cell cultures have already been performed to examine the toxicity of the soluble components of root canal sealers. Since cell cultures are exceedingly delicate, sheer weight of a metallic cone could cause cell death from pressure necrosis
rather than from toxicity of the metal itself. No other single test allows evaluation of materials used together as a system as well as the procedure described in this study.

Assuredly, alteration of the length of the experimental period could yield a different histologic result since the time orientation would be changed. Additionally, other sealers used with the same metals would result in testing of a different root canal filling material system and most likely a different histologic result.
CHAPTER VI

SUMMARY AND CONCLUSION

This study histologically compared tissue reaction to two metal cone and root canal sealer endodontic filling systems. Using two rhesus monkeys as experimental animals, twelve roots were prepared and filled past the apices with Ti-6Al-4V and Proco-Sol non-staining root canal sealer and twelve roots with silver points and Proco-Sol non-staining root canal sealer.

After the 155-day experimental period, the twenty-four roots and periapical areas were examined. The animals were sacrificed and the teeth along with the associated structures were removed, fixed and decalcified. Semi-serial sections were stained with hematoxylin and eosin and evaluated microscopically.

The overall histologic picture of the silver plus sealer group was noticeably less satisfactory than that of the Ti-6Al-4V plus sealer group. Root resorption, connective tissue capsule, inflammatory cells, bone spicule arrangement and the surrounding marrow spaces were all scrutinized and each root response was graded. The average grade of the silver plus sealer group was 3.33 while the average grade of the Ti-6Al-4V group was 1.75.

On the basis of this study, Ti-6Al-4V plus Proco-Sol sealer would appear to have less inflammatory potential than silver cones plus Proco-Sol sealer when used as an endodontic filling material system and extended into the periapical tissues.
CHAPTER VII

REFERENCES


18. Doisy, H.L.: De la prothese interno au moyen d'atteles d'aluminum dans le trailement des pseudo arthrosis. Lille, p. 113, 1894.


CHAPTER VIII

APPENDIX

TABLES - FIGURES
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<th>TOOTH</th>
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<th>ADJACENT MARROW SPACES</th>
<th>GRADE OF ROOT SPECIMEN</th>
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<td>Alpha maxillary left central incisor</td>
<td>angular resorption 1-2 mm.</td>
<td>inflammatory infiltrate 3-4 mm. into pdl</td>
<td>thick, loose fibrous capsule, dark pigment lining channel, heavy infiltrate of macrophages &amp; giant cells</td>
<td>random bone pattern active osteoclasis cortical plate perforated by abscess</td>
<td>inflammatory response spreads almost to adjacent normal tooth</td>
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<td>random bone pattern active osteoclasis</td>
<td>Inflammation spreads apically 5-6 mm. residual granulomatous response</td>
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<td>same</td>
<td>same</td>
<td>random bone pattern active osteoclasis perforation of lingual cortical plate</td>
<td>inflammatory response spreads almost to adjacent normal tooth</td>
<td>IV</td>
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<td>TOOTH</td>
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<td>SILVER GROUP</td>
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<td>more regular arrangement of bone</td>
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<td>minimal residual granulomatous response</td>
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TABLE II
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<th>ROOT-END</th>
<th>PERIODONTAL LIGAMENT</th>
<th>CHANNEL AND SURROUNDING (CAPSULE)</th>
<th>ADJACENT MARROW SPACES</th>
<th>GRADE OF ROOT SPECIMEN</th>
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<tr>
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<td>Alpha mandibular root</td>
<td>mesial appears normal</td>
<td>inflammation 3-4 mm. along pdl</td>
<td>thick, loosely arranged fibrous capsule - many macrophages and giant cells with brown-pigmented granules in cytoplasm</td>
<td>lateral spread of inflammatory and granulation tissue replacing fatty marrow</td>
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<tr>
<td>TOOTH</td>
<td>Ti-6Al-4V GROUP</td>
<td>SURROUNDING BONE</td>
<td>ADJACENT MARROW SPACES</td>
<td>GRADE OF ROOT SPECIMEN</td>
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<tr>
<td>Gamma maxillary left central incisor</td>
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<td>minimal inflammation</td>
<td>little inflammation</td>
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<td>regular arrangement along long axis of cone</td>
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</table>
Figure 1: A typical grade III silver specimen. Note the darkly pigmented material lining the channel. The fibrous capsule is thick and heavily infiltrated by inflammatory cells. Note inflammation in adjacent bone marrow. (40 X magnification).

Figure 2: A typical grade IV silver specimen. Note the dark granules within the thick fibrous capsule near the root apex (right). The inflammatory response is widespread and frank abscess reaction perforates the cortical plate (left). (40 X magnification).
Figure 3: A typical grade III silver specimen. Note the lack of organization of trabecular bone along the implant channel and the numerous dark staining granules within the loosely arranged fibrous capsule. (40 X magnification).

Figure 4: A grade I Ti-6Al-4V specimen demonstrating the rather longitudinal arrangement of bone parallel to the implant channel. Reversal lines are in evidence as well as a compact well-organized fibrous capsule. (40 X magnification).
Figure 5: A typical grade II Ti-6Al-4V specimen. Note the somewhat thicker capsule than the one in Figure 4. Also the dense dark lining characteristic of the silver specimens is absent. (40 X magnification).

Figure 6: An unusual specimen which was found to be perforated by the Ti-6Al-4V implant through the cortical plate. This grade I response reveals a remarkably thin fibrous capsule and lack of inflammation. (40 X magnification).
This thesis, submitted by Gene R. Palmer, has been read and approved by four members of the faculty of the Graduate School.

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

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

5/17/74

Date

Franklin S. Weine, D.D.S., M.S.D.

Signature of Advisor