RAT CONNECTIVE TISSUE REACTIONS TO IMPLANTS OF CERTAIN PULP CAPPING AND CAVITY LINING MATERIALS

An Experimental Study

by

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF THE LITERATURE</td>
<td>5</td>
</tr>
<tr>
<td>Pulp Capping Materials</td>
<td>5</td>
</tr>
<tr>
<td>Post-foetal Osteogenesis</td>
<td>18</td>
</tr>
<tr>
<td>STATEMENT OF THE PROBLEM</td>
<td>27</td>
</tr>
<tr>
<td>MATERIALS</td>
<td>28</td>
</tr>
<tr>
<td>EXPERIMENTAL PROCEDURES</td>
<td>32</td>
</tr>
<tr>
<td>RESULTS</td>
<td>40</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>65</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>71</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>74</td>
</tr>
<tr>
<td>VITA</td>
<td>81</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1. The commercial products tested. 37
Figure 2. The armamentarium and areas implanted. 38
Figure 3. The procedure of implantation. 39
Figure 4. Severe connective tissue reaction around a two day Cavit implant. 51
Figure 5. Osteoid material and giant cells within a fibrous capsule surrounding a 16 day Serocalcium paste implant. 52
Figure 6. Osteoid remnants 32 days after an implant of Serocalcium paste. 53
Figure 7. Osteoid material and giant cells 32 days after an implant of Serocalcium paste. 54
Figure 8. Osteoid remnants 32 days after an implant of Serocalcium paste with von Kossa stain. 55
Figure 9. Mild connective tissue reaction around a two day Pulprotex implant. 56
Figure 10. A two day Hydroxyline implant showing pathological calcification. 57
Figure 11. A two day Hydroxyline implant with von Kossa stain. 58
Figure 12. Moderate connective tissue reaction around a two day Chembar implant. 59
Figure 13. Coagulation necrosis separating a two day calcium hydroxide and water implant from calcified muscle bundles. 60
Figure 14. A 16 day calcium hydroxide and water implant showing osteoid in association with fat. 61
Figure 15. A 16 day calcium hydroxide and water implant with von Kossa stain. 62
Figure 16. Osteoid material in association with fat within a fibrous capsule surrounding a 16 day calcium hydroxide and water implant. 63
Figure 17. Normal dermal and subdermal areas of a rat two days after it was injected with saline. 64
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table I.</td>
<td>Composition and Reactions of Implants</td>
<td>30</td>
</tr>
<tr>
<td>Table II.</td>
<td>Gross Observations at Sacrificing</td>
<td>36</td>
</tr>
</tbody>
</table>
INTRODUCTION
Reports in the dental literature offer considerable controversy on the effects of various cements, pulp capping and restorative materials on the pulp. Most of these opinions are based on animal experiments or inadequately controlled clinical observations. Almost all the techniques employed in the practice of dentistry have been evolved on the basis of clinical experience. Those operations that made the patient comfortable and restored masticatory efficiency were retained, while those that proved failures were discarded. There is a dire need now to place our procedures on a more scientific basis rather than employing the trial and error method.

Often reliable and experienced research workers have presented contradictory findings and the severity of response of the pulp to the same material has varied with different investigators. These diversities may be explained by the introduction of extraneous factors, since the investigators used similar restorative materials, but the techniques of cavity preparation and teeth employed (rats, dogs, monkeys or humans) often were not standardized.

Some of the variables affecting pulpal reaction that have existed and which have not been adequately controlled by all investigators are:

1. Changes in permeability of the dentin with age.
2. Irritation of caries.
3. Attrition.
5. Depth of the cavities prepared.
7. Misinterpretation of results.

Other problems have been related to the technique often encountered in the difficult and time consuming decalcification procedure.
Hence the inconsistency that exists in the tooth tissue and the variations in technique employed often alter the histologic pulp picture, tending to furnish false conclusions when different investigations are correlated.

Although results obtained from animal experiments are difficult to evaluate and must be accepted with reservation, Massler\(^1\) commented favorably on the use of rat molars and incisors for the histopathological study of pulpal effects of various filling materials. Maurice\(^2\) described a technique for employing the mesial surface of the rat molar to test the effect of filling materials on the pulp.

**The Objective of Vital Pulp Therapy**

The objective of vital pulp therapy is to maintain partial vitality of the pulp by warding off further infection. However, as pointed out by McDonald\(^3\) "one procedure should not be adapted to all vital exposures in deciduous teeth," and this applies to permanent teeth, too.

When a vital pulp exposure is discovered and an accurate clinical and roentgenographic assay signifies that vital pulp therapy may be employed, the operator has three choices of treatment.

**Pulp Capping.** In spite of the controversy in the literature on the value of this method of treating vitally exposed pulps, most authors are in agreement that should the exposure be "pin point", traumatic during operative procedures, and no history of toothache is presented by the patient, pulp capping is the treatment of choice.

Rosenstein\(^4\) and Wittich\(^5\) in individual studies reported success with pulp capping of deciduous teeth and both authors are in agreement that a small exposure, hemorrhage and sensitivity of the pulp at the site of exposure are good prognostic signs.
Pulp Curettage. This method was advocated by Chatterton in 1952. He recommends partial removal of the coronal portion of the pulp after the tooth has been isolated and the carious dentin removed; and reported 72% success in 71 cases observed for 1 to 2 years.

Pulpotomy. As with pulp capping and pulp curettage a careful selection of cases is essential. Teuscher reported significant success in the preservation of young teeth by means of pulpotomy. Pulp amputation has been conventionally recommended for use in vitally exposed permanent teeth in young people or for the deciduous dentition, but not generally for adults.

More recently Berk has shown that age is relatively of little importance, since pulp amputation in patients up to 59 years of age were followed roentgenographically for approximately 5 years, with an appreciable degree of success.

Today we are no longer concerned with the question of whether the pulp is capable of healing or not, but rather with finding those conditions which might promote or retard healing of the injured pulp. The effect of currently used medicaments on pulpal repair should be studied to establish adequate proof of whether calcium salts are essential for the formation of a calcific bridge over the site of amputation or not.

Once a tooth has been selected for vital pulp therapy, the question arises as to what medicament will restore a healthy pulp with expediency and least discomfort to the patient. The severity of response to the same material varies with different investigators. Diversities may be explained by the introduction of extraneous factors due to inadequate controls. In addition, modifications in tooth tissues exist which may alter the histologic pulp picture.

When clinical studies without histologic investigations are made,
it is difficult to observe positive evidence of true healing, for example, "dentin bridge" formation. The evaluation of such results must therefore depend on negative evidence alone—that is, negative radiographic findings, persistence of normal response to thermal and electrical tests, and absence of other symptoms. These criteria have limitations since a non-vital pulp may remain asymptomatic and radiographically negative for some time.

In view of the contradictory results reported by different investigators, and realizing the importance of preservation of the vitality of the injured pulp, it was felt that a histological investigation of the effects of materials available on the market, implanted in the connective tissue of the rat would serve as an efficient screening technique.

Just as all comparisons of the effect of materials on animal pulp tissue with that on human pulp should be made with reservation, similarly, the exact correlation between connective tissue reaction of the rat with human pulp will be difficult. However, this investigator believes that the fundamental reaction of connective tissue, whether in the pulp or subcutaneously, is basically the same and can be employed to determine the relative irritant and other effects of dental materials.
REVIEW OF THE LITERATURE
Since a complete review of the recorded literature dealing with the
effect of restorative and pulp capping materials is beyond the scope of
this thesis, the author has restricted the review to pertinent pulp
capping and cavity lining agents having ingredients common to those
available commercially and studied by this investigator.

As some of the materials implanted in the connective tissue of the
rat caused the appearance of osteoid material it was felt that a brief
review of the literature concerned with studies in bone development and
growth should be included.

Hence, to establish the present status of our knowledge with clarity,
this section has been divided into two main parts:

1. A review of recorded dental literature concerning pulp capping
materi als.

2. A brief review of the literature explaining the theories of post-
foetal osteogenesis.

Pulp Capping Materials

The writer feels justified in recommending the comprehensive review
of Massler\(^1\) on the effect of restorative and pulp capping materials on
the dental pulp.

Glass and Zander\(^10\) have made a very complete review of the literature
and reported that over the years a wide variety of materials have been
advocated to promote pulp healing.

White\(^11\) and later Grove\(^12\) believed the dental pulp to be incapable
of regeneration and they discarded pulp capping attempts as impractical.
Berman\(^13\) reports that Rebel in 1922 concluded "it is impossible to get
recovery of the pulp and one must consider an exposed pulp a lost organ."
However, during the past two decades there gradually arose the realization
that the dental pulp does possess powers of recuperation and repair like any other organ in the body, provided the adverse conditions that over-
whelmed it were removed. Histological studies by Teuscher and Zander as early as 1938 showed definite evidence that a pulp can heal with a bridge of dentin forming at the line of amputation.

The Effect of Calcium Hydroxide and Similar Calcium Containing Materials

Zander reported the formation of a dentin bridge with Calxyl (calcium hydroxide in combination with other salts) and calcium hydroxide. According to his theory on the possible mechanism of the dentin bridge formation, the blood is normally saturated or supersaturated with calcium and phosphate ions, and any increase in either of these ions would cause a precipitation of calcium salts. At an alkaline pH, calcium phosphate is precipitated if an organic matrix and calcium ions are available. Since calcium hydroxide has an approximate pH of 12, the conditions for calcification are at an optimum. This occurs since "bone phosphatase is known to act best in an alkaline medium and as the solubility product decreases with increased alkalinity, the conditions here approach an optimum."15

Saltzer made an extensive review of the literature on the effect of restorative materials and cavity liners on the pulp. He reported that the severity of the pulpal reaction is due either to the depth of the cavity or to the closeness of the filling material to the pulp as well as the type of material used. Once the pulp has been irritated, ultimate recovery may take place or an abscess may form and the whole pulp may be ultimately destroyed.

Fish demonstrated in histologic sections that the formation of secondary dentin in the teeth of dogs and monkeys results from peripheral
injury and when this injury is severe, the secondary dentin is delayed for several months because of necrosis of the affected area of the pulp. Bevelander and Benzer\textsuperscript{18} confirmed this observation in a study of 300 extracted human teeth. Manley\textsuperscript{19} believed that indiscriminate cavity preparation may be responsible for hemorrhage and necrosis of the pulp.

Shoemaker\textsuperscript{20} reported the results of 28 pulpotomies where the pulp was covered with calcium hydroxide and bone meal (by insufflation), and only 31% of these cases were successful. He commented that "the profession has been and is advocating pulpotomy with more optimism and less proof than is justified."

In 1960, Hess\textsuperscript{21} confirmed the earlier reports of Zander\textsuperscript{15} and Glass and Zander\textsuperscript{10} that a paste of calcium hydroxide and water promotes the healing of an exposed vital pulp by forming a layer of calcified secondary dentin over the exposure.

Berk and Cohen\textsuperscript{22} reported that a paste of methyl cellulose and calcium hydroxide is equally as effective as calcium hydroxide and water in the production of secondary dentin. A control tooth treated with zinc oxide eugenol showed extensive necrosis of the remaining pulp tissue.

Via\textsuperscript{23} evaluated 103 pulpotomized deciduous molars treated with calcium hydroxide for periods ranging from 9 to 72 months and judged 68.9% as failures. This higher percentage of failures is not in accord with earlier work of other investigators\textsuperscript{10, 14, 15, 21}

Although much research on pulp capping and vital amputation has been undertaken and a mass of statistics has been compiled on the subject, and although these methods have advanced beyond the experimental stage, a number of commercial preparations have not been critically evaluated. Manufacturing companies often present emphatic testimony as to the ease of manipulation and successful results, but there is little or no controlled
experimental evidence to substantiate the use of their product for the promotion of pulp healing. A few years later the same companies may offer new materials and techniques with more emphatic testimonies.

Castagnola and Orlay24 reviewed the commercial preparations Calxyl, Serocalcium, Dentinigene, Pulpatect, Vitapulp, Endoxyl, and Citronellol. Serocalcium, a Swiss product, was made when the war interrupted the supply of the original German preparation Calxyl, while Dentinigene is a French product. These three, for all practical purposes, have the same constituents. The authors believed that Calxyl has a high bactericidal action due to its high hydroxyl ion content. They commented that the alkalinity is important not only because of the bactericidal action, but because it influences the injured surface of the pulp favorably. The authors quoted Czernyel and Fischer, stating that the pulp has a slight alkaline reaction similar to any other healthy tissue, and any inflammation results in a shift towards acidity, while neutralization of the acid by-products which follow an injury will facilitate healing. They concluded that calcium has a stimulating effect on the odontoblasts and a local surplus of calcium should facilitate the precipitation of calcium phosphate by the alteration of the factors of the calcium-phosphorous solubility product. Castagnola and Orlay quote Berger, stating that when Serocalcium was used in the pulp amputation of 22 teeth, 21 showed normal radiological evidence two months to two years later, while histologically 20 teeth showed a complete dentin bridge with normal odontoblasts and pulp tissue.

Jensen25 making a histologic comparison of calcium hydroxide and a commercial mixture containing zinc oxide, calcium hydroxide, iodoform, eugenol and phenol, reported similar results with either material. An area of necrosis was found adjacent to the pulp capping agent in all instances, and this necrotic area was separated from vital pulp tissue.
by an irregular dentin bridge and a continuous layer of odontoblasts. Wittich in an earlier clinical study with the same commercial preparation reported success in 87% of 243 cases.

Berk reported that the use of calcium hydroxide and methyl cellulose paste, marketed as 'Pulpdent' by the Rower Dental Manufacturing Company and classified by the council of Dental Therapeutics in Group A, resulted in a dentin bridge in dogs' teeth after a period of two and a half months. Using the same material in a clinical study of 120 cases of pulp capping and pulpotomy studied for a year, success was obtained in all but 3 cases according to clinical and roentgenographic observations.

Cabrini, Maisto and Manfredi reported a high rate of internal resorption of dentin coinciding with pulp amputation in teeth treated with calcium hydroxide. In cases of pulp capping without amputation, there was no internal resorption. They commented that "in pulp tissue it is difficult to accurately differentiate bone tissue from secondary dentin, especially when it is nucleated. We were guided by the presence, within the basic substance, of cells of the osteocyte type placed parallel to the opposed surface."

It seems necessary to differentiate between pulpal reactions caused by infection, the trauma of amputation and those due to the ingredients of a pulp capping material. The subdermal connective tissue of the rat seems to be an ideal screening site for various materials with the trauma factor minimized and standardized.

Easlick reported that pulps capped with a mixture of calcium hydroxide as the powder, and a suspension of powdered silver nitrate in approximately equal parts of Canada balsam and oil of cloves as the liquid, showed the presence of a bridge of secondary dentin seven weeks later. The pulp beneath this was normal except for slight irritation immediately beneath the silver impregnated dentin.
Hunter in an attempt to study the mechanism concerned in the deposition of lime salts in bridging over a pulp exposure reported that calcium oleate, cholesterol, zinc oxide-phosphoric acid cement stimulated no calcific bridging. Of five pulps treated with zinc oxide and eugenol, only one showed extensive bridging and two showed depositions about dentin fragments. Six out of ten pulps capped with calcium hydroxide showed bridging, while five out of ten capped with magnesium hydroxide showed bridging. The author postulated that the "similar dentin stimulating effect of both calcium and magnesium may be due to their having an elevated pH. Cation appears to be unimportant as long as it is bland."

To determine the effectiveness of calcium hydroxide mixed with methyl cellulose as a dentin liner, the material as a thin suspension was used in deep seated cavities under silicate cements by Berk. After ten weeks an inflammatory response was observed in a nonlined control cavity, while the lined cavity showed a healthy pulp.

Zander recommends the use of calcium hydroxide as a cavity lining material to protect the pulp from the "free" acid of cements. He states that calcium hydroxide can "react with free phosphoric acid of cement liquid to form Ca₃(PO₄)₂, neutralizing it at the same time preventing the penetration of further free acid that may not have been neutralized. This is usually accomplished by suspending calcium hydroxide in a membrane which then becomes coated with Ca₃(PO₄)₂. Ca₃(PO₄)₂ is not readily soluble in acid present and therefore acts as a physical barrier to any unneutralized acid."

Zander and Pejko testing the effectiveness of various cavity varnishes on dog and human teeth, found that all teeth whether lined or not with cavity varnish showed similar pulpal reaction, although occasional differences were noted in the severity of reaction. Pulps protected by
application of cavity varnishes before the insertion of silicate cements tended to show a less severe reaction.

Zander and Vissotsky testing three cavity varnishes in dog and human teeth reported that the teeth showed similar inflammatory pulp reactions with or without liners under silicate restorations. However, the severity of reaction was not as marked underneath varnished cavities as underneath the ones without varnish.

Since cavity varnishes were not effective in preventing pulpal irritation, Zander, Glenn and Nelson decided to test the following cavity liners:

1. Aluminum hydroxide in polystyrene in benzene
2. Aluminum hydroxide in polyvinyl-vinylidene chloride in acetone and methyl ethyl ketone
3. Aluminum isopropylate in polystyrene in benzene
4. Calcium hydroxide in polystyrene in benzene
5. Aluminum hydroxide and zinc oxide in polystyrene in benzene and ethyl ether
6. Calcium hydroxide and zinc oxide in polyethylene glycol (Carbowax)

Experimental and control teeth were filled with silicate cement and viewed histologically after two months. The authors found that no liner, per se, gave complete pulp protection. One liner, calcium hydroxide and polystyrene in benzene marketed by L. D. Caulk Co. as "Chembar" gave less irritation than the others. Besides, the liners containing calcium hydroxide, zinc oxide and polystyrene were not penetrated by P. Testing "Chembar" on 33 human teeth, the authors found that 31 teeth showed normal pulps except for vacuolization in 19 teeth which they attributed to the chloroform in the liner. The authors postulated that the calcium combined with "free" phosphoric acid of the cement to form $Ca_5(PO_4)_2$, "which
provides mechanical plugs in the polystyrene network and prevents further penetration of free phosphoric acid."

Stinnett and Carter\(^{37}\) advocated the following ingredients for a cavity liner: Polystyrene, yellow zinc cement powder, chloroform, zinc oxide, calcium hydroxide and thymol. The authors commented that when this liner is applied chloroform evaporates rapidly leaving a hard plastic film "impermeable to orthophosphoric acid and resistant to thermal stimuli."

Paynter, Nikiforuk, and Wood\(^{38}\) reported no inflammatory reaction in pulps of rats' teeth lined with methyl silicone resin when applied to the base of cavities as 30% solution in methylmethacrylate monomer.

Massler and Silberkweitz\(^{39}\) found that cavity varnish was completely ineffective in protecting the pulp against injury by silicates. However, they reported that calcium compound liners were very effective in this respect.

Barker\(^{40}\) reported a severe pulpal response beneath a cavity lined with sodium fluoride and glyceine paste as a liner and covered with cement. He commented that hydrofluoric acid may be the cause. He found a similar severe pulpal response when formalin in a cotton pellet was placed in a cavity and covered with cement.

As early as 1934, Orban\(^{41}\) believed that low concentrations of paraformaldehyde produced secondary dentin, while larger percentages caused metaplasia of the pulp.

Massler\(^{1}\) reported that the protection (approximately 65%) offered to the pulp by Pulpdent was less than that offered by Chembar (approximately 80%), but more than resin based varnishes (approximately 20-40%). He concluded that "at the present time, therefore, only Chembar and Pulpdent Paste have been shown to be effective in protecting the pulp against the noxious action of the silicate and phosphate cements."
In 1956 Massler suggested that a protective base or cavity liner should be used under all permanent filling materials placed in deep cavities, since in such cavities even silver amalgam may be injurious to the odontoblasts. A protective base or liner should be used even in shallow cavities filled with silicate cement. He found that zinc oxide and eugenol could not be used as a cavity liner in some cases since acrylic resins and silicate cements become discolored by eugenol and acrylics did not polymerize in the presence of essential oils. The resin based varnishes were ineffective under silicate cements, but the calcium hydroxide - polystyrene and calcium hydroxide - methyl cellulose liners were effective in protecting the pulp against injury by silicate cement and copper cements.

Shroff studying the pulpal effects of silicate cements reported the disintegration and even degeneration of the odontoblastic layer with the formation of a collagen plug at the severed ends of the dentin tubules. This collagen later calcifies (Fish's "calcific scar tissue"). If the injury has not been too severe, the odontoblasts reorganize and produce a tubular form of secondary dentin. Subsequently, edematous and inflammatory reactions tend to subside. It is generally agreed that the greater the thickness of the dentin between the pulp and the cavity floor, the greater are the reparative chances of the odontoblastic layer and pulp tissue.

Kramer, McClean and Mackenzie decided to test plastic emulsion paints as possible cavity liners, since they can be applied to the skin repeatedly without causing irritation and adhere firmly to most surfaces, forming a slightly pliable film. They concluded that the particular plastic emulsion paint that they employed, though free from significant irritant properties itself, did not form an effective barrier against
the irritant properties of two self polymerizing resins, one activated by paratoluene sulphonic acid and the other by a benzoyl peroxide-lauryl system and also containing methacrylic acid.

Dillon\textsuperscript{45} reported an experimental study in pulp therapy by the local application of calcium. He employed two pastes, one containing calcium phosphate and calcium carbonate with oil of cloves, and the other calcium carbonate, calcium phosphate, magnesium phosphate and calcium fluoride with oil of cloves. He found that calcium hydroxide gave better clinical results than either of these pastes.

Quigley's\textsuperscript{46} review of the English literature on the history of the treatment of pulpal exposures showed that the work published has been voluminous and equally contradictory. As early as 1860, Taft\textsuperscript{47} stated that "the pulp does not die of exposure" and reported bony deposition in 50\% of his carious exposures capped with a temporary filling. However, Westcott\textsuperscript{48} denied the possibility of the continuity of dentin being restored once a pulp exposure has occurred.

Zander and Law\textsuperscript{49} reviewing the use of phenol, hydrogen peroxide and cautery, zinc oxide and formoresol, and zinc oxide and eugenol, concluded that these antiseptic drugs were too powerful to be used, while they advocated calcium hydroxide as antiseptic but mild. Rosenstein\textsuperscript{50} reported a clinical study of 1,232 pulp capped deciduous teeth using zinc cement and silver nitrate liquid, copper cement and regular liquid, copper cement and silver nitrate liquid, zinc oxide and thymol, and zinc oxide. He obtained over 85\% success with each material, and without any statistical difference between the materials. His conclusion was that the choice of material was not as important as the other factors in diagnosis and treatment. Repeated studies by Berk\textsuperscript{26} Berk and Cohen,\textsuperscript{22} Brindsden,\textsuperscript{51} Glass and Zander,\textsuperscript{10} Patterson and Van Huysen,\textsuperscript{52} Teuscher and Zander,\textsuperscript{14} and Zander\textsuperscript{15} demonstrated that the pulp can heal under a dressing of
calcium hydroxide. Restarski,53 Marmasse,54 and Castagnola and Orlay24 demonstrated healing of pulp under "Calxyl," a combination of calcium hydroxide, and sodium, potassium and calcium chlorides, and sodium bicarbonate. Wheeler55 reported 95% success in 60 cases of pulp capping with calcium phosphate and calcium hydroxide. Wittich5 in a clinical study of 243 cases of pulp capping observed over a period of 6 months to 4½ years, reported a success of 87% with a paste of calcium hydroxide, iodoform, zinc oxide, eugenol and phenol. Law56 using calcium hydroxide in 256 cases of vital pulpotomy obtained a rather low percentage of 49% success. However, Strange57 obtained 85% success with 45 cases of vital pulpotomy with calcium hydroxide.

The Effect of Zinc Oxide and Eugenol Mixtures

A great deal of work has been devoted to the comparing of the effect of calcium hydroxide and zinc oxide in the treatment of exposed pulps.

In a recent study of experimental pulpotomies on rat molars, Berman13 and Berman and Massler58 reported no basic differences in pulpal reactions to calcium hydroxide, and zinc oxide and eugenol at 21 and 28 days after amputation. Both materials stimulated the formation of a dentin bridge. There were some differences in the histological picture and rate of healing at 7 and 14 days. The authors noted that calcium hydroxide has a necrotizing or coagulating effect on the superficial pulp tissue and on any intervening blood clot or exudate. This appeared to cause an early appearance (7 days) of a zone of dystrophic calcification and this was associated with an early appearance (14 days) of a dentin bridge. At 7 days the pulp tissue under zinc oxide and eugenol appeared normal or showed evidence of a large number of polymorphonuclear leucocytes. These cells were absent at 21 days. Besides, the rate of healing appeared slower in the early stages of zinc oxide and eugenol.

As early as 1936, Manley59 studied the effect of zinc oxide and eugenol
on dogs' teeth and reported that it had no deleterious effect on the pulp. Later, he demonstrated similar results in humans.  

Gurley and Van Huysen in an experimental study found no disturbance in the pulp of dogs' teeth after they were treated with zinc oxide and eugenol.  

Harvey, LeBrocq and Rakowski in a thorough and critical survey of dental cements suggested that a valuable measure of pulpal protection is afforded by using zinc oxide and eugenol to isolate dentinal tubules from such cements.  

James and Schour reported that under cavities lined with zinc oxide and eugenol the pulp showed minimal or occasional inflammation and concurrently an absence of reparative dentin.  

Nygaard-Ostby reported normal pulps in human teeth under cavities where zinc oxide and eugenol was used as a base material.

Seelig and Lefkowitz reported normal odontoblasts with no inflammatory reaction in the underlying pulp when zinc oxide and eugenol was used in cavities of 10 to 12 year old monkeys.  

James and Diefenbach in their study on the effect of zinc oxide and eugenol on the teeth of young dogs reported no deleterious effect on the pulp, and recommended it as a protective lining under permanent fillings.  

Shroff reported that zinc oxide and eugenol had a protective effect on the pulp against the action of silicate cement.  

Kramer and McClean asserted that zinc oxide and eugenol had a protective effect on the human pulp against the action of self curing resins.  

The exact mechanism by which a zinc oxide and eugenol mixture acts on the pulp is not known, but Massler suggests that it "promotes healing
by reducing the inflammatory reaction in connective tissue."

Although it is generally accepted that the active ingredient in the mixture is the eugenol, Turkheim in a bacteriological investigation on dental materials found that zinc oxide itself is antiseptic.

The Role of Antibiotics in Pulp Therapy

As early as 1960, Kutscher and later Gilberg in 1951, reported 98% success with penicillin incorporated as a pulp capping material. Rosen in a four month observation period in the pulp capping of 40 deciduous teeth reported 100% success.

Grossman developed a mixture of antibiotics which proved to be effective against all organisms isolated from infected root canals. The mixture consisted of penicillin, streptomycin, bacitracin and sodium caprylate in a vehicle of DC 200 silicone fluid. The compounds were compatible in silicone and the mixture was stable. Seelig, Fowler and Tanchester demonstrated that normal healing can occur under a dressing of calcium carbonate and penicillin G potassium, when the dental pulps of the rhesus monkey were surgically exposed.

Via reported that a dentin bridge formed under dry barium sulphate when pulpotomies were performed in the incisors of monkeys. This bridge was approximately equal to that formed with calcium hydroxide, but no necrotic pulpal tissue was observed between the barium sulphate and the dentin bridge as commonly found with calcium hydroxide. The author found that a more regular bridge formed with barium sulphate and an antibiotic paste, and concluded that a layer of necrosis is not necessary for the production of a dentin bridge and that the presence of artificially placed calcium ions has little to do with the production of secondary dentin.
Feitelson\textsuperscript{79} reported 91% success in pulp capping 85 teeth with a dressing material containing a suspension of calcium hydroxide and penicillin G in water, or in an aqueous suspension of methyl cellulose. On the other hand, Kutscher and Yigdall\textsuperscript{80} asserted that the antibacterial activity of penicillin and chloromycetin was nearly completely destroyed by calcium hydroxide. Some antibacterial activity remained following the addition of calcium hydroxide to aureomycin, streptomycin or terramycin.

Roberts\textsuperscript{81} advocated the adjunctive use of intramuscular penicillin injections in the treatment of exposed pulps capped with calcium hydroxide and water, and reported 94% success.

The precise role of antibiotics in vital pulp therapy has still not been determined, and since the literature at the moment reveals adequate success without the addition of antibiotics to pulp capping materials, the necessity of using antibiotics is questionable.

\textbf{Post-Fetal Osteogenesis}

"Osteogenesis, the formation of bone tissue occurs always and everywhere in the same way. Whether we deal with the first appearance of bone spicules in the mesenchyme of the embryo at the vault of the skull or with the appearance of bone trabeculae around a cartilagenous model of a bone, whether we deal with the development of bone inside the destroyed cartilagenous model or with the appositional growth of spongy or compact bone, whether we observe the healing of fractures or the ectopic or neoplastic formation of bone, the process of osteogenesis is in principle identical. The mother tissue is always loose connective tissue."

\textsuperscript{82} Bertelsen\textsuperscript{83} in an experimental investigation of post-fetal osteogenesis presented three types:
1. Homoiotopic osteogenesis, associated with the osseous system.

2. Heterotopic osteogenesis, bone formation in soft tissue.

3. Induced osteogenesis, caused by the transplantation of osseous or osteogenic substances.

A review of the literature reveals that three theories are available to explain post-fetal osteogenesis:

1. The osteoblastic theory.

2. The metaplastic theory.

3. The theory of osteogenic dualism.

**The Osteoblastic Theory**

According to the Osteoblastic Theory, there are within the soft parts surrounding the skeleton—the periosteum, marrow and the content of the Haversian canals—specific cells, osteoblasts, which are osteogenetic and can develop bone tissue.

Keith\(^8\) cited Duhamel, who studied bone growth in madder fed animals, as the originator of the osteoblastic theory of osteogenesis. Duhamel concluded that the periosteum is the most important tissue concerned with osteogenesis. This concept was supported by Ollier\(^8\) who carried out extensive studies with periosteum. Ollier conclusively proved the osteogenic ability of periosteum when bone was produced around periosteal implants in the subcutaneous tissue of rabbits.

Ham and Gordon\(^8\) in experiments to determine the origin of bone forming in association with cancellous chips transplanted into muscle, reported that no new bone formed in association with thrice frozen and thawed chips, while new bone was found in each animal implanted with fresh cancellous chips. Therefore, the authors concluded that the bone growth did not occur due to the activity of the muscle, but due to the specific osteogenic cells of fresh bone.
Hudak, Blount and Darby\textsuperscript{87} reported that denatured bone powder was incapable of producing bone in their study.

In spite of the wide support in the literature of the Osteoblastic Theory, several investigators have refuted it. Baetzner\textsuperscript{88} could not obtain new bone formation in a series of experiments where he transplanted periostea. His reports are in agreement with earlier observations by Good sir\textsuperscript{89} and Macewen\textsuperscript{90, 91}

### The Metaplastic Theory

According to the Metaplastic Theory, osteogenesis is not related to the existence of specific cells, osteoblasts, but may occur when an adequate stimulus induces the undifferentiated cells in the young connective tissue to differentiate into osteogenetic cells.

Bertelsen\textsuperscript{83} cites Baschkirzew and Petrow as the propagators of the Metaplastic Theory of Osteogenesis. They reported similarity of results in bone formation when bone with or without periostea and marrow was transplanted in the gluteal musculature. Nageotte\textsuperscript{92} obtained bone formation even with dead bone which had been fixed in alcohol and formalin before transplantation. Leriche and Policard\textsuperscript{93} supporting the Metaplastic Theory, reported that the matrix was the predominant tissue involved in the metaplasia while the cells took a secondary role. They theorized that the factors essential for bone formation are: (a) an irritant acting as a stimulus to convert the connective tissue into "pre-osseous tissue" and (b) an adequate supply of calcium salts.

Investigations by Levander\textsuperscript{94} gave support to the Metaplastic Theory. The author made observations as early as one or two days after implantations to observe the reactions taking place in both the graft and in its surroundings. Results showed that implanted skeletal parts died after a brief period, while surrounding the graft new bone arose from mesenchymatous tissue.
The Theory of Osteogenic Dualism

Bertelsen\textsuperscript{83} cites Bier as propagator of the Theory of Osteogenic Dualism. According to this concept, osteoblastic cells in the periosteum, bone marrow, or Haversian canals, as well as the undifferentiated mesenchymal cells of the connective tissue are osteogenic. Auxhausen\textsuperscript{85} reported two distinct phases in the regeneration of bone: (1) earlier phase, activity of the pre-existing specific cells, osteoblasts, and (2) later phase, activity of the undifferentiated connective tissue cells.

Bancroft\textsuperscript{86} theorized that when the bridging of a bony defect occurs, osteoblasts are responsible for the periosteal and intermediate callus that is laid down, whereas, when heterotopic bone formation occurs, it is a result of the metaphasic activity of undifferentiated mesenchymal cells.

The Role of Calcium Salts in Osteogenesis

In an extensive review of the literature on osteogenesis, Shankwalker\textsuperscript{87} observed no uniformity of results relating the role of calcium salts to osteogenesis.

Shands\textsuperscript{88} in a series of experiments to study the effect of the local presence of calcium salts in various combinations, had promising but inconsistent results. He reported that calcium triple phosphate (3 parts) and calcium carbonate (1 part) stimulated bone growth in the dog ulna, while calcium glycerophosphate, selected due to its action upon phosphatase, showed no stimulation of bone.

Wells\textsuperscript{89} theorized that calcium salts caused metaplasia of the connective tissue with the subsequent appearance of osteoblasts and a regular bony system including bone marrow, with its hematogenic function.

Albee and Morrison\textsuperscript{90} made a study of fracture healing in the radius of rabbits injected with 1 cc of a 5% solution of triple calcium phosphate.
compared to control animals with similar defects. They reported that the experimental fractures injected with tricalcium phosphate healed within an average of 31 days compared to the controls which healed within an average of 42 days. The authors commented that various materials "osmic acid, fibrin, blood, gelatine and lime salts, zinc chloride, thyroidin, glacial acetic acid, iodine tincture, adrenalin, hypophysis extract, bone marrow acid, silver nitrate solution, alcohol, carabolic acid, oak bark extract, vaccines and sera" have been tried to stimulate bone growth without success.

Haldeman and Moore\textsuperscript{101} in their studies of the effect of monocalcium phosphate, dicalcium phosphate, tricalcium phosphate and calcium glycerophosphate reported that tricalcium phosphate actually retarded the healing of experimental fractures, while the other materials had no essential effect when compared to controls. The authors concluded that a local excess of calcium and phosphorus had no appreciable effect on the healing of fractures in rabbits. Similar results were obtained by Key,\textsuperscript{102} and Eden.\textsuperscript{103}

Stewart\textsuperscript{104} reported that lime salts and autogenous boiled bone were unsuccessful as a source of supply of calcium in the healing of bone defects.

Although some investigators have reported discouraging results with the effect of local concentrations of calcium and phosphate, several others have obtained accelerated healing of fractures under the same conditions. Murray\textsuperscript{105} reported that calcium carbonate, calcium phosphate, and boiled bone resulted in rapid osteogenesis to form a bony deposit in experimentally prepared bony defects in the radii of dogs. When the bone was decalcified prior to insertion within the defects, osteogenesis failed to occur and the author concluded that the presence of calcium salts is essential. Ray and Ward\textsuperscript{106} studied the effect of basic calcium phosphate, which has almost the same chemical composition as bone salts,
on bone defects. They reported that the crystals of basic calcium phos-
phate appeared to exert no specific influence on osteogenesis and healing
was not as rapid as when fresh autogenous cancellous bone grafts were used.

Weil, Schram and Fosdick, Costigan, and Welborn reported favorable results with the use of synthetic bone pastes consisting of
calcium salts inserted in bony defects.

More recently Peltier and co-workers confirmed earlier reports
by Edberg that plaster of Paris stimulated osteogenesis when used to
fill defects in bone.

Of the eleven materials tested by Mitchell and Shankwalker only
calcium hydroxide, magnesium hydroxide and plaster of Paris gave any
evidence of osteogenesis when embedded in the connective tissue of the
rat. The authors found that despeciated calf-bone paste, and calcium
hydroxide and gelatin mixtures appeared to delay the healing of small
surgical wounds in monkey tibiae. Anorganic bone is rather inert when
implanted in the connective tissues of the rat, and in the surgical
wounds of the tibiae. Earlier Mitchell and Amos reported heterotopic
bone formation with calcium hydroxide implants in the subcutaneous tissues
of the rat.

Urist and McClean defined osteogenic potency as the capacity of
a tissue to produce new bone. This potency becomes manifest as osteogenic
activity, expressed as actual formation of bone by survival and prolifer-
tion of transplanted cells. However, when the ingrowing cells of the
host are induced to form bone by contact with the transplanted material,
the process is termed induction and it is this process that elicits a
potency in the ingrowing connective tissue cells which might otherwise
have remained latent.
Weinmann and Sicher\textsuperscript{116} expressed the opinion that the "physiologic development of bone in the tissues of so many organs proves that, phylogenetically at least, the potency of bone formation is universal in the connective tissue of the entire body. The potency of differentiation of connective tissue was not lost during the evolution of the highest mammals, in which, under pathologic condition or in experimentation, formation of bone occurs in many areas outside the skeleton."

The authors believed that "formation of bone can occur only if two conditions are fulfilled: the presence of cells of low differentiation and therefore of high potentiality, and an adequate stimulus to induce these pluripotential cells to differentiate into osteoblasts. Loose connective tissue contains, probably everywhere, reserve cells, the undifferentiated mesenchymal cells of Maximow, whose pluripotentiality is well known."

According to Asboe-Hansen\textsuperscript{117} "the formation of bone is essentially a patterned calcification of ground substances associated with increased vacuolisation, and although as a histologist one distinguishes bone formation in fibre, by apposition and endochondral ossification, yet the changes in all three are really the same; there is an increase in mucopolysaccharides in the ground substance with a separation of the collagen fibres and a morphological modification of the connective tissue cells, there is a change to an alkaline pH within depolymerization of mucopolysaccharide...."

The part played by mucopolysaccharides in the fibroplasia around gelfoam pledges implanted subcutaneously in rats is emphasized by Taylor and Saunders\textsuperscript{118}

In 1935, Dixon and Rickert\textsuperscript{119} studied the effect of several dental materials implanted in the subcutaneous tissue of rabbits. They reported that zinc oxide showed chronic productive inflammation with fibroblastic
proliferation and a moderate cellular exudate.

Schaad, Carter and Myers\textsuperscript{120} observed a similarity in the reactions in the abdominal connective tissue of rats to dental base materials with the results obtained with similar materials in rat incisors. Their study was on a short term basis of 24, 48 and 96 hours and they reported the most severe reaction was observed with red copper cement which tended to get more severe at 96 hours than at 24 hours. Zinc oxide and zinc acetate with eugenol gave a moderate reaction, while both dicalcium phosphate with saline and calcium hydroxide with saline showed slight reactions.

According to Hill\textsuperscript{121} calcification within the pulp arises from three different sources:

1. Metabolic disturbances such as vitamin deficiency and the resulting infolding of the odontoblastic layer to form islands of dentin.
2. Calcification subsequent to and associated with hyalinization of connective tissue.
3. Calcification of amyloid bodies.

He cites Weber and Euler as having demonstrated that the presence of fat precedes calcification. Mitchell and Shankwalker\textsuperscript{113} found fat tissue in close association with heterotopic bone deposits. Tunbridge\textsuperscript{122} occasionally noted fatty material with evidence of calcification in the vicinity.

Kreshover and Bevelander\textsuperscript{123} failed to disclose any radiographic evidence of pulp calcification or periapical pathosis in dogs following pulp exposure.

Kalmnis\textsuperscript{124} studied the effect of pressure by means of a thick paste composed of calcium hydroxide, sulphasthiazole and strontium salts on exposed healthy human teeth. Histologically, the effects of pressure
were compacting of the connective tissue stroma in the superficial portion of the pulp which showed fibrous metaplasia, below which the tissue was undisturbed. The author observed that the restoration of a dentinal wall developed in two ways:

1. formation of a bridge directly below the compacted portion of the pulp, and

2. establishment of a dentinal bridge preceded by formation of a dysfunctional zone of pulp tissue.
STATEMENT OF THE PROBLEM
Although there have been exhaustive studies of the effects of various filling materials upon the pulp, there have been no comprehensive studies made on the effect of various commercial cavity lining and pulp capping drugs. Realizing the importance of preservation of the vitality of the injured pulp, the author hopes that a histological investigation of the effect of these commercial preparations on the connective tissue of the rat would be a contribution to the literature. Use of the subcutaneous connective tissue of the rat is offered as an adequate medium for mass screening of cavity lining and pulp capping agents. The purpose of this investigation is to study the effects of various implants on the connective tissue of the rat to classify them according to their irritant qualities, and to discuss the possible correlation of these results with earlier reports of other investigations dealing with the dental pulp.
MATERIALS
Animals

Forty-one young adult Wistar rats, 17 females and 24 males, all of which appeared clinically healthy were used in the study.

Implant Materials

The pH of the test materials was determined by preparing a suspension of 1 gm by weight of the paste in 100 ml of liquid. The liquid employed with Cavit, Cargenol, Gardenier Sedative Cement, Vitec, Caveline, Cavitec, and Pulprotex was 95% alcohol (pH 7.0) since their ingredients tended to go into solution more easily with alcohol than with water. The liquid employed with Serocalcium paste, Hydroxyline, Chembar and calcium hydroxide was triple distilled water (pH 6.0). A standard laboratory Beckman pH meter was used to determine the pH of the suspensions 24 hours after preparation.

Cavit. A product of Premier Dental Products Company is supplied in paste form and is composed of the following ingredients: zinc oxide, zinc salts, calcium salts, vinyl polymers and oxysters. (pH 3.1)

Serocalcium paste. A product of Dr. Wild and Company, Basel, is supplied in paste form. It is a "white, slightly radio-opaque paste, composed of calcium hydroxide, Ca(OH)₂, with some salts of the human blood serum NaHCO₃, CaCl₂, and KCl."²⁴ According to Castagnola and Orlay, the serum salts bring about a better tolerance of the calcium hydroxide. (pH 12.2)

Cargenol. A product of King's Specialty Company is available in powder and liquid form. The powder is composed of zinc and magnesium oxides, calcium phosphate, and barium phosphate. The liquid contains eugenol, thymol and carbolic acid. (pH 3.9)

Gardenier Sedative Cement and Pulp Protector. A Densco product is supplied in powder and liquid form. The powder is composed of zinc oxide and bismuth
subnitrate, while the liquid contains eugenol and thymol. (pH 8.3)

Vitec. A Kerr product is supplied in powder and liquid form. The main ingredients are zinc oxide, eugenol, oleoresins and sulphathiazole. (pH 6.9)

Caviline. A Kerr product, two pastes--a base and an accelerator, are supplied. The main ingredients are zinc oxide, eugenol, rosin, and chlorobutanol. (pH 7.7)

Cavitec. A Kerr product, consists of two pastes--a base and an accelerator. The main ingredients are zinc oxide, eugenol, sulphathiazole with fillers and modifiers. (pH 7.6)

Pulprotex. A product of L. D. Caulk Company, is supplied in powder and liquid form. The powder is composed of zinc oxide and rosin. The liquid contains eugenol, chlorothymol and chlorobutol 1%. (pH 6.9)

Hydroxyline. A product of George Taub Inc. is supplied in liquid suspension form, consisting of a suspension of calcium hydroxide, 30.4% with methyl methacrylate polymers 29.1% in methyl ethyl ketone. It is classified in the B Group by the council of Dental Therapeutics. (pH 12.1)

Chembar. A product of L. D. Caulk Company is supplied in liquid suspension form, consisting of a suspension of calcium hydroxide. Massler\(^1\) reports that this product consists of calcium hydroxide 5.0, zinc oxide 5.0, pigment 0.1, polystyrene 2.0, and chloroform 87.9. A solvent is supplied with the suspension. (pH 12.2)

Calcium Hydroxide. Pure Ca(OH)\(_2\) by Baker Chemical Company, New Jersey, was mixed with distilled water to form a paste. (pH 12.2)

Saline. Normal physiological saline. (pH 7.4)
EXPERIMENTAL PROCEDURES
<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturing Co.</th>
<th>Ingredients</th>
<th>Form</th>
<th>Interval</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavit</td>
<td>Premier Dental Products</td>
<td>Zinc oxide, Zinc salts, Calcium salts, Vinyl polymers</td>
<td>Paste</td>
<td>2 days</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 8.1</td>
<td></td>
<td>16 days</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 12.2</td>
<td></td>
<td>16 days</td>
<td>Ulcerating. Severe. Osteoid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Ulcerating. Severe. Osteoid remnants.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 8.9</td>
<td></td>
<td>16 days</td>
<td>Plenty polymorphonuclear leucocytes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Coagulation necrosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe. Thick capsule.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe. Thick capsule.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J N I Gard</td>
<td>Powder</td>
<td>2 days</td>
<td>Moderate. Fibrin and cellular capsule.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E Denso Cement and Pulp Protector</td>
<td></td>
<td>16 days</td>
<td>Severe. Thick capsule.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 8.3</td>
<td></td>
<td>32 days</td>
<td>Severe. Ulcerated.</td>
</tr>
<tr>
<td>Vitee</td>
<td>Kerr</td>
<td>Zinc oxide, Eugenol, Sulphathiazole</td>
<td>Powder &amp; Liquid</td>
<td>2 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 6.9</td>
<td></td>
<td>16 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Moderate.</td>
</tr>
<tr>
<td>Caviline</td>
<td>Kerr</td>
<td>Zinc oxide, Eugenol, Rosin, Chlorobutanol</td>
<td>Paste</td>
<td>2 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td>Base &amp; Accelerator</td>
<td>pH 7.7</td>
<td></td>
<td>16 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Moderate.</td>
</tr>
<tr>
<td>Material</td>
<td>Manufacturing Co.</td>
<td>Ingredients</td>
<td>Form</td>
<td>Interval</td>
<td>Reaction</td>
</tr>
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</tr>
<tr>
<td>Cavitec Base &amp; Accelerator</td>
<td>Kerr</td>
<td>Zinc oxide</td>
<td>Paste</td>
<td>2 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eugenol</td>
<td></td>
<td>16 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulphathiazole with modifiers &amp; fillers</td>
<td></td>
<td>32 days</td>
<td>Mild.</td>
</tr>
<tr>
<td>pH 7.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulprotex</td>
<td>L. D. Caulk Co.</td>
<td>Zinc oxide</td>
<td>Powder</td>
<td>2 days</td>
<td>Mild.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosin</td>
<td></td>
<td>16 days</td>
<td>Severe.</td>
</tr>
<tr>
<td>pH 6.9</td>
<td></td>
<td>Eugenol</td>
<td>Liquid</td>
<td>32 days</td>
<td>Severe.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlorothymol, Chlorbutol 1%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyline</td>
<td>George Taub, Inc.</td>
<td>Calcium hydroxide suspended in copolymer plastic</td>
<td>Suspension</td>
<td>2 days</td>
<td>Moderate. Coagulation necrosis. Calcification in panniculus carnosus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solvent: Methylethyl ketone</td>
<td></td>
<td>16 days</td>
<td>Moderate. Calcification in panniculus carnosus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Moderate. No calcification or osteoid.</td>
</tr>
<tr>
<td>pH 12.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chembar</td>
<td>L. D. Caulk Co.</td>
<td>Calcium hydroxide</td>
<td>Suspension</td>
<td>2 days</td>
<td>Moderate. Coagulation necrosis. No osteoid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zinc oxide</td>
<td></td>
<td>16 days</td>
<td>Severe. No osteoid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polystyrene</td>
<td></td>
<td>32 days</td>
<td>Severe. Giant cells plentiful. No osteoid.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloroform</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium Hydroxide &amp; distilled water</td>
<td>Baker Chemical Co.</td>
<td>Pure calcium hydroxide</td>
<td>Paste</td>
<td>2 days</td>
<td>Moderate. Osteoid and calcification in panniculus carnosus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 days</td>
<td>Moderate. Osteoid near fat and calcification in panniculus carnosus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Moderate. Material shed by ulceration. Remnants of osteoid.</td>
</tr>
<tr>
<td>pH 12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>Solution</td>
<td>2 days</td>
<td>Normal.</td>
<td></td>
</tr>
<tr>
<td>pH 7.4</td>
<td></td>
<td></td>
<td>16 days</td>
<td>Normal.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32 days</td>
<td>Normal.</td>
<td></td>
</tr>
</tbody>
</table>
Three areas were selected for implantation in each animal; the two on the dorsal aspect were the shoulder area and the pelvic area, and one on the ventral aspect was the abdominal area. Each animal was implanted in three areas and three animals were used with each material. The animals were sacrificed at three intervals, 2 days, 16 days and 32 days. These intervals were selected since earlier work by Mitchell using implants in the subcutaneous tissue of the rat, showed that the acute reaction to the implant could be observed at 2 days, the chronic reaction at 16 days, and the reparative or chronic reaction at 32 days. Hence, three specimens from one animal were available at each interval with each material. A total of nine specimens was sectioned per material, and approximately four sections of each specimen were studied microscopically.

Two animals, one implanted with Kerr's Vitec and another with Caulk's Chembar, died during the experiment and the experiments were re-done. The cause of death was not related to the material implanted. Two series of experiments were carried out at each interval with Serocalcium paste since it was observed macroscopically immediately after implantation that the skin above the material discolored.

**Surgical Procedure**

The animal was put in a glass jar containing a towel on which ether had been poured. The lid was held over the jar until it appeared that the animal was anesthetized. The animal was then removed from the jar and its head was placed within a paper cone containing ether on a paper towel.

The areas to be implanted were shaved with a pair of electric shears. At this time the material, if it was in powder and liquid form was mixed and an empty carpule was partly filled with the paste. The carpule was
then placed in the plastic syringe, and the nozzle screwed on. Materials that were in base and accelerator tubes were mixed according to the manufacturers directions. Some paste was primarily ejected from the syringe to obtain a steady flow. The technique was standardized, so that approximately 0.1 cc. of paste was implanted in each area. Figure 1 shows the amount of material implanted in each area.

A clean transverse incision was made with a Bard Parker knife in the shaved shoulder area. The skin was lifted and a blunt pair of tweezers was inserted cephalically to permit easy access for the syringe nozzle. The tweezers were removed and replaced by this syringe nozzle, the material was implanted in the subcutaneous connective tissue 15 mm. cephalic to the incision and the nozzle was retracted. The incision was sutured. The dorsal pelvic and abdominal areas were similarly implanted with the same material. See Figure 3.

Chamber and Hydroxyline, being in suspension form, and normal saline, were injected subcutaneously without an incision. A 20 gauge, 1\(\frac{1}{2}\) inch needle was used and 0.1 cc. was injected.

The ether cone was removed from around the animal's head. A rubber dam punch was used to punch the required number of holes in the animal's ears, as a means of numbering.

**Gross Post-Operative Observations**

All the animals came out from under the effect of the anesthetic within a few minutes of completion of the surgical procedure.

The areas implanted with Seroalcium paste discolored immediately after implantation. A 3 mm. diameter patch was observed 10-15 mm. cephalic to the incision indicating that the skin directly exterior to the implant was affected. The discoloration was most apparent in the abdominal
region where the epidermis and dermis are thinner than in the pelvic and shoulder regions, and least apparent in the pelvic area. Similar dis-
colorations were observed in the second series of animals, when the experiment was repeated. The animals (numbers 4, 37) sacrificed at 2 days showed an ulcerating patch 5 mm. in diameter in the implanted area. At 16 days, the implanted area was ulcerated and the skin was adherent to the deeper tissue. At 32 days, the ulcerated area showed evidence of healing.

At 16 days rats implanted with Cargenol showed 12 x 14 mm. swellings in the shoulder and pelvic regions, and an 8 x 9 mm. swelling in the abdominal region. At 2 days and 32 days no gross changes were apparent.

When rat No. 19, implanted with Cavitec, was sacrificed at 2 days, excessive hemorrhage was observed in incising the tissue in the region implanted. Swellings, 5 mm. in diameter were also observed, and were more apparent in the abdominal and pelvic regions. At 16 days, the swellings were 10 x 15 mm. in the shoulder and pelvic regions and 6 x 7 mm. in the abdominal region. Excessive hemorrhage was similarly observed at 16 days. No gross changes were apparent at 32 days.

At 16 days, rats implanted with Chembar showed 7 x 8 mm. swellings in the shoulder and abdominal regions and a 10 x 15 mm. swelling in the pelvic region. No apparent changes were observed at 2 days and 32 days.

Implants of Cavit, Gardenier Sedative Cement, Vitec, Pulprotex, Hydroxyline, Saline, and calcium hydroxide and water showed no gross changes in the implanted areas. (Table II).

Sacrificing of the Animals

The animals were sacrificed at three intervals, 2, 16 and 32 days. Careful clinical observations were made before sacrificing the animals
(Table II).

The animals were sacrificed by subjecting them to an overdose of chloroform in a glass jar.

A 30 sq. mm. area of skin and underlying connective tissue containing the implant was excised and immediately transferred to a solution of 10% formalin for fixation. At the 16 and 32 day sacrificing intervals, the areas implanted were reshaved before the tissue was excised.

There was no difficulty in locating the implants, since the incision scar was apparent at all intervals. In the case of Hydroxyline, Chembar and normal saline where the material was directly implanted without an incision, the area had been marked with an indelible pencil and this was continuously checked and remarked if necessary.

After fixation the tissue was cut directly through the implant and trimmed. Later it was dehydrated in ethyl alcohol, cleared with naphtha and embedded in paraffin.

Sections were made 3 microns in thickness and stained by the standard hematoxylin and eosin technique.
Table II

GROSS OBSERVATIONS AT SACRIFICING

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Material</th>
<th>Interval Sacrificed</th>
<th>Gross Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cavit</td>
<td>2 days</td>
<td>Ulcerating patch 5 mm. in diameter.</td>
</tr>
<tr>
<td>2</td>
<td>Cavit</td>
<td>16 days</td>
<td>Ulcerated. Skin adherent to deeper tissues.</td>
</tr>
<tr>
<td>3</td>
<td>Cavit</td>
<td>32 days</td>
<td>Ulcerated area tending to heal.</td>
</tr>
<tr>
<td>4, 37</td>
<td>Sero-calcium Paste</td>
<td>2 days</td>
<td>Swelling. 12 x 14 mm. in the shoulder and pelvic regions, 3 x 9 mm. in the abdominal region.</td>
</tr>
<tr>
<td>5, 38</td>
<td>Sero-calcium Paste</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 5 mm. in diameter. More apparent in abdominal and pelvic regions.</td>
</tr>
<tr>
<td>6, 39</td>
<td>Sero-calcium Paste</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 10 x 15 mm. in shoulder and pelvic regions, 6 x 7 mm. in abdominal region.</td>
</tr>
<tr>
<td>7</td>
<td>Cargenol</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>8</td>
<td>Cargenol</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>9</td>
<td>Cargenol</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>10</td>
<td>Gardenier Sedative Cement</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>11</td>
<td>Gardenier Sedative Cement</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>12</td>
<td>Gardenier Sedative Cement</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>13</td>
<td>Vitee</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>14</td>
<td>Vitee</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>15</td>
<td>Vitee</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>16</td>
<td>Caviline</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>17</td>
<td>Caviline</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>18</td>
<td>Caviline</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>19</td>
<td>Cavitec</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>20</td>
<td>Cavitec</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>21</td>
<td>Cavitec</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>22</td>
<td>Pulprotex</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>23</td>
<td>Pulprotex</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>24</td>
<td>Pulprotex</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>25</td>
<td>Hydroxyline</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>26</td>
<td>Hydroxyline</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>27</td>
<td>Hydroxyline</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>28</td>
<td>Chembar</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>29</td>
<td>Chembar</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>30</td>
<td>Chembar</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>31</td>
<td>Normal Saline</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>32</td>
<td>Normal Saline</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>33</td>
<td>Normal Saline</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>34</td>
<td>Calcium hydroxide + water</td>
<td>2 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>35</td>
<td>Calcium hydroxide + water</td>
<td>16 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
<tr>
<td>36</td>
<td>Calcium hydroxide + water</td>
<td>32 days</td>
<td>Excessive hemorrhage. Swelling, 7 x 8 mm. in shoulder and abdominal regions, 10 x 15 mm. in pelvic region.</td>
</tr>
</tbody>
</table>
Figure 1. The commercial products that were tested in this study, the syringe used, and the amount of material implanted in each area.
Figure 2. The areas implanted and the armamentarium necessary for the experimental procedures.
Figure 3. The shoulder area has been incised, implanted and sutured. The pelvic area has been incised and the skin flap is raised while the material is being implanted.
In the evaluation of the results, the inflammatory response of
the host tissues to the implants was of primary importance. An arbitrary
classification of mild, moderate and severe was made. The factors con-
sidered in this classification were:

1. types and numbers of leucocytes in the tissue surrounding the
   implant
2. the degree of vascularity of the area
3. "membranes" actually contacting the implant, and
4. the fibroblastic capsule surrounding the implant. The thickness
   of the capsule and the degree of fibroplasia were taken into account.

A marked difference in the healing capacity was observed between the
various materials implanted.

Cavit

Two Day Experimental Period.

Figure 4 is a photomicrograph of a two day implant of Cavit. A
severe inflammatory reaction was observed in the connective tissue surround-
ing the implant. A heavy infiltration of polymorphonuclear leucocytes
showing necrosis and karyorrhexis, denoted the formation of micro-abscesses.
Fat cells were noted in abundance in the surrounding tissue. Extravasation
of red blood cells into the tissue spaces and hyperemia was noted.

Sixteen Day Experimental Period.

At this period the inflammatory changes were of a chronic nature.
Foam cells, plasma cells and lymphocytes were the predominating inflam-
matory cells. The reaction was classified as moderate. A few red blood
cells were still observed in the tissue spaces and a slight hyperemia
persisted.
Thirty-two Day Experimental Period.

A moderate connective tissue reaction persisted at this period. The implant was encapsulated with granulation tissue, the predominating cell being the fibroblast. The panniculus carnosus above the implant was interrupted due to the reaction to the material.

Serocalcium Paste

Two Day Experimental Period.

A severe inflammatory reaction with a tendency to ulcerate was noted at this interval. A thick fibrin membrane surrounded the implant, and around this there was a thick inflammatory zone. Osteoid material was noted in association with adipose tissue. The inflammatory cells were predominantly lymphocytes.

Sixteen Day Experimental Period.

A severe connective tissue reaction persisted at this interval. The material either had been shed by ulceration or the tissues were tending to ulcerate. When present the material was surrounded by a scanty layer of fibrin, and granulation tissue was observed enmeshing the material. Lymphocytes and plasma cells were the predominating inflammatory cells. Figure 5 is a photomicrograph showing cracked osteoid material in the fibrous capsule surrounding a sixteen day Serocalcium paste implant. The osteoid material presumably fractured during sectioning indicating its harder consistency. Multinucleated giant cells were noted surrounding the osteoid material. This material also stained black by the selective von Kossa silver nitrate stain suggesting calcification. When the implant was still present, it was noted that the osteoid material was approximately 500 microns away from the implant.
Thirty-two Day Experimental Period.

At this interval this material had already been shed by ulceration as shown in Figure 6. The severe reaction was tending to subside and the skin had healed over the ulcerated area entrapping remnants of osteoid material within the connective tissue. The subdermal muscle, panniculus carnosus, appeared normal.

Figure 7 is a photomicrograph showing the giant cells surrounding the osteoid material and the persisting fibroplasia.

Figure 8 shows the remnants of osteoid accentuated by the selective von Kossa silver nitrate stain.

Cargenol

Two Day Experimental Period.

A thick fibrin layer surrounded by an equally thick inflammatory zone was observed around the implant at this experimental interval. The predominating inflammatory cells were polymorphonuclear leucocytes which showed karyorrhexis. In some areas coagulation necrosis was observed in close association with the implant. The connective tissue reaction was severe.

Sixteen Day Experimental Period.

The implant was surrounded by a very thick capsule of granulation tissue. Adjacent to the material was an inflammatory zone with plasma cells and mononuclear lymphocytes predominating. On the periphery fibrous tissue predominated. Hyperemia was observed in the surrounding connective tissue. There was slight disruption of the panniculus carnosus. The overall picture of the connective tissue reaction appeared severe.

Thirty-two Day Experimental Period.

A severe reaction persisted in the connective tissue surrounding the implant. A chronic inflammatory reaction was observed. The implant was
well encapsulated by fibroblasts and chronic inflammatory cells—foam cells, plasma cells and mononuclear leucocytes. Precipitated fibrin was also observed in the area surrounding the implant.

Gardinier Sedative Cement

Two Day Experimental Period.

The connective tissue reaction at this interval appeared moderate. The implant appeared fractured, apparently due to sectioning. A thin, irregular, fibrinous layer was found in close association with the implant. The inflammatory capsule surrounding the implant was moderately thick, with mononuclear leucocytes being the predominating type of cells.

Sixteen Day Experimental Period.

A wide and dense inflammatory zone, with lymphocytes and plasma cells predominating, surrounded the implants. A thin fibrin "membrane" was found in close association with the implant. The connective tissue reaction was severe.

Thirty-two Day Experimental Period.

The implanted areas were ulcerated. A wide inflammatory zone was noted with foam cells, plasma cells, lymphocytes and polymorphonuclear leucocytes in equal distribution. Hyperemia was observed in the surrounding tissues. The panniculus carnosus appeared disturbed. The over-all connective tissue reaction was severe.

Vitec

Two Day Experimental Period.

A severe reaction was observed at this interval. In close association with the implant was a wide cellular area, with a dense distribution of polymorphonuclear leucocytes. These cells exhibited necrosis and karyorrhexis. Around the cellular zone was an acellular area which was surrounded by another cellular zone. The latter showed some mononuclear
leucocytes, but polymorphonuclear leucocytes predominated. The panniculus carnosus appeared disrupted immediately overlying the implant.

**Sixteen Day Experimental Period.**

The material was observed adhering to an irregular cellular zone surrounding it. Mononuclear leucocytes were the predominating inflammatory cells. This wide inflammatory zone was surrounded by a wide fibrous capsule containing numerous young fibroblasts. The reaction was severe.

**Thirty-two Day Experimental Period.**

At this interval the connective tissue reaction appeared moderate. The implant was infiltrated by young granulation tissue. A moderate zone of inflammatory cells, with mononuclear leucocytes predominating, surrounded the implant. The panniculus carnosus appeared disturbed.

**Cavilinc**

**Two Day Experimental Period.**

A severe acute inflammatory reaction was observed in the connective tissue at this interval. The polymorphonuclear leucocytes surrounding the implant showed necrosis and karyorrhexis. Hyperemia was observed in the surrounding tissues and extravasation of red blood cells into the tissue spaces was noted in certain areas. The panniculus carnosus was disturbed.

**Sixteen Day Experimental Period.**

A wide and dense inflammatory zone was observed surrounding the implant at this interval. The predominating inflammatory cells were plasma cells and lymphocytes. A monocellular darkly stained layer adjacent to the material with a brush-like border towards the implant was believed to be due to contraction of the material during fixation and other procedures. A fibrous capsule surrounded the inflammatory zone. The connective tissue
reaction was severe at this experimental period.

**Thirty-two Day Experimental Period.**

An inflammatory zone of irregular width encapsulated the material and the connective tissue reaction appeared moderate. The predominating inflammatory cells were foam cells, plasma cells, and mononuclear lymphocytes.

**Cavities**

**Two Day Experimental Period.**

A severe inflammatory connective tissue reaction was observed at this interval. A thin, irregular fibrin layer was noted adjacent to the implant. This was surrounded by a dense inflammatory zone in which the predominating cells were polymorphonuclear leucocytes exhibiting necrosis and karyorrhexis. The cellular zone was surrounded by a thin fibrin and monocellular layer. Around this the tissues were slightly diffused with inflammatory cells, in which mononuclear leucocytes predominated. Hyperemia was observed in the surrounding tissues and in certain areas red blood cells had extravasated into the tissue spaces.

**Sixteen Day Experimental Period.**

A severe inflammatory reaction was observed in the connective tissue at this interval. A thin, irregular layer of fibrin was noted adjacent to the implant. This was surrounded by a wide inflammatory zone with polymorphonuclear leucocytes, plasma cells, foam cells and mononuclear lymphocytes in equal distribution. A fibrous capsule with young fibroblasts encapsulated the inflammatory zone.

**Thirty-two Day Experimental Period.**

The connective tissue reaction appeared mild at this interval. The implant was infiltrated by young granulation tissue. Fat cells were
observed within the granulation tissue and surrounding the material. A slight inflammatory reaction with mononuclear lymphocytes, foam cells, and plasma cells predominating was noted in the surrounding tissues. The panniculus carnosus overlying the implant was disturbed.

**Pulprotex**

**Two Day Experimental Period.**

Figure 9 illustrates a mild connective tissue reaction at this interval. An irregular thin, fibrin layer was noted adjacent to the implant. This was surrounded by a thin irregular inflammatory zone with mononuclear leucocytes predominating. Necrosis was minimal. A fibrous capsule surrounded the inflammatory zone.

**Sixteen Day Experimental Period.**

The connective tissue reaction at this interval appeared severe. The implant was infiltrated with young granulation tissue. A thin irregular fibrin layer was observed adjacent to the implant. Surrounding this was a wide inflammatory zone where the predominating cells were mononuclear leucocytes, foam cells, and plasma cells. A fibrous capsule of young fibroblasts surrounded the inflammatory zone. Hyperemia and extravasated red blood cells were noted in certain areas of the surrounding tissue.

**Thirty-two Day Experimental Period.**

The connective tissue reaction appeared severe at this experimental interval. A tendency to ulcerate was observed. A wide inflammatory zone surrounded the material. Adjacent to the implant the predominating inflammatory cells were polymorphonuclear leucocytes exhibiting karyorrhexis and necrosis. Surrounding these were mononuclear lymphocytes, foam cells and plasma cells. Extravasation of red blood cells into the tissue spaces was noted in the surrounding tissues. The panniculus carnosus was disturbed.
Figure 10 illustrates a moderate connective tissue reaction at this experimental period. Immediately adjacent to the implant was an area of coagulation necrosis. Surrounding this was an inflammatory zone where the predominating cells were mononuclear leukocytes. Pathological calcification of the bundles of the panniculus carnosus overlying the implant was observed. Slight hyperemia was observed in the surrounding tissues. Figure 11 illustrates a von Kossa stain accentuating the pathological calcification.

Sixteen Day Experimental Period.

A moderate inflammatory reaction was observed around the implant at this interval. The inflammatory cells adjacent to the material were predominantly mononuclear lymphocytes, plasma cells and foam cells. Osteoid material was observed in close association with fat cells. Calcification was noted within the bundles of the panniculus carnosus. Multinucleated giant cells surrounded the osteoid material which was in the midst of the fibrous capsule consisting predominantly of young fibroblasts.

Thirty-two Day Experimental Period.

The connective tissue reaction at this experimental period was moderate. The material was impregnated with young granulation tissue and multinucleated giant cells. The predominating inflammatory cells were mononuclear lymphocytes, plasma cells, and foam cells. The panniculus carnosus overlying the implant appeared disturbed. No osteoid or calcification was observed around the implant at this experimental interval.
Two Day Experimental Period.

Figure 12 illustrates a moderate connective tissue reaction surrounding a two day Chembar implant. A thin, irregular fibrin layer was observed adjacent to the implant. An even distribution of polymorphonuclear leucocytes, lymphocytes and plasma cells was noted in the surrounding tissues. There was more fibrin than cellular exudate in the tissues around the implant. No osteoid or calcification was found.

Sixteen Day Experimental Period.

At this interval, a severe reaction was observed in the connective tissue surrounding the implant. An irregular thin fibrin layer surrounded the implant and this was encapsulated by a wide inflammatory zone. The predominating inflammatory cells were mononuclear lymphocytes, foam cells, and plasma cells. Around the inflammatory zone, fibroblasts tended to encapsulate and localize the reaction. Hyperemia was noted in the surrounding tissues. The panniculus carnosus was disturbed. No osteoid or calcification was found.

Thirty-two Day Experimental Period.

The implant was infiltrated with young granulation tissue and multinucleated giant cells. The surrounding inflammatory reaction appeared severe. The predominating inflammatory cells were mononuclear lymphocytes, plasma cells and foam cells. No osteoid or calcification was found.

Calcium Hydroxide and Water

Two Day Experimental Period.

Figure 13 illustrates a moderate connective tissue reaction surrounding a two day calcium hydroxide and water implant. Adjacent to the implant
was an area of coagulation necrosis. A tendency to ulcerate was also observed. A moderate fibrin and cellular exudate surrounded the implant, with an even distribution of polymorphonuclear leucocytes, lymphocytes and plasma cells. Calcification of the muscle bundles of the panniculus carnosus overlying the implant was noted. Some hyperemia was observed in the surrounding areas.

Sixteen Day Experimental Period.

Figure 14 illustrates the moderate connective tissue reaction surrounding a sixteen day calcium hydroxide and water implant. An area of coagulation necrosis separated the implant from a rim of osteoid material. The osteoid was found to be in close association with fat cells. The predominating inflammatory cells were plasma cells, foam cells, and lymphocytes. Multinucleated giant cells and clumps of fat cells were observed in the surrounding tissues. Figure 15 shows a von Kossa stain which accentuates the osteoid material in close association with fat and separated from the implant by an area of coagulation necrosis. Figure 16 is a higher magnification of the fractured osteoid material with the surrounding fat within the fibrous capsule of a sixteen day calcium hydroxide implant.

Thirty-two Day Experimental Period.

At this experimental interval the connective tissue reaction appeared moderate. The implant had been shed by ulceration and remnants of osteoid were observed within the connective tissue. There was a great deal of reorganization by fibroplasia with young fibroblasts predominating.

Normal Saline

Two Day Experimental Period.

Figure 17 illustrates the normal appearance of the dermal and subdermal areas of a rat two days after a saline injection. The panniculus carnosus
was undisturbed. No inflammatory infiltration was observed.

Sixteen Day Experimental Period.

The connective tissue and overlying skin appeared normal.

Thirty-two Day Experimental Period.

The connective tissue and overlying skin appeared normal.
Figure 4. Photomicrograph of a 2 day implant (A) of Cavit. Note the severe connective tissue reaction with a heavy infiltration of polymorphonuclear leucocytes (B) showing necrosis and karyorrhexis.

H and E stain. Magnification 20X.
Figure 6. Photomicrograph showing cracked osteoid material (A) surrounded by giant cells (B). Note the fibroplasia in the surrounding tissue. The osteoid material was in the fibrous capsule surrounding a 16 day Serocalcium paste implant, and was at a distance of approximately 500 microns from the implant material. H and E stain. Magnification 75X.
Figure 6. Photomicrograph showing the area of a 32 day Seroalcium paste implant. The material was shed by ulceration and the skin has recently healed (A) over remnants of osteoid material (B). The panniculus carnosus appears normal (C). H and E stain. Magnification 20X.
Figure 7. Photomicrograph showing cracked osteoid material (A) surrounded by giant cells (B). The osteoid material was part of remnants after ulceration of a 32 day Serocalcium paste implant. H and E stain. Magnification 75X.
Figure 3. Photomicrograph of an area 32 days after a Serocalcium paste implant. von Kossa stain accentuates the remnants of osteoid material (A).

Magnification 20X.
Figure 9. Photomicrograph of a 2 day implant(A) of Pulprotex. Note the mild connective tissue reaction with few inflammatory cells and slight necrosis.

H and E stain. Magnification 20X.
Figure 10. Photomicrograph of a 2 day Hydroxyline implant(A). Note the apparent pathological calcification of the bundles of the panniculus carnosus(B). H and E stain. Magnification 20X.
Figure 11. Photomicrograph of a 2 day Hydroxyline implant (A). von Kossa stain accentuates the calcification of the bundles of the panniculus carnosus (B). Note the undisturbed bundles of the panniculus carnosus (C).

Magnification 20X.
Figure 12. Photomicrograph of a 2 day implant(A) of Chembar. This illustrates a moderate connective tissue reaction with an even distribution of polymorphonuclear leucocytes, lymphocytes and plasma cells.

H and E stain. Magnification 20X.
Figure 13. Photomicrograph of a 2 day calcium hydroxide and water implant (A) with a tendency to ulcerate (B). Note the moderate reaction in the connective tissue and the area of coagulation necrosis (C) separating the implant from the calcified muscle bundles of the panniculus carnosus. H and E stain. Magnification 20X.
Figure 14. Photomicrograph of a 16 day calcium hydroxide and water implant (A). Note the osteoid material (B) in close association with fat. H and E stain. Magnification 20X.
Figure 15. Photomicrograph of a 16 day calcium hydroxide and water implant (A). von Kossa stain accentuates the osteoid material (B) in close association with fat. Magnification 20X.
Figure 16. Photomicrograph showing cracked osteoid material (A) in close association with fat (B) within the fibrous capsule surrounding a 16 day calcium hydroxide and water implant. H and E stain. Magnification 75X.
Figure 17. Photomicrograph of the dermal and subdermal areas of a rat 2 days after it was injected with saline. Note the normal appearance of the dermis(A), subdermal fat(B), and the panniculus carnosus(C).

H and E stain. Magnification 75X.
DISCUSSION
The applicability of findings in animals to human beings cannot be ascertained until comparable results are also obtained in experiments with humans or clinical observations. The exact correlation between the results obtained by the author and earlier studies on the human pulp will be difficult, but it seems that the connective tissue of the rat can be used as an efficient screening site to determine the relative irritant qualities of cavity lining and pulp capping materials. The connective tissue system is interconnected throughout the animal body and is formed of similar elements, cells, collagen, elastic and reticular fibers, and ground substances, hence, maintaining an overall comparable identity. Therefore, the connective tissue reactions to dental materials could be used to evaluate the relative irritability and perhaps reparative properties of these materials.

The superiority of calcium hydroxide as a pulp capping agent has been claimed by Glass and Zander\textsuperscript{10} and Zander\textsuperscript{16} and confirmed by Hess\textsuperscript{21}. It was interesting to observe that their results could be correlated with the author's results in the connective tissue of the rat. Whereas, they observed the formation of a dentin bridge over pulps capped with calcium hydroxide, the author noted the formation of osteoid material within the connective tissue and in close association with fat as early as two days after implantation. Pathological calcification was also observed within the bundles of the panniculus carnosus muscle. Similar osteoid formation and calcification was observed with Serocalcium paste and Hydroxyline, both of which contain calcium hydroxide as a principal ingredient.

Castagnola and Orlay\textsuperscript{26} reported the formation of a dentin bridge over pulps capped with Serocalcium and similar products.

Berk and Cohen\textsuperscript{22} and Berk\textsuperscript{26} reported that a paste of calcium hydroxide and methyl cellulose is as effective as calcium hydroxide and water in
the production of secondary dentin.

This author's results indicate that calcium hydroxide is capable of retaining its osteogenic influence in some variable media and the presence of odontoblasts or osteoblasts is not essential for post-foetal osteogenesis.

However, under the experimental conditions employed in this study, Chembar had no osteogenic influence on the connective tissue surrounding the implant. Zander, Glenn and Nelson\textsuperscript{36} showed that Chembar gave the maximum pulpal protection as a cavity liner, under silicate restorations, but no secondary dentin was deposited. In this study, no osteoid material or calcification was observed around the Chembar implant in spite of its calcium hydroxide content. At two days the connective tissue reaction was moderate, but at 16 and 32 days the connective tissue surrounding the implant was in a severe state of inflammation. The author postulates that the presence of the other ingredients, zinc oxide, polystyrene and chloroform is in some manner related to the non-osteogenic effect of this material.

The results of the present study also seem to indicate that the pH of the material is not directly related to osteogenic ability, since theoretically Chembar would be expected to be osteogenic, because it has an alkaline pH and provides a local surplus of calcium salts.\textsuperscript{24} Chembar has a pH of 12.2 and calcium hydroxide is one of its ingredients, but the author did not observe osteoid material or calcification in the connective tissue around a Chembar implant.

Sericalcium paste, pH 12.2, besides containing calcium hydroxide, contains salts of human serum, NaHCO\textsubscript{3}, CaCl\textsubscript{2}, and KCl. In this study it was found to produce osteoid, but the inflammatory reaction in the surrounding connective tissue was severe at 2, 16 and 32 days with ulceration. The skin overlying the implanted area discolored immediately upon implantation, denoting the severity of reaction. Because of marked species difference,
human blood salts may account for the severity of reaction in the rat connective tissue. Hence comparisons between this study and earlier reports in humans should be made with reservation.

Although osteoid was observed around Hydroxyline implants at 2 and 16 days, no osteoid was observed at 32 days. It has been noted in this study that osteoid material is surrounded by multinucleated giant cells which are probably responsible for the disappearance of osteoid at the latest interval used in this experiment. It was consistently noted that when the material ulcerated as in the case of calcium hydroxide and Serocalcium paste, remnants of osteoid were found in the connective tissue even though the overlying skin had healed. This study also confirmed earlier observations by Mitchell and Shankwalker\(^{113}\) who found adipose tissue in close association with heterotopic bone deposits and calcification of the bundles of the panniculus carnosus muscle. It is difficult to evaluate the exact significance of the osteoid occurring in close association with fat. In an attempt to evaluate its significance, an extensive review of the reported literature on post-foetal osteogenesis was made but few investigators have observed it. Tunbridge\(^{122}\) occasionally noted fatty material with evidence of calcification in the vicinity. Hill\(^{121}\) cites Weber and Euler as having demonstrated that the presence of fat precedes calcification.

Cavit, which contains calcium salts, in addition to other ingredients, did not show any osteogenic ability.

Cargenol, which contains calcium phosphate as one of its ingredients, showed a severe connective tissue reaction at 2, 16 and 32 days, without any apparent osteogenic ability.

This author feels justified in stating that the presence of calcium salts \textit{per se} is not the exclusive factor which promotes post-foetal osteogenesis. In earlier experiments it was not possible to determine whether
bone formation following implantation of certain materials in a favorable environment was the result of osteogenic activity of the implanted material, or whether the cells of the host under the influence of induction as a result of the implant are responsible for the appearance of young bone. From this study, the author believes that the implant has an effect of induction on the surrounding tissues of the host resulting in post-foetal osteogenesis. This belief arose from the fact that osteogenesis was observed at some distance from the implant. Generally, a zone of coagulation necrosis separated the implant from the osteoid or calcified material. Although the von Kossa staining technique demonstrated the presence of calcific material in both the implant and osteoid material it cannot be postulated that the calcium ions of the heterotopic calcification had not come directly from the implant. The author can only theorize that the calcium ions of the heterotopic calcification may have been contributed by the implant, but Mitchell and Shankwalker\textsuperscript{113} reported induction of osteogenesis in the connective tissue of rats surrounding magnesium hydroxide implants, indicating that calcium ions are not essential for osteogenesis. Hunter\textsuperscript{30} also reported that the deposition of a dentin bridge occurred over pulps capped with magnesium hydroxide.

Several investigators\textsuperscript{58-71} strongly advocate the use of zinc oxide and eugenol as a pulp capping and cavity lining material. Berman\textsuperscript{5} and Berman and Massler\textsuperscript{58} reported in a recent study of experimental pulp-tomies on rat molars that there were no basic differences between calcium hydroxide, and zinc oxide and eugenol at 21 and 28 days after amputation when both materials showed the presence of a dentin bridge. All the commercial preparations containing zinc oxide and eugenol as basic ingredients that were tested in this study showed no osteogenesis in the surrounding tissues. Cavit, Cargenol, Vitac, Caviline and Cavitec,
containing zinc oxide and eugenol, among other ingredients, all showed a severe reaction in the connective tissue two days after implantation. This reaction tended to become moderate or mild in all but Cargenol. The author believes that the severity of the connective tissue reaction persisted with Cargenol, since it is the only preparation tested containing carbolie acid. The severity of reaction with carbolie acid was also noted by Mitchell when he injected 0.1 cc. subcutaneously.

None of the materials containing zinc oxide and eugenol exhibited any osteogenic potentiality within the surrounding connective tissue of the rat. In fact, it seems that the presence of zinc oxide actually inactivates the ability of calcium salts to cause heterotopic calcification. Could the presence of zinc oxide in Chembar and Cavit inactivate the calcium salts?

Although the reactions with Gardenier Sedative Cement and Pulprotex, both of which contain zinc oxide and eugenol among other ingredients, were moderate and mild, respectively, at two days, both exhibited severe connective tissue reactions at the later intervals.

The author would like to comment that several materials exhibited a more severe reaction at the later intervals than at the earlier two days interval, indicating that the full irritant qualities of the material may not be observed within 48 hours with all materials. At the present time it can only be postulated that either the resistance of the host is decreasing, or the irritation of the implant is increasing. This author is apt to believe the latter is less plausible since no additional material is implanted to reinforce the original material.

Vitec and Cavitec both contain sulphathiazole as one of their ingredients, but the severity of the connective tissue reaction could not be specifically correlated with the presence of this ingredient, since other materials which did not contain sulphathiazole showed a similar severe
reaction. However, no advantage could be demonstrated in incorporating sulphathiazole as an ingredient. Kalnins\textsuperscript{124} reported the formation of a dentin bridge over pulps, capped with a paste containing calcium hydroxide, sulphathiazole and strontium salts, but similar bridge formation occurs with calcium hydroxide alone, so this author believes that at the present time the role of antibacterial agents in vital pulp therapy is uncertain. Results obtained in this study indicated that calcium hydroxide was the only material tested that was capable of osteogenic induction in the surrounding tissues of the host.

The results of this study indicate that of all the pulp capping and cavity lining agents tested, Hydroxyline and calcium hydroxide and water produce only a moderate connective tissue reaction and are also osteogenic. Hence, Hydroxyline may well be the material of choice for cavity lining. Calcium hydroxide and water may be recommended as a pulp capping material because of its ability to stimulate the deposition of a dentin bridge.

As previously stated, the author emphasizes that reservation should be used in correlating results obtained in the connective tissue of the rat, with other studies on human pulps, although this technique seems to be adequate in evaluating the relative irritant qualities of different materials.
SUMMARY AND CONCLUSIONS
Twelve materials, Cavit, Serocalcium paste, Cargenol, Gardenier Sedative Cement and Pulp Protector, Vitec, Caviline, Cavitec, Pulprotex, Hydroxyline, Chembar, calcium hydroxide and water, and saline were implanted in the connective tissue of 41 young adult Wistar rats. Each animal was implanted in two dorsal and one ventral areas. Specimens for histological study were obtained at the experimental periods of 2, 16 and 32 days. Three specimens of each material were available at each interval.

It would be premature to draw too many conclusions from the results obtained in this study, particularly as to their correlation with the important problem of maintaining the vitality of human pulps. They do, however, serve to emphasize the fact that the connective tissue of the rat can be used as an initial mass screening medium to determine the reaction of vital tissues to certain pulp capping and cavity lining materials.

From the results of this study, the following conclusions were drawn:

1. Under the conditions employed in this study certain correlations could be detected between the results obtained by the author in the connective tissue of the rat and earlier reports by other investigators with similar materials used on the human dental pulp.

2. Pure calcium hydroxide and the commercial preparations, Serocalcium paste and Hydroxyline both containing calcium hydroxide as a main ingredient, were osteogenic and caused the appearance of osteoid or calcification in the tissues surrounding the implant.

3. Osteoid material was observed in close association with adipose tissue, while calcification was observed within the bundles of the subdermal muscle, the panniculus carnosus.

4. Multinucleated giant cells surrounded the osteoid material at the 16 day experimental period and probably this explains the disappearance
of osteoid at the 32 day experimental period as observed in the case of Hydroxyline.

5. When the implant containing calcium hydroxide and water ulcerated and was shed, osteoid remnants were observed in the rat connective tissue, although the overlying skin had healed.

6. Osteoid or calcification was observed as early as two days after implanting in the connective tissue of the rat.

7. Chembar which also contains calcium hydroxide as a basic ingredient did not induce osteogenesis in the tissues surrounding the implant.

8. The osteogenic influence of a material was independent of its pH.

9. The inflammatory reaction surrounding the implant at any experimental period was completely independent of its pH.

10. Commercial preparations containing zinc oxide and eugenol as basic ingredients were incapable of inducing osteogenesis in the connective tissue of the rat.

11. Three products, Vitec, Caviline, and Cavitec showed similar severe connective tissue reactions which tended to become mild to moderate at 32 days. The addition of sulphathiazole as an ingredient did not effect the severity of the connective tissue reaction.

12. Cargenol which contains zinc oxide, eugenol and carbolic acid among other ingredients showed a severe inflammatory reaction at each of the experimental periods. It was postulated that carbolic acid could be the ingredient responsible for the persisting severe inflammatory reaction.

13. Cavit which contains zinc oxide and calcium salts as basic ingredients exhibited a severe connective tissue reaction at two days, but the reaction tended to become moderate at 16 and 32 days.

14. A mild connective tissue reaction was observed with Pulprotex at two days, but at 16 and 32 days the reaction appeared severe.
15. Hydroxyline produces only a moderate inflammatory reaction and is osteogenic in nature. Since it is a suspension, it may well be the material of choice for living cavities. By the same reasoning, calcium hydroxide and water is recommended as a pulp capping material because of its ability to stimulate the deposition of a dentin bridge.

16. All the materials implanted in this study were irritating except saline which served as a control.

17. The results of this study warrant further investigations to detect a possible correlation between the connective tissue reaction of the rat and human pulpal reactions, using not only pulp capping and cavity lining agents, but all the other dental materials in common use today.

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ABSTRACT
Although there have been extensive studies on the effects of various filling materials on the pulp, there have been no comprehensive studies made on the pulpal effect of various commercial cavity linings and pulp capping agents. Realizing the importance of preservation of the vitality of the pulp, a histological investigation of the effect of certain cavity lining and pulp capping agents was carried out. The subcutaneous connective tissue of the rat is offered as an adequate medium for mass screening for dental materials. Under the conditions employed in this study certain correlations could be detected between the results obtained by the author in the connective tissue of the rat and earlier reports by other investigators with similar materials in the human pulp.

Twelve materials, Cavit, Serocalcium paste, Cargenol, Gardenier Sedative Cement, Vitec, Cavitec, Caviline, Pulprotex, Hydroxyline, Chembar, calcium hydroxide and water, and saline were implanted in the subcutaneous connective tissue of young Wistar rats. Specimens for histological study were obtained at the experimental periods of 2, 16, and 32 days.

Pure calcium hydroxide and water, and the commercial preparations Serocalcium paste and Hydroxyline both containing calcium hydroxide as a main ingredient were osteogenic and caused the appearance of osteoid in close association with fat, or were responsible for calcification within the bundles of the panniculus carnosus muscle.

The inflammatory reaction surrounding an implant or its osteogenic influence were independent of the pH of the material. The addition of sulphathiazole to a material did not effect the severity of the connective tissue reaction.

Hydroxyline produced only a moderate inflammatory reaction and was osteogenic. Since it is a suspension, it may well be the material of choice for cavity lining. By the same reasoning, calcium hydroxide and water
is recommended as a pulp capping material, because of its ability to stimulate the deposition of a dentin bridge. All the materials implanted in this study were irritating except saline which served as a control.