Outcomes of a Modified Pulpotomy Technique

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science in Pediatric Dentistry
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Title: OUTCOMES OF A MODIFIED PULPOTOMY TECHNIQUE
Year of Convocation: 2009
Degree: Master of Science
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Abstract

Background: Despite the high success rates reported with the use of a five minute application of formocresol it has been postulated that it may be applied for a lesser amount of time and still achieve equivalent results. Few studies have adequately addressed the effects of the medicament on permanent successors and exfoliation times. Furthermore, the effects of shorter application times on success rates have not been adequately reported. Objectives: To assess the clinical and radiographic outcomes of a one minute application of full strength Buckley’s formocresol with concurrent hemostasis using the medicated cotton pledget in human primary teeth. To evaluate the effect of this technique on their successors and to evaluate the exfoliation times in comparison to the contralateral non-pulpotomized tooth. Methods: Using a retrospective chart review, clinical and radiographic data were available for 557 primary molars in 320 patients. Descriptive statistics and regression analysis were used to assess outcomes. Results: 99.3% clinical and 89.8% radiographic success rates were obtained. Internal root resorption (4.85%) and pulp canal obliteration (1.97%) were the most frequently observed radiographic failures. Sixty-five and half percent exfoliated at the same time as their contra-lateral counterpart and 28.8% exfoliated earlier (p<0.001). There was no difference in the number of enamel defects of succedaneous teeth between treated and control teeth (p>0.05). Conclusions: Success rates for the modified technique are comparable to techniques that use the five-minute dilute or full strength solutions reported in the literature. The one minute technique had no clinical effect on exfoliation times or incidence of enamel defects on succedaneous teeth. The one minute full strength formocresol technique is an acceptable alternative to published traditional techniques.
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This is a dedication to the memory of my late father who was the first to believe in me. His passion for knowledge is something I hope to live up to. His love and strength will always be an inspiration.
ACKNOWLEDGEMENTS

I would like to extend my heartfelt appreciation to the following people who made this project possible. I am a result of all your efforts, teachings, and faith in me!

To Dr. Michael Sigal, Dr. Paul Andrews and Dr. Keith Titley for helping me through the challenges of this education.

To Dr. Michael Sigal for his never ending enthusiasm and integrity, passion for teaching and education, all of which far exceed the call of duty and is a continuous inspiration to me.

To Dr. Paul Andrews for his continuous advice, support and never ending encouragement in my journey through the past three years.

To Dr. Keith Titley for his additions to the research, invaluable editorial assistance and academic insights.

To Dr. Julia Rukavina for her time in the standardization process of radiographic observations.

To Farida Ghany who has been a remarkable source of personal support and motivation.

To James Fiege, Christine Nicolau, Bruno Rakiewicz and Jeff Comber, who provided me with continuous help on short notice, always with smiles.

Finally, a very special thanks to Jordan Fingard for standing by me, whose unwavering love and support carried me through the trying times and who never doubted it could be done.
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A. INTRODUCTION

Caries involvement or traumatic exposure of pulps that remain vital in primary teeth can be treated by a technique called vital pulpotomy. The treatment involves removal of the coronal pulp, application of medicaments and restoring the tooth to maintain function and arch length until its exfoliation and the eruption of its successor.

Since the introduction of vital pulpotomy, various medicaments have been used and various alterations to the technique itself have been advocated. The most common pulpotomy technique involves amputation of the coronal pulp followed by the application of full-strength formocresol to the radicular pulp stumps for a period of five minutes. Histologic, radiographic and clinical success and failure rates have been reported for this technique and its modifications. The use of formocresol has been associated with high clinical and radiographic success rates that are due to the potent germicidal and tissue fixative qualities of formaldehyde. Over the years modifications of the five minute formocresol technique have included varying concentration levels (Sweet 1956, Loos et al. 1973), application times (Venham 1967, Garcia-Godoy et al. 1982, Hyland 1986), incorporation of formocresol into the sub-base (Beaver et al. 1966) and hemostasis with the formocresol dampened cotton pellet (Thompson et al. 2001).

The use of formocresol has raised various concerns that include its mutagenic, carcinogenic and allergenic potentials. Furthermore, its potential effect on the exfoliation times of treated teeth and on succedaneous teeth have also been raised. In light of this, alternative agents such as ferric sulphate (Fei et al. 1991, Ranly & García-Godoy 1991, Ranly 1994, Ibricevic et al. 2003, Casas et al. 2004), osteogenic proteins (Ranly & García-Godoy 1991, Ranly 1994, Rutherford et al. 2001).

Despite the high success rates reported with the use of a five minute application of formocresol it has been postulated that it may be applied for a lesser amount of time and still achieve equivalent results. It is also of interest that few studies have addressed the effects of the medicament on permanent successors and exfoliation times. Furthermore, the effects of shorter application times on success rates have not been adequately reported. The aim of this retrospective study is to examine the outcomes of a one minute pulpotomy using full strength Buckley’s formocresol and concurrent hemostasis with the medicated cotton pledget.
B. REVIEW OF LITERATURE

1. Development of the Technique

In 1872, Nitzel advocated the amputation of pulpal tissue as a method of treating the exposed vital pulp (Hess 1929). Hess (1929) reported that Witzel in 1872 was the first to suggest the devitalization of the coronal pulp by using arsenic followed by placement of a medicated paste.

Lepkowski, in 1897, used a 40% formaldehyde solution to exposed pulp and this resulted in “intolerable pain” (Lepkowski 1897). Thereafter, formagen, a formalin-treated enterotoxin from Vibrio cholera was placed on inflamed pulpal tissue. This resulted in the maintenance of pulp vitality with a reported 99% clinical success rate (Lepkowski 1897).

Bossard (1929) referred to the work of Gysi, who introduced Trio Paste in 1898. This paste was used to mummify radicular pulp tissue after the coronal portion had been devitalized by cobalt and amputated. Trio Paste was composed of tricresol (10 c.c), creolin (20 c.c), glycerin (4 c.c), paraformaldehyde (20 grams), and zinc oxide (60 grams)(Bossard 1929).

In 1904, Buckley introduced the use of formocresol (a mixture of formalin and tri-cresol) for the treatment of necrotic pulps. The formaldehyde component of formocresol was intended to produce “odorless and non-infectious compounds”. Buckley believed that the degenerating tissue produced ammonia and hydrogen sulfide gases. The sole function of the formaldehyde was to chemically combine with these gases to produce urotropin, sulfur and wood alcohol. According to Buckley, these substances, being non gaseous, would not force any poisonous pulp decomposition products into the periapical regions where they had been proved capable of setting up inflammation and suppuration (Buckley, 1905). Tri-cresol was used to dilute the formalin
since it had the property of being miscible with formalin in all proportions. Tri-cresol was also a good germicide and it was suggested that the “fats” in the degenerating pulp would be reduced by it to lysol. This was said to be a more desirable reaction (Buckley 1904).

In the early 1920’s Davis introduced the concept of amputation of vital coronal pulps (Davis 1923). This was a departure from the earlier procedure that entailed the amputation of coronal pulps that had been previously devitalized by cobalt or arsenic (Bonsack 1930) (Rzeszotarski 1939). Davis attempted to maintain the remaining radicular pulp tissue in a vital condition after amputation.

In the 1920s Sweet introduced a five appointment pulpotomy procedure using arsenic followed by formocresol as the devitalizing agent (Sweet 1923). In 1930, Sweet modified this to a four appointment technique without the use of local anesthetic (Sweet 1930). The first appointment involved placing phenol on the pulp for forty-eight hours; the second involved application of formocresol on the pulp and left for another forty-eight hours; the third involved amputation of the coronal pulp followed by a temporary restoration that contained formocresol and this was left in place for three or four days. During the fourth appointment, the fixed radicular pulps were capped with zinc-oxide eugenol and the tooth restored.

By 1937, Sweet was advocating a three appointment procedure. At the first appointment the pulp was amputated, under anesthesia, and a dressing of formocresol was sealed in for two to three days. This procedure was repeated at a second appointment. At the third appointment the temporary filling was removed, a dressing of zinc oxide and eugenol placed over the radicular pulp stumps and a final restoration placed.

In the late 1930s and 1940s, the use of calcium hydroxide as a capping agent following pulpal amputation was being investigated. The work of Zander (1939) and Glass & Zander (1949) demonstrated that pulp tissue would repair itself by formation of a bridge under calcium
hydroxide. A one appointment pulpotomy treatment was advocated whereby hemostasis was obtained by sterile cotton pellets soaked in a solution of calcium hydroxide followed by use of a calcium hydroxide paste over the radicular pulpal tissue. The purpose was to maintain pulpal vitality and induce the pulp to produce reparative dentin. It was felt that calcium hydroxide was a more biocompatible material than formocresol. Shoemaker (1955), Via (1955), Law (1956) and Sweet (1956) showed that the use of calcium hydroxide as a capping agent following pulpal amputation resulted in a high incidence of internal resorption leading to a high rate of clinical failures. Interest revived in the empirically successful formocresol pulpotomy technique.

Over a thirty-five year period Emmerson et al. (1959) demonstrated a ninety-seven percent clinical success rate using Sweet’s formocresol pulpotomy technique. Over the years, however, the so called Sweet technique had been slightly modified. In 1956, Sweet advocated a three appointment procedure. The initial appointment involved a pulpotomy and placement of cresolated formaldehyde on a cotton pellet under zinc-oxide-eugenol cement for three to five days. At the second appointment beechwood creosote dressing (cresol 13%, guaicol 47%, and other phenols 40%) was sealed into the chamber for three to five days. The third appointment involved the removal of the dressing and a final pulpal dressing with a portion of formocresol added to the zinc oxide and eugenol.

By 1960, the Sweet Technique was a two-appointment procedure. After coronal amputation of the pulp, formocresol was sealed into the pulp chamber for three to four days. At the second appointment the final pulpal dressing consisted of zinc oxide mixed with two parts eugenol and one part formocresol (Sweet 1960). Redig (1968) advocated the one-appointment pulpotomy technique with similar results and it is this technique that is commonly in use today.
2. Formocresol

In a 2005 survey reported by Dunston & Coll (2008) of pediatric dentistry program directors (76%) and board certified pediatric dentists (81%) from Canada and United States, both full-strength and dilute formocresol remained the medicament of choice for pulpotomy procedures.

Avram & Pulver (1989) reported that the majority of pediatric dental practitioners in Canada (92.4%) and dental schools worldwide (76.8%) use formocresol as the preferred pulpotomy agent for vital primary teeth. The most widely used formulation of formocresol is Buckley’s 19% formaldehyde, 35% cresol, and 15% glycerin in a water base (ADA 1984). The active ingredients of formocresol are formaldehyde and cresol. Glycerin is used as an emulsifier and to prevent polymerization of the formaldehyde to paraformaldehyde (‘s-Gravenmade 1975). Water and glycerin are also used as vehicles for the application of formaldehyde and tricresol to the pulp.

a. Formaldehyde.

The credit for the discovery and first synthesis of formaldehyde (HCHO) is attributed to Hofmann who in 1867, passed methanol/air vapors over a hot platinum wire and documented the formation of formaldehyde (Lepkowski 1897). HCHO is the simplest member of the aldehydes. HCHO is a gas that is readily soluble in water to a maximum concentration of 37% and belongs to the therapeutic category of disinfectants (Granath 1982). The concentrated aqueous solution of formaldehyde is called formalin. In its most concentrated solution, the formaldehyde in formalin precipitates to a polymerized form, paraformaldehyde. If the solution is further diluted with water, the precipitate dissolves once again into formaldehyde (Berger 1965).

Most people come into contact with formaldehyde daily. Formaldehyde is used in the manufacture of products such as plywood, paper, resins, leather, agricultural products,
fabrics, preservatives, embalming fluids, drugs and cosmetics. Owen and others (1990) estimated daily formaldehyde intake from food in a North American diet is 11 mg/day.

As part of normal cellular metabolism, formaldehyde is formed during amino acid metabolism, oxidative demethylation, and purine and pyrimidine metabolism (Squire et al. 1984). Endogenous levels of formaldehyde produced range from three to twelve nano-grams per gram of tissue (Hileman, 1984). The principal oxidative product of formaldehyde is formate, which is further oxidized to carbon dioxide and water. Formate can also be converted to a soluble sodium salt that is excreted in urine or it can be used in biosynthesis (Bardana & Montanaro, 1991). As it is a necessary component in the synthesis of biochemical compounds and a metabolite, it is not considered toxic at low levels of exposure.

Formalin is a tissue fixative and a strong germicide. Both qualities are dependent upon the chemical bonding of formaldehyde with proteins in both host tissues and bacteria. This bonding may take place on the side chain amino acids of the protein at the peptide groups. Formaldehyde links the proteins by the formation of methylene bridges between these peptide groups (Berger 1965).

As a fixative, formaldehyde prevents tissue autolysis because it binds to protein. Bonding of the amino side-group of protein is the most common reaction and stabilization of the proteins is accomplished through the formation of inter- and intra-molecular bonds. While reaction with a single amino group produces an unstable intermediate methylol compound, cross-linking of adjacent amino acids results in more stable compounds. The latter prevents alteration of the basic protein structure and cross-linking with adjacent amino acids prevents enzymatic degradation of proteins.
b. Cresol.

According to Dorland’s Medical Dictionary (Dorland, 2003), tricresol, derived from coal tar, is an antiseptic and a germicidal compound. It is a compound of the three isomeric forms of cresol: ortho-cresol, meta-cresol and para-cresol. It is a hydrophobic, lipophilic compound that requires glycerol to enable its mixing with the water in formocresol. Cresol has an antimicrobial effect (Mejáre et al. 1978), and has been shown to be cytotoxic (Massler & Mansukhani, 1959) and cause tissue necrosis (Mejáre et al. 1979).

‘s-Gravenmade (1975) suggested that cresol may react with formaldehyde and form large hemiacetal molecules. The formation of these molecules would result in its reduced diffusion out of the root canal. On the other hand, Ranly & Pope (1979) did not find that there was a significant reaction between cresol and formaldehyde.

Fulton & Ranly (1979) suggested that following a formocresol pulpotomy, cresol diffused in advance of formaldehyde through pulpal tissue. Ranly et al. (1988) showed that cresol extracted lipids from pulpal tissue. The loss of cellular detail observed in tissue after exposure to cresol and its ability to dissolve cell membranes and release lipids was given as an explanation to explain the toxicity attributed to cresol. Mejáre & Mejáre (1978) assessed the rate and duration of diffusion of the components of formocresol when incorporated in different vehicles. Cresol diffused more slowly than formaldehyde. The lack of systemic exposure to cresol was attributed to more rapidly diffusing formaldehyde causing the shut down of the uptake by the pulpal vessels (Mejáre & Mejáre, 1978).
3. Histologic Effects of Five-Minute Application of Formocresol

a. Human Studies Using Routine Histology.

It was not until the late 1950s that the histologic effects of formocresol on dental pulp tissue were known. Massler & Mansukhani (1959) investigated the histologic effects of formocresol on dental pulp. The other histologic study carried out at the same time was by Emmerson and his co-workers in 1959.

Emmerson et al. (1959) amputated the coronal pulps of 20 human primary canine and molar teeth. The pulps were treated with formocresol for five, ten, 15 minutes and from three days to 21 days. The pulp stumps were covered with either a paste of zinc oxide eugenol with formocresol or dusted with calcium hydroxide powder. This was then followed by a zinc oxide and eugenol paste dressing. All teeth were covered with oxyphosphate cement and restored with amalgam or stainless steel crowns. The teeth were extracted one to eight weeks following treatment. All specimens were fixed in 10% formalin solution. The specimens were decalcified and processed for celloidin sections, prepared, and stained with hemotoxylin and eosin for microscopic evaluation.

Emmerson and his co-workers (1959) found that the pulpal response depended on the total amount of time formocresol was in contact with the pulpal tissue. In the shorter application times, three days or less, two histologic zones were observed: a superficial zone of “fixed” pulp tissue under which was normal radicular pulp tissue. There was no evidence of inflammatory cells. In application times of over three days’ duration, a surface zone of fixed tissue with intact cellular components was seen. Below the fixed zone was a zone that showed “complete degeneration of odontoblasts and calcification”. This observation was seen in a vertical pattern parallel to the long axis of the root canal.
From these findings, Emmerson and his co-workers (1959) concluded that brief periods of formocresol application resulted in pulp tissue remaining vital whilst application beyond three days resulted in non-vital pulp tissue.

Massler & Mansukhani (1959) performed histologic examination of human primary and permanent pulps that had been treated with formocresol for one to 36 minutes, seven, 14, 30 days and longer. The teeth were extracted and fixed in Zenker's formol solution for sixteen hours and washed under running water for 24 hours. They were then decalcified in five percent nitric acid for 48 hours, and again washed in running water overnight, dehydrated and embedded in paraffin. The sections were cut serially through the exposure at 8 to 10 microns thickness and stained with hematoxylin and eosin or Mallory's trichrome stain. In teeth treated for 36 minutes or less, there was a narrow eosinophilic surface layer representing fixed tissue. The underlying normal pulp tissue was clearly demarcated from this eosinophilic fixed zone. In teeth treated for seven and 14 days there was a fixed layer of tissue on the surface followed by a broader pale-staining zone in which there was a marked reduction in the number of cells and fibers. Following the pale-staining zone, there was a dense layer of inflammatory cells which gradually diffused into normal appearing pulp tissue. In teeth treated for 30 days, the surface fixed zone was broad and extended to the apex of the root in some instances. The pale-staining zone, if present, was seen only near the apex and the inflammatory zone seen in the seven and 14 days of application was not seen in this group. In teeth treated for 30 days, the eosinophilic zone was larger and the pale staining zone extended more apically. In teeth treated for 60 days to one year, the pulp was “progressively fixed with ultimate fibrosis of the entire pulp” (Massler & Mansukhani, 1959).

Massler & Mansukhani (1959) concluded that formocresol produced a progressive fixation and degeneration of pulpal tissue. The progressive fixation was considered destructive and it was suggested that formocresol be placed for two to three days followed by a more palliative
dressing so as to allow for pulpal healing. As a result, Massler & Mansukhani (1959) recommended that to prevent progressive fixation formocresol should be applied for less than seven days.

Beaver et al. (1966) reported that Dietz (1961) investigated the histologic effects of formocresol on amputated pulps of primary cuspids. Formocresol was placed in the coronal pulp chamber for seven days. The teeth were extracted at periods ranging from 24 hours to 16 weeks. Dietz (1961) noted that after 24 hours, a surface layer of necrosis followed by a collagenous-like band was produced. After seven days, the specimens showed a more highly organized collagenous-like band. The odontoblasts adjacent to this band appeared normal but they had lost their integrity as the middle portion of the pulp was approached. The middle portion of the pulp showed signs of advanced degeneration, hemorrhage, and extreme blood vessel engorgement. The apical portion of the pulp was normal. In the 14 day specimens the collagenous-like band was further strengthened with a zone of proliferating fibroblasts immediately below.

As reported by Beaver et al. (1966), Dietz (1961) concluded that the pulpal tissue attempted to wall off the surface necrosis with a collagenous band and further noted an attempt at pulpal repair by the network of proliferating fibroblasts.

Beaver et al. (1966), in their study, reported on 60 formocresol pulpotomies. Half of the teeth were capped by a zinc oxide and eugenol mix. The other half was capped by a zinc oxide and eugenol-formocresol mix. Although investigators noted areas of fixation, coagulation necrosis, and fibrosis there seemed to be no difference in the pulpal response to the two different capping agents.

Doyle and his co-workers, in 1962, performed two-stage formocresol pulpotomies in which amputated pulps received a four to seven day application of formocresol prior to the second
appointment where the teeth received a dressing of zinc-oxide-eugenol and formocresol. In five cases, the formocresol containing cotton pledget remained in place for periods of eight to 42 days. Histologic evaluations took place on teeth extracted anywhere from four to 380 days after treatment. After decalcification in formic acid the specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Doyle et al. (1962) observed superficial debris and dentin chips at the amputation site. This was followed by a fibrous, dark-staining zone. Beneath the dark-staining zone was a pale-zone in which the cells were indistinct and odontoblasts were scarce or missing. The pale-zone was most apparent in the middle third of the root canal. Inflammation was not seen in specimens with the longest time interval following treatment. Doyle et al. (1962) concluded that no matter how long the formocresol was applied its primary effect took place in four days or less.

In the same investigation, Doyle et al. (1962) followed a group of 28 formocresol pulpotomies clinically for one to 18 months. Clinically all teeth were asymptomatic. Radiographically, 26 treated teeth appeared normal, a 93 percent success rate. In the same study a histologic comparison between calcium hydroxide treated teeth and formocresol treated teeth revealed a success rate of 50 percent and 92 percent respectively.

Berger (1965) studied the histologic effects of 30 formocresol pulpotomies. In this investigation, amputated pulps had been treated with full strength formocresol for five minutes and then followed with placement of a mix of zinc oxide and eugenol-formocresol paste. The teeth were extracted from 22 to 263 days post-operatively and examined microscopically. The tissues were stained with hematoxylin and eosin or Mallory trichrome stain.

In the 21 day specimens, Berger (1965) observed well defined cellular detail in the coronal third of the pulp tissue with intact red blood cells and odontoblasts. The middle third
showed less affinity for stain and the red blood cells seemed to be decomposing. This was called early coagulation necrosis. The apical third was fibrous, granular with no cellular detail and stained lightly eosinophilic. This was called late coagulation necrosis. The 49 day specimens had the same histologic picture as the 21 day specimens except granulation tissue appeared to be growing into the root canal from the periodontal membrane. The older the specimens the further coronally the granulation tissue was situated. Teeth extracted at 245 days to 266 days post-operatively showed granulation tissue approximating the amputation sites. This contradicts the findings of Beaver et al. (1966) cited previously.

In 49 day and later specimens, Berger (1965) observed internal resorption and subsequent repair by reparative dentin apical to the advancing front of granulation tissue. There was also a consistent observation of a slight accumulation of inflammatory cells at the junction of the granulation and necrotic tissues. He regarded this as normal and as evidence that debris removal and repair was taking place. Berger (1965) concluded that radiographically, the pulpotomies in his study were 97 percent successful and histologically, 82 percent successful.

Rølling & Lambjerg-Hansen (1978) assessed the pulpal condition of clinically and radiographically successfully treated primary teeth 90 to 730 days after formocresol treatment. The teeth were sectioned and stained with hematoxylin and eosin or by Movat’s connective tissue stain and examined by light microscopy. Further, the presence of microorganisms in the pulp tissue was investigated by means of a modified Gram staining method. Thirty-one of the 40 roots showed vital cell-rich tissue in varying extension from the apical area to the amputation zone. The pulpal tissue of the remaining nine roots showed partial or complete necrosis. Complete in vivo fixation was not observed as reported by Berger (1965).
Waterhouse *et al.* (2000) investigated the histologic features of formocresol treated teeth which had failed clinically or radiographically. Sections were stained using haematoxylin and eosin. The investigators found that reactionary dentin deposition occurred in all teeth examined. Post-extraction radiographs and histological sections demonstrated that bridge and appositional dentin were deposited in teeth classified as clinical failures. Amorphous, reactionary dentin, purulent exudate and chronic apical abscesses were identified in radiographically failed teeth.

*b. Human Studies Using Enzyme Histo-Chemical Technique.*

In 1976 Mejáre *et al.* investigated the effect of formocresol on human dental pulp using an enzyme histochemical technique. They believed that the effect of formocresol on dental pulp could not be explicitly established as the tissue was exposed to the same fixative during histologic preparation. Using enzyme histochemical studies, they set out to determine whether an inactivation of enzymes could be used to detect tissue effects of formaldehyde on the human dental pulp. Five minute formocresol pulpotomies were performed on healthy human permanent teeth. The treated teeth were extracted after one to 16 days, freeze-sectioned, freeze-dried, and then incubated for histochemical demonstration of oxidative and hydrolytic enzymes. It was found that lactate dehydrogenase was affected by formaldehyde. A clearly demarcated border was found between the tissue penetrated by formaldehyde and the unaffected tissue. The former was indicated by an absence of enzymatic activity. The distinct border moved apically depending on the concentration and duration of formocresol used. The apex was always characterized by vital tissue.

Using a similar technique as Mejáre *et al.* (1976), Rølling *et al.* (1976) found that in 25 of 27 teeth, vital tissue was observed in the apical third three to five years after formocresol treatment. The investigators in this study felt that their findings demonstrated that the
original pulpal tissue was still present at the apex. In contrast, Berger (1965) believed that vital tissue had grown into the canals from the periodontal ligament due to prolonged exposure to formocresol. Longer-formocresol exposure times correlated with a greater degree of coronal extension of granulation tissue.

c. Animal Studies.

Spedding et al. (1965) reported on an evaluation of pulp and periapical tissues following coronal pulpal amputation, five-minute formocresol application and placement of zinc oxide powder mixed with one drop of formocresol and eugenol in rhesus monkeys. Serial sections of specimens were prepared and stained with hematoxylin and eosin. Microscopic examination led them to conclude that after two hundred eighty-six days formocresol had no effect on the periapical tissues. The investigators noted that the apical portion of the pulp retained vitality and the microscopic appearances resembled those found by Doyle (1962). A histological success rate of seventy percent was reported.

Kelley et al. (1973) histologically evaluated five minute formocresol pulpotomies on primary and permanent teeth in monkeys examined after twenty-two to two-hundred sixty days. The extracted teeth were sectioned and stained with hematoxylin and eosin. The investigators found coagulation necrosis in the coronal portion of the canals and granulation tissue in the remaining apical region. The granulation tissue seemed to arise from periapical connective tissue and growing towards the coronal portion. This observation coincided with the granulation in-growth found in Berger’s 1965 investigation and the prolonged exposure time to formocresol.

Rølling & Melsen (1979), using three Macaca monkeys, investigated the rate of dentin formation and collagen synthesis through the use of tetracycline and $^3$H-proline labels in twenty-four primary pulpal tissue(s) after formocresol treatment. The labels were injected
into the animals after pulpotomies were performed in non-caries teeth. The treated teeth were extracted after periods ranging from 22 to 607 days. The investigators found that 11 of the 25 root canals deemed clinically successful showed dentin formation after treatment. All areas with apparently normal pulpal tissue were labeled with proline. The results indicated that dentin formation and collagen synthesis could occur subsequent to formocresol application, which confirmed that vital pulpal tissue was present after pulpotomy.

Ranly & Fulton (1983) used $^3$H-thymidine as a marker for mitosis in order to study the response of rat pulpal cells to formocresol. The teeth were treated for five minutes with formocresol and covered with a zinc oxide-eugenol and formocresol paste. The animals were sacrificed from three hours to 28 days after treatment. One hour prior to sacrifice, $^3$H-thymidine was injected intraperitoneally. After in vivo fixation, the teeth were washed, dehydrated, embedded in paraffin. Those teeth not used for autoradiography were stained with hematoxylin and eosin. Three day specimens demonstrated three histologic zones; deeply stained area of debris and fixed tissue, followed by a wider, pale-staining, ill-defined zone which blended into a third zone of viable pulp with inflammatory cells. The results showed that formocresol suppressed mitotic activity in the pulp for about three days after application. After three days, mesenchymal cells began dividing and migrating into the necrotic zone. By seven days, this zone was infiltrated by viable labeled cells, forming a “cellular bridge” which isolated the wound site. Condensation of the cells, matrix deposition, odontoblast differentiation and reparative dentin formation occurred by twenty-eight days. Rats have a different pulpal response than humans, specifically of active mitosis and migration of cells and were capable of rebounding from the effects of formocresol (Massler & Mansukhani, 1959, Ranly & Fulton, 1983).

Russo et al. (1984) studied the in vivo fixative effect after formocresol pulpotomy on primary canine and molar dog teeth. The animals were sacrificed 15 days following treatment. The
pulp was removed from the tooth and either freeze-dried for immediate examination or incubated to determine resistance to necrosis. The serial sections were stained with hematoxylin and eosin or by the van Gieson technique prior to histologic examination. The specimens capped with zinc oxide-eugenol-formocresol following treatment with a formocresol pellet did not demonstrate increased fixation but rather a “more intense inflammatory reaction” than the pulps capped with zinc oxide-eugenol paste alone (Russo et al., 1984).

4. Histologic Effects of Less than Five Minute Formocresol Application Times

Few investigations (Venham 1967, Hyland 1969, García-Godoy et al. 1982) studied the histologic effects of applying formocresol for less than five minutes on radicular pulpal tissue after the amputation of coronal pulp.

a. Animal Studies.

Venham (1967) compared the histologic effects of application times of formocresol of less than five minutes in 24 primary cuspids and molars of rhesus monkeys. Formocresol application times and the concentration of formaldehyde in formocresol were varied. In addition, incorporation of formocresol into the zinc-oxide-eugenol paste was carried out for some specimens but not in others. Twenty-eight days following the procedure the treated teeth were extracted, prepared, sectioned, and stained with haematoxylin and eosin in preparation for histologic examination. Venham (1967) found no histologic difference between 15 second and five-minute application times. In all specimens normal-appearing tissue was found in the apical portion of the canals.

García-Godoy and his co-workers (1982) compared pulpal response of one, three, and five minute applications of formocresol followed by plain zinc-oxide eugenol in young adult dogs.
The teeth were extracted 30 days after treatment and serial sections prepared and stained with hematoxylin and eosin. The investigators found that a one-minute application of formocresol produced the least inflammatory response and tissue reaction when compared with three- and five-minute applications. The middle and apical thirds of the radicular pulp in the one minute application showed no inflammation. “Some inflammatory response” was evident in specimens in which the five minute formocresol application was used (García-Godoy et al. 1982). In all experimental groups, the apical third showed absence of inflammation.

b. Human Studies.

Hyland (1969) investigated the histologic effects on human primary teeth treated with two-minute formocresol application time. The teeth were extracted for orthodontic reasons at intervals ranging from two to sixty-six days post-operatively. After fixation in ten percent formalin, the teeth were decalcified, dehydrated, embedded in paraffin, sectioned, mounted, and stained with haematoxylin and eosin. While other investigations reported varying degrees of pulpal fixation following the pulpotomy procedure, Hyland’s investigation (1969) demonstrated “very little that could be interpreted as fixed tissue”. The consistent finding in this investigation was of “intense inflammation” (Hyland 1969). Hyland (1969) explained this finding as a reaction to the zinc oxide and eugenol in the dressing, in light of the apparent failure of the formocresol in fixing the pulpal tissue at the exposure site.
5. Effect of Formocresol on Connective Tissue

The effects of formocresol on connective tissue have been studied through various techniques. Torneck (1961) and Powell & Marshall (1973) found severe cellular damage with necrosis and abscess formation following the use of formocresol injected into subcutaneous tissues of hamsters (Torneck 1961) and rats (Powell & Marshall, 1973). Powell & Marshall (1973) showed that thirty day specimens showed partial recovery.

Simon et al. (1979) sealed formocresol pellets into pulpectomized teeth of Rhesus monkeys for two, seven and forty-two days. The treated teeth were sectioned and stained with haematoxylin and eosin or Brown and Brenn dye. Simon and his co-workers (1979) found that formocresol had a toxic effect on the periapical tissue. However, the observed inflammation decreased with time.

Straffon & Han (1968) used sponge implants placed on the dorsum of the neck of hamsters and found that a 1:50 dilution of formocresol caused effective fixation of cells near the implant. Further, formocresol may have reduced the inflammatory response through its cytostatic and cytotoxic effects. The connective tissue surrounding the implant had fewer inflammatory cells when compared to a control group. Connective tissue ingrowth was seen by ten days. Straffon & Han (1970) using hamsters and Loos & Han (1971) using rats placed sponge implants and demonstrated cytotoxic effects associated with formocresol use. The cytotoxic effects resulting from formocresol use included pyknosis, karyolysis, damaged oxidative enzyme activity and reduced synthetic activity.

Autoradiography performed on monkey teeth by Myers et al. (1978) illustrated high concentrations of $^{14}$C-formaldehyde in the periodontal ligament and bone after formocresol pulpotomy. Fulton & Ranly (1979) obtained similar results in rats using formocresol containing $^3$H-formaldehyde on rats.
Pashley et al. (1980) performed pulpotomies in sixteen maxillary and mandibular anterior teeth in rhesus monkeys. After obtaining hemostasis and collecting control samples of blood, urine and expired air, cotton pellets containing Buckley’s formocresol were placed for five minutes on the pulp stumps. Cerebrospinal fluid and blood samples were collected at 15, 30, 45 and 60 minutes following the pulpotomies. At 60 minutes the dogs were sacrificed and tissue samples from the lung, liver, spleen, skeletal muscle, heart and kidney were examined. Five to ten percent of the formaldehyde placed in the pulpotomy sites was actually absorbed systemically. The investigators concluded that formocresol was absorbed and distributed rapidly (within minutes) throughout the body (Pashley et al. 1980). Myers et al. (1978) observed plasma levels of formaldehyde were similar regardless whether the cotton pellet with formocresol was left in the tooth for five minutes or for 120 minutes. The authors concluded that formocresol compromised the micro-circulation and that absorption was reduced after five minutes (Myers et al. 1978).

Chiniwalla & Rapp (1982) studied the effect of formocresol on pulpal blood vessels. Eighty-four days after formocresol pulpotomy, the investigators perfused monkey primary teeth with India ink-sodium citrate. The vascular architecture was found to be intact throughout the pulp except immediately under the amputation site where there was a reduction in vascularity.

Van Mullem & Van Weelderen (1983) examined vascular changes in the pulp tissue of formocresol treated teeth of Rhesus monkeys by perfusing them with physiological saline. The parts of the jaws containing the teeth were dissected. Histological sections were prepared and stained with hematoxylin and eosin, hematoxylin only or with Massons’s trichrome. Where vessels remained filled with erythrocytes it was concluded that thrombosis had occurred. Thrombi were found in twenty-one of twenty-three teeth where the formaldehyde concentration was greater than 8.75%. It was theorized that areas of autolysis were caused by ischemia which originated from the thrombosed blood vessels. The investigators further speculated that thrombi could enhance the penetration of formaldehyde into radicular tissues since the formaldehyde was
not carried away from the affected area by circulation. It was felt that thrombus formation which occurred towards the apical region would eventually disappear because of formaldehyde dilution and its chemical bonding to tissue components.

6. Histological Techniques Employed In Pulp Examination

Pulp reactions may be affected by the histological technique used. In highly calcified tissues such as teeth, difficulty may arise from lack of penetration by the fixative, especially into the pulp. Histologic studies of demineralized teeth represent by far the most commonly employed technique for the evaluation of pulp reactions (Mjör 1980). Hematoxylin and eosin-stained section of demineralized teeth are usually employed. The main artefacts are due to poor fixation resulting in vacuolization or the presence of empty spaces on histological sections. Such vacuoles may be interpreted as degenerative changes. Human teeth must be decalcified during processing for histological analysis. Rapid fixation of all dental elements is difficult to obtain because penetration of the fixating agent through enamel and dentin is a slow process (Mattuella et al. 2007). In such cases, the tissues in the center of the specimen may undergo some alterations before fixation is completed. The most seriously affected tissue is the pulp tissue (Morse 1945). ‘General hyperemia’ based on the presence of large blood vessels in the pulp may have been caused by trauma applied during extraction of the teeth or by undue stretching of the sections after cutting, prior to mounting on the slide (Mjör 1980). Most teeth used for pulp studies are demineralized in some acid. Part of the tooth’s organic material will be lost during demineralization.
7. General Findings

Previous investigations have demonstrated localized fixation following short application times of up to five minutes. Widespread fixation followed by connective tissue ingrowth and recovery of the tissue have generally followed larger application times up to several days. The presence of extensive necrosis and intraradicular resorption occurred with application times of weeks to months.

The currently accepted technique involving the five minute application of formocresol to radicular pulp stumps results in pulp fixation after formocresol use in the coronal third of the pulp tissues. Immediately apical to this level a broader band of pale, eosinophilic fibrotic tissue is observed. The apical one-third of the canal contains vital tissue (Fig. 1).

Figure 1. Histologic Zones of Radicular Pulp after Formocresol Treatment (adapted from Ranly & Fulton, 1983).
8. Success Rates of the Formocresol Pulpotomy

A number of studies have been performed evaluating the clinical, radiographic, and histologic success rates of formocresol as a medicament agent (Table 1). Various radiologic features have been evaluated including the presence or absence of internal and or pathologic external resorption, radiolucency of the supporting alveolar bone and the invasion of the follicle of the succedaneous tooth. Clinical evaluation considered features such as the presence of pain, the appearance of surrounding soft tissue, reaction of the treated tooth to percussion and the degree of pathologic mobility.

In a landmark study reported by Emmerson et al. (1959), Sweet in 1953 reported a 97 percent clinical and radiographic success rate after a three-appointment formocresol pulpotomy technique of 16,651 human primary teeth.

Doyle et al. (1962) evaluated the results of a two-step formocresol technique in 30 human primary molar teeth and found a 77 percent histologic and 93 percent radiographic success rate with periods of observation ranging from one to 18 months. In the same study, based on clinical observation periods from five to 18 months, a 100 percent clinical success rate was reported (Doyle et al. 1962). Law & Lewis (1964) used a two-step procedure on 324 primary teeth and determined a 90 percent clinical and radiographic success rate after one year. Berger (1965) used a one appointment technique on 30 human primary teeth and found an 82 percent histologic, a 97 percent radiologic and a 100 percent clinical success rate.

Redig (1968) reported that after a period of 18 months clinical and radiographic success rates for one- and two-step pulpotomies were 82 and 90 percent respectively.
Hyland (1969) showed that application of full-strength formocresol directly over the amputated radicular pulp in primary teeth for two minutes with a cotton pellet produced a 97 percent clinical success after six months.

Morawa et al. (1975) evaluated 125 pulpotomies using a one-step appointment and a one fifth dilution of formocresol. Clinical and radiographic examinations were made at six-month intervals. The treated teeth were examined for any evidence of internal and/or external resorption, the appearance of the supporting alveolar bone and the position of the underlying succedaneous tooth. The range of time between pulpotomies and final examinations was six months to five years. The investigators found that 1.62 percent of the pulpotomies were considered unacceptable.

Rølling & Thylstrup (1975) used one or two appointment procedure on 98 primary molars and found on clinical and radiographic examination that the success rates ranged from 91 percent at three months to 70 percent at three years.

Willard (1976) analysed 30 human primary molars which had been treated with a four-minute application of formocresol in a one appointment pulpotomy procedure. The postoperative period ranged from six months to thirty-six months. Using preoperative and postoperative periapical radiographs to evaluate the effects of the treatment he reported that post-operative calcification of root canals was present in 24 of the 30 teeth. This was interpreted by the investigator to represent odontoblastic activity, indicating retained vitality and function within the pulp tissue. The conclusion was that formocresol did not result in complete loss of pulp vitality.

García-Godoy in 1983 used two radiographic views to examine ten extracted and ten in situ primary molars which were previously treated with a formocresol pulpotomy. He concluded that because of overlapping canals, a periapical radiograph, as used in his investigation, was an
unreliable method of analyzing post-operative calcification of root canals. He proposed a second radiograph with a mesial or distal angulation taken for comparison.

García-Godoy (1984), evaluated 45 pulpotomized primary molars treated with a paste of zinc oxide powder mixed with a drop of eugenol and a drop of 1/5 diluted formocresol. At regular six-month intervals clinical and radiographic examinations were made. The treatment was considered a failure when one or more of the following signs were present: internal root resorption, furcation and or periapical bone destruction, pain, swelling, sinus tract or mobility. Clinical and radiographic follow-up ranged from six to eighteen months. The investigator found a success rate of 96 percent.

Hicks et al. (1986) evaluated the radiographic appearance of 164 human primary molars using the five-minute application of formocresol. The post-treatment time ranged from 24 to 87 months. The investigators evaluated post-operative radiographs to determine the presence or absence of radiolucencies in the apical or bifurcation areas, the integrity of the lamina dura in the furcation area, presence or absence of pathologic root resorption, and the incidence of calcific metamorphosis. Based upon the radiographic findings, the pulpotomy procedure was considered to be successful in 89 percent of the cases.

Verco & Allen (1984), clinically and radiographically examined 1246 teeth using a one- or two-stage, five-minute formocresol pulpotomy technique over a five-year period. The clinical criteria to determine failure were pathologic mobility of the tooth and abscess or sinus formation. The radiographic criteria of failure included the presence of a granuloma [sic], internal resorption or ankylosis of the tooth. There was no significant difference between the success and failure rates of the one or two-stage pulpotomies. The investigators reported a 98 and 92 percent clinical and radiographic success rate, respectively.
Roberts (1996), in a prospective study, carried out 142, one-visit, formocresol pulpotomies using full-strength formocresol and evaluated the clinical and radiographic success of the treatment. Failure constituted the presence of pain, swelling, abscess or fistula formation or the radiographic presence of internal root resorption and or evidence of furcation or periapical radiolucency. A 99 percent success rate was reported (Roberts 1996).

Thompson et al. (2001) evaluated 194 primary molars which had undergone a formocresol pulpotomy technique in which hemostasis was obtained with the same formocresol dampened cotton pellet used to medicate the radicular pulp. Radiographic success was defined as the absence of internal or pathologic external root resorption, furcal or periapical radiolucency and absence of root perforation. The radiographic success rates ranged from 91 percent at five to twelve months to 97 percent at more than five years. Teeth were scored as a clinical success if they had no symptoms of pain, tenderness to percussion, swelling, fistulation, or pathologic tooth mobility. The clinical success rate was reported to be 98 percent.

Overall, the majority of the radiographic success rates reported in the literature for formocresol pulpotomies range from 85 to 98 percent (Redig 1968, Berger 1965, Morawa et al. 1975, Beaver et al. 1966, Verco & Allen, 1984). The majority of the clinical success rates reported in the literature range from 88 percent to 100 percent (Redig 1968, Berger 1965, Beaver et al. 1966, Thompson et al. 2001). A summary of these studies is presented in Table 1.
<table>
<thead>
<tr>
<th>Investigation</th>
<th>N</th>
<th>Formulation of Formocresol (Full strength/1:5 dilution)</th>
<th>Observation Period</th>
<th>Histologic Success (%)</th>
<th>Radiographic Success (%)</th>
<th>Clinical Success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet (1953)</td>
<td>16,651</td>
<td>Full strength (3 step)</td>
<td></td>
<td></td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>Doyle et al. (1962)</td>
<td>30</td>
<td>Full strength (2 step)</td>
<td>1-18 months</td>
<td>77</td>
<td>93</td>
<td>100</td>
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<tr>
<td>Law &amp; Lewis (1964)</td>
<td>324</td>
<td>Full strength (2 step)</td>
<td>12 months</td>
<td></td>
<td></td>
<td>90</td>
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<tr>
<td>Berger (1965)</td>
<td>30</td>
<td>Full strength (1 step)</td>
<td>3-38 weeks</td>
<td>82</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>Beaver (1966)</td>
<td>30</td>
<td>Full strength (1 step)</td>
<td>1-3 months</td>
<td></td>
<td>96</td>
<td>-</td>
</tr>
<tr>
<td>Redig (1968)</td>
<td>20</td>
<td>Full strength (1 and 2 step)</td>
<td>18 months</td>
<td></td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>Hyland (1969)</td>
<td>34</td>
<td>Full strength</td>
<td>6 months</td>
<td></td>
<td></td>
<td>97</td>
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<tr>
<td>Magnusson (1977)</td>
<td>48</td>
<td>Full strength (5 minute)</td>
<td>6-36 months</td>
<td></td>
<td>Both groups:53%</td>
<td></td>
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<tr>
<td></td>
<td>36</td>
<td>Full strength (3-5 day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Morawa (1975)</td>
<td>125</td>
<td>One-fifth dilution (one step)</td>
<td>60 months</td>
<td></td>
<td>98.4</td>
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<tr>
<td>Rolling &amp; Thylstrup (1975)</td>
<td>98</td>
<td>Full strength</td>
<td>3 months</td>
<td></td>
<td>91</td>
<td>91</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3 years</td>
<td></td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Willard (1976)</td>
<td>30</td>
<td>Full strength</td>
<td>6-36 months</td>
<td></td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Fuks &amp; Bimstein (1981)</td>
<td>70</td>
<td>One-fifth dilution</td>
<td>4-36 months</td>
<td></td>
<td>65.7</td>
<td>94.3</td>
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<tr>
<td>Garcia-Godoy (1984)</td>
<td>45</td>
<td>One-fifth dilution</td>
<td>6-18 months</td>
<td></td>
<td>96</td>
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<tr>
<td>Hicks et al. (1986)</td>
<td>164</td>
<td>Full strength</td>
<td>24-87 months</td>
<td></td>
<td>89</td>
<td>-</td>
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<tr>
<td>Verco &amp; Allen (1984)</td>
<td>1,246</td>
<td>Full strength</td>
<td>72 months</td>
<td></td>
<td>92</td>
<td>98</td>
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<td>Roberts et al. (1996)</td>
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<td>Full strength</td>
<td>30 months</td>
<td></td>
<td>99</td>
<td>99</td>
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<tr>
<td>Thompson et al. (2001)</td>
<td>194</td>
<td>Full strength</td>
<td>5-12 months</td>
<td></td>
<td>91</td>
<td>98</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 5 years</td>
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<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>
9. Effect of Formocresol Pulpotomies on Succedaneous Teeth

The effects of abscessed primary teeth on succedaneous teeth have been reported to include a significantly higher incidence of enamel defects, early eruption, rotations (McCormick & Filostrat, 1967) and developmental arrest of the succedaneous tooth when compared to controls (Brook & Winter, 1975).

Numerous investigations attribute enamel hypoplasias of bicuspids (Binns & Escobar, 1967) to periapical or inter-radicular infection of primary molars (Shiere & Frankl, 1961). Shiere & Frankl (1961) reported a positive correlation between rotation of premolars and pathological involvement of roots of primary teeth. The effect of a primary molar pulpotomy on the condition of the succedaneous premolar has been less well studied.

Kaplan *et al.* (1967), using rhesus monkeys, studied the effects of inflammation in the pulps of primary teeth on the developing permanent successors. Inflammation was induced in the experimental teeth by surgical exposure of their pulps. 19 of the experimental teeth were further treated with one drop of hydrochloric acid. Although the effects on the permanent teeth were noted, the investigators concluded that they were irregular and unpredictable in occurrence.

Matsumiya (1968) studied the effect of exposing 462 root canals in primary teeth of healthy dogs to the oral environment. A proportion of the canals were subsequently sterilized and filled with various materials. The animals were sacrificed and their periapical tissues were examined histologically. Matsumiya (1968) reported evidence of periapical inflammation in all instances but destruction of the enamel organ had occurred in less than one-fourth of the sample. Upon treating the infected canals the periapical inflammation was resolved while damage to the tooth germ remained unrepaired.
Several *in vitro* investigations (‘s-Gravenmade *et al.* 1981, Wemes *et al.* 1982) using permanent teeth, showed that formocresol can diffuse through dentin and cementum. The presence of accessory canals in the bifurcation and radicular areas of primary teeth can conceivably aid in dispersion of medicament (Winter 1962, Winter & Kramer, 1965).

Binns & Escobar (1967) used dogs’ primary teeth to examine the effect of injury and infection of the primary pulp on succedaneous teeth. Pulp chambers of primary teeth were exposed and ‘macerated’ with an explorer. Pus from infected pulps was used to inoculate the uninfected teeth. The puppies were sacrificed, fixed in 10 percent formalin, and blocks of tissue for histological sectioning were decalcified in formic acid for three weeks. Small blocks were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Binns & Escobar (1967) reported that intentional mechanical exposure of the pulps in primary teeth in dogs resulted in hypoplasia and hypocalcification of the permanent successors. The authors do not mention whether the teeth once operated on were restored or left exposed to the oral environment.

Pruhs *et al.* (1977) evaluated 25 premolars for enamel defects. Their antecedents had been successfully treated with one-visit, five-minute formocresol pulpotomies. The premolars on the treated side were clinically and radiographically compared to their antimeres for enamel defects. Enamel defects were defined as any abnormality in surface morphology or color. The teeth were dried for a minimum of one and half minutes prior to clinical examination for enamel defects. Morphologic defects were determined by passing an explorer over the entire enamel surface of the tooth. Abnormalities in color were determined by visual examination. The investigators reported that 24 of the 25 premolars on the treated side showed enamel defects, and concluded a “definite” relationship existed between formocresol pulpotomies in primary teeth and enamel defects on their successor (Pruhs *et al.* 1977).
Rølling & Poulsen (1978) evaluated 52 permanent tooth pairs for enamel opacities and hypoplasias. Each tooth pair had antecedents that consisted of one primary tooth that had received a formocresol pulpotomy and a contralateral primary tooth with no pulp exposure. Both successful and unsuccessful pulpotomies established by clinical and radiographic criteria, were included. Of the 52 formocresol pulpotomized primary teeth, five teeth had pathological peri-radicular conditions and 36 were clinically and radiographically successful at the time of exfoliation or extraction three to five years following treatment. Prior to clinical examination the teeth were air-dried for a minute. The number, type and location of enamel defect were recorded clinically using transillumination by a fibre optic light. Rølling & Poulsen (1978) concluded that the application of formocresol on pulpal tissue in primary teeth had no effect on the mineralization of the succedaneous permanent tooth germs. Even when the stage of development of the permanent tooth germ at the time of pulpotomy was taken into account, no correlation could be found.

Messer et al. (1980) studied 43 premolars which replaced successfully treated vital or non-vital primary molars for enamel defects and position. In the same investigation, Messer et al. (1980) also examined 20 premolars for evidence of enamel defects and eruption abnormalities following unsuccessful pulpotomies. The premolars on the treated side were compared to the contra-lateral untreated side. The investigators reported on the positional changes affecting 40 percent of premolars that succeeded primary molars treated with either a successful or unsuccessful pulpotomy. They concluded that test premolars showed an increased prevalence of positional alteration and an increase in the prevalence of hypoplastic and or hypomineralization defects.

Mulder et al. (1987) evaluated 139 pairs of premolars for evidence of opacity or enamel hypoplasia. The investigators reported that there were no demonstrable differences between a formocresol pulpotomy in a primary tooth and formation of the permanent successor in terms of
hypoplasia and opacities. Their data also suggested that the presence of enamel lesions was unrelated to the patients’ age at the time of pulpotomy.

Thompson et al. (2001) found no hypoplastic or hypocalcified areas in the premolars that succeeded the treated primary molars when hemostasis and medication were applied with the same cotton pellet.
10. Effect of Formocresol Pulpotomy on Exfoliation and Life-Span of Primary Molars

There is conflicting evidence concerning the effect of pulpotomies on the age at which primary molars exfoliate. In a longitudinal study, Lauterstein et al. (1962) found that infection and pulpotomy of the overlying primary tooth altered the eruption pattern of the succedaneous tooth. In their group of 28 children, the premolar under the pulpotomy treated tooth erupted earlier than the contralateral in 13 cases. In two cases, a delay in eruption was noted (Lauterstein et al. 1962).

Van Amerongen et al. (1986) compared the life-span of 152 primary teeth that underwent five-minute formocresol pulpotomies with corresponding teeth on the contralateral side. The investigators reported that the mean life-span of treated primary molars was 35 months compared to 42 months on the control side. Van Amerongen et al. (1986) concluded that there was no significant difference in life-spans between primary teeth with or without pulpotomies. In another study of 27 primary molar pulpotomies, three teeth erupted earlier than, 15 teeth later than, and nine teeth at the same time as the succedaneous teeth (Loevy & Crawford, 1991). Hobson (1970) and Morawa et al. (1975) both reported that pulpotomized molars exfoliated six to twelve months earlier than usual.

In their retrospective investigation, Hicks et al. (1986) reviewed 164 primary molars treated by using dry cotton pellet for hemostasis without any medicament. This was followed by a zinc oxide paste with a drop each of eugenol and formocresol over the radicular pulp. The investigators reported that 47.2 percent of the pulpotomized molars exfoliated approximately six months prior to exfoliation of the antimeres. Delayed exfoliation occurred with 11.3 percent of the treated teeth. 41.5 percent of the pulpotomized teeth exfoliated at a similar time as the antimeres.
Fuks & Bimstein (1981) using a one-fifth dilution, and Fuks et al. (1983), using full strength and the one–fifth dilution determined that the pulpotomy led to enhanced resorption of the primary tooth. The authors thought the early resorption occurred because the formocresol acted as an irritant to normal periodontal tissue stimulating a cell-mediated response which resulted in external root resorption.

Roberts (1996) carried out a one-visit prospective study of 142 primary molars using full strength formocresol for five minutes and evaluated exfoliation times. Roberts (1996) concluded that there were no significant differences in the ages at which the pulpotomized and non-pulpotomized teeth exfoliated.

Thompson et al. (2001) in their investigation used the same cotton pellet technique to apply medication and obtain hemostasis. They found that six of the 194 treated molars exfoliated earlier than contralateral teeth that had not been treated. There was no clinical significance because the eruption of the succedaneous teeth followed and space maintenance was not required.

Vargas et al. (2005) evaluated the radiographic findings with formocresol and ferric sulfate pulpotomies in relation to early tooth loss in 85 molars followed between six to 61 months. Eleven of the 85 teeth were lost prematurely: four in the ferric sulfate group; four in the formocresol group; and three in the combined ferric sulfate-formocresol group (Vargas et al. 2005).
11. Modifications to the Formocresol Technique

a. Number of Appointments.

As stated previously the number of appointments taken to perform a formocresol pulpotomy has been reduced from four (Sweet 1930) to two (Sweet 1960). Berger (1965) and Beaver et al. (1966) utilized a one-appointment formocresol pulpotomy technique and demonstrated excellent clinical success. Redig (1968) compared the one appointment with the two-appointment technique and found that after a period of 18 months clinical and radiographic success rates for one- and two-step pulpotomies were 82 and 90 percent respectively.

Presently, the preferred technique is the one appointment formocresol pulpotomy (Avram & Pulver, 1989, and Dunston & Coll, 2008) on teeth judged to be vital with little or no inflammation in the radicular tissue.

b. Modification to the Zinc-Oxide Eugenol Sub-Base.

Once the coronal pulp has been amputated and placement of a formocresol moistened cotton pellet completed, the current generally accepted technique involves placement of pure zinc-oxide eugenol sub-base over the remaining radicular pulpal stumps.

Magnusson (1971) evaluated zinc-oxide-eugenol as a medicament for pulpotomized primary human teeth for up to 39 months post operatively. He reported a 45 percent rate of internal resorption as well as a histologically demonstrable chronic inflammation of the residual pulp.

Hume (1986), in reviewing the pharmacologic and toxicological properties of zinc oxide-eugenol, explained that set zinc oxide-eugenol cement consists of zinc oxide particles within a matrix of zinc eugenolate. The presence of water from tissue fluid allows immediate release of eugenol from the zinc oxide-eugenol combination in concentrations sufficient to kill mammalian cells (Hume 1986) therefore, direct contact with vital cells is not desirable.
Beaver et al. (1966) investigated the effects of incorporating formocresol into the zinc-oxide-eugenol sub-base. The pulp responses to the zinc oxide-eugenol sub-base and to the sub-base with the addition of a drop of formocresol did not differ. The investigators concluded that once formocresol has initiated a pulpal response, it was not necessary to incorporate it into the subbase, as it was probably too dilute to exert a further beneficial effect.

Ranly et al. (1975) analyzed the loss of formaldehyde from zinc oxide-eugenol cement in an in vitro investigation in order to determine if the drug was available in a fluid environment. The investigators noted that there was little or no binding of formaldehyde by zinc oxide-eugenol. The loss of formaldehyde from the subbase led the investigators to conclude that the application of formocresol on a cotton pellet may be an unnecessary step in the pulpotomy technique.

García-Godoy (1981), in a study on baboons demonstrated that there was no histological difference in the pulpal response whether formocresol was applied with a cotton pellet or incorporated as a component in the subbase.

Strange et al. (2001) investigated the success of incorporating formocresol in the sub-base and omitting the five-minute application of formocresol in 196 primary molars. Using radiographic assessment criteria that included internal resorption as a failure yielded a radiographic success rate of 79%. In including internal resorption as a success, the technique resulted in a 99% success rate.

c. Concentration of Formocresol.

Buckley’s formulation of commercially available formocresol contains 19 percent formaldehyde, 35 percent cresol in a water and glycerine base. Various investigations have evaluated the biologic effects of varying concentrations of formocresol. Dilute formocresol is prepared as suggested by Morawa et al. (1975) by mixing three parts of glycerin with one part of distilled
water. After the diluent is made, four parts of it are added to one part of full-strength formocresol (containing 19% formaldehyde and 35% cresol) and mixed thoroughly.

Straffon & Han (1968) investigated a one in fifty dilution of formocresol in sponge implants placed in the dorsum of the neck and in the right femur of hamsters. The investigators concluded that formocresol in this relatively low concentration did not interfere with the recovery of connective tissue and appeared to suppress the initial inflammatory response. In a separate study, Straffon & Han et al. (1970) found that a one-fifth dilution was as effective as full strength formocresol. The functional recovery of cells occurred more rapidly after exposure to the one-fifth dilution when compared with full strength formocresol.

Loos & Han (1971), using similar implants in rats demonstrated a reduction in the respiratory enzyme activity of fibroblasts and found that the one-fifth dilution closely matched the effects of full strength formocresol. Also, the time required for tissue recovery was directly proportional to the concentration of formocresol. In light of the faster recovery of affected cells, Loos et al. (1973) concluded that the one-fifth dilution was as effective as the full-strength Buckley’s formula.

Morawa et al. (1975) performed 125 pulpotomies using the one-fifth dilution of formocresol over a six-month to five-year period in human primary teeth with a carious exposure. The clinical and radiographic success of the one-fifth dilution was as good as, or better than, the full strength formocresol. In light of this, the investigators recommended the use of one-fifth concentration of formocresol as the preferred concentration. Escobar (1972) concluded that there were no deleterious effects with the use of a one-fifth formocresol concentration when compared with the full strength solution. Fuks & Bimstein (1981) reported a 94.3 percent clinical success rate and a 65.7 percent radiographic success rate using a one-fifth dilution of formocresol.
Fuks *et al.* (1983) showed that the full strength and one-fifth dilution formocresol produced similar radiographic and histological results in monkeys. By using the dilute form, a milder degree of inflammation was found. Using baboons, García-Godoy (1981) found that the pulpal response to the one-fifth dilution was comparable to that of the full-strength formocresol. Thomas *et al.* (1980) and Verco (1985) reported in bacteriologic studies that formocresol in ten to twenty percent concentration is bactericidal and therefore clinically useful.

*d. Application Time of Formocresol.*

Emmerson evaluated the pulps of 20 primary teeth treated with formocresol for five, ten and fifteen minutes, and from three days to 21 days (Emmerson 1959). The investigator concluded that the pulpal response depended upon the application time.

The five minute application time of formocresol has been arbitrarily assigned (García-Godoy, 1981). Avram & Pulver (1989) reported that 42.2% and 50% of respondents in a world-wide survey apply full-strength and one-fifth dilution respectively for a period of five minutes. Dunston & Coll (2008) reported that 92% of the pediatric dental program directors and 76% of the diplomats of the American Board of Pediatric Dentistry used a medicated pellet in the pulp chamber for two to five minutes.

Venham (1967) found no histological difference between fifteen second and five minute formocresol application times when followed by zinc oxide-eugenol containing formocresol.

Hyland (1969) showed that full strength formocresol directly applied over a pulpal exposure for two minutes with a cotton pellet produced a high clinical success rate (97%).

García-Godoy (1981) compared one-, three- and five-minute applications of formocresol followed by a pure zinc oxide-eugenol. The investigators concluded that the one-minute
application of formocresol in a cotton pellet produced the least inflammatory response when compared to the three- and five-minute applications.

Aktören (1998) in twenty primary molars applied full strength formocresol on pulp stumps for one minute followed by plain zinc oxide eugenol. Radiographic signs of failure included presence of internal root resorption, inter-radicular or periapical destruction of bone. Clinical signs of failure included presence of pain, swelling, sinus tract or mobility. Clinical and radiographic success rates were 90% and 85% respectively after six months. A two year investigation by Aktören & Gençay (2000) reported a clinical success rate of 88% and a radiographic success rate of 80% in 24 molars.

e. Omission of a Separate Cotton Pledget to Obtain Hemostasis.

Thompson et al. (2001) used 194 primary molars with follow up times ranging from five to 109 months to evaluate a formocresol pulpotomy technique in which hemostasis was obtained with the same formocresol dampened cotton pellet used to medicate the radicular pulp stumps. The investigators reported a radiographic success rate of 87 percent and a clinical success rate of 98 percent (Thompson et al. 2001).
12. Systemic Effects of Formocresol

Formaldehyde has been implicated in a wide variety of acute and chronic health effects.

Malaka & Kodama (1990) investigated the adverse effects of formaldehyde exposure in the workplace and community. The respiratory status of 186 plywood workers was evaluated by spirometric tests, respiratory questionnaires and chest x-rays. The concentrations of formaldehyde in the work environment ranged from 0.28 to 3.48 parts per million (ppm). The results of the study supported the hypothesis that chronic exposure to formaldehyde induced signs and symptoms of chronic obstructive lung disease.

Using respiratory symptom questionnaires and spirometry, Horvath et al. (1988) evaluated formaldehyde levels in workers who had experienced occupational exposure to airborne formaldehyde. Low level exposures to formaldehyde were associated with dose-dependent irritation of the eyes and mucous membranes. However, after a mean exposure of ten years there was no evidence of permanent respiratory impairment. Sim & Pattle (1957) demonstrated in healthy volunteers exposed to 13.8 ppm formaldehyde for 30 minutes that there was initial irritation to the eyes and nose but the effects rapidly wore off with no signs of eye irritation observed after ten minutes.

Kilburn et al. (1987) showed an association between neuro-behavioural function and occupational exposure to formaldehyde. Increasing exposure times and age were correlated with poor memory, poor dexterity and poor equilibrium. The results implied that chronic low level exposure to formaldehyde impaired function of the nervous system.

High oral doses of formaldehyde (up to 150 and 100 mg/kg/day) given to rats and dogs for 91 days did not produce specific treatment-related effects on any organ or tissue (Johannsen et al. 1986). Therefore, Johannsen et al. (1986) concluded that formaldehyde possesses little subacute toxicity.
The systemic effects of formocresol after pulpotomies have been studied. Myers et al. (1978), using a radioactive tracer (\(^{14}\)C) to identify and quantify formaldehyde demonstrated that \(^{14}\)C-formaldehyde was distributed in the systemic circulation after single or multiple formocresol pulpotomies of five rhesus monkeys. The investigators reported that a five-minute exposure of pulpal tissue to \(^{14}\)C-formocresol resulted in the systemic absorption of approximately one percent. Two hours of exposure of pulpal tissue to \(^{14}\)C-formocresol did not increase the amount of systemic absorption. In the same investigation \(^{131}\)NaI (Iodine isotope tracer) was sealed in teeth to determine whether the microcirculation was capable of absorption after it was exposed to formocresol. The investigators determined that when \(^{131}\)I was applied to formocresol-treated pulpotomy sites, it was absorbed at a moderate rate whereas \(^{131}\)I applied to sites not previously treated with formocresol resulted in increased systemic absorptions. This indicated that formocresol compromised the microcirculation of the dental pulp. In the same study Myers et al. (1978) showed, through timed urinalysis, that a substantial renal excretion of \(^{14}\)C-formaldehyde occurred. According to the authors, this indicated that \(^{14}\)C-formaldehyde was filtered at the glomerulus and not all was protein-bound (Myers et al. 1978).

Pashley et al. (1980) confirmed these findings and demonstrated that \(^{14}\)C-formaldehyde is absorbed from pulpotomy sites and subsequently appears in body fluids. In this study, 32 five-minute formocresol pulpotomies were performed in two dogs and the distribution of \(^{14}\)C-formaldehyde was evaluated. Between five and ten percent of the labeled formaldehyde was rapidly absorbed into the systemic circulation. The liver, kidney, lung, heart and spleen were found to bind formaldehyde and the marker was also found in bile, urine, pulmonary excretions and the cerebrospinal fluid. Twenty to twenty-six percent of the \(^{14}\)C-formaldehyde filtered by the kidney was excreted in the urine. The remainder was either re-absorbed or bound to renal tissue. Most of the absorbed formaldehyde was bound to tissue. The liver showed high levels of bound
formaldehyde. The amount of labeled formaldehyde that was absorbed was small and it was rapidly distributed throughout the body within minutes of the pulpotomy procedure.

In order to determine the nature of an acute toxic reaction to systemically administered formocresol, Myers et al. (1981) intravenously injected formocresol at 0.048ml/kg and 0.149 ml/kg in two dogs. The basis for the administration of the doses selected stemmed from the works of Tani et al. (1978) where it was determined that intravenous formaldehyde administration to dogs at levels above 1mg/kg resulted in hypotension. Blood samples were taken at zero, 30, and 60 minutes and then hourly for six hours following formocresol application. Urine collections were obtained, blood pressure measurements performed and heart rates were recorded for six hours. After six hours the dogs were sacrificed and tissue samples taken from the heart, kidney and lung for histological examination. The degree of tissue injury appeared to be dose-dependant as the dog that received the higher dose of formocresol demonstrated more marked biochemical and histologic evidence of tissue injury. The authors noted that the nature of the cellular injury would likely be reversible in the early stages (Myers et al. 1981). It has been calculated that over 3000 pulpotomies would have to be performed at the same time in order for formocresol to reach comparable exposure levels (Ranly 1984).

Myers et al. (1983) investigated whether cellular injury could be detected following formocresol application to the vital pulp tissue of five dogs. Twenty-one five-minute, full-strength formocresol pulpotomies were performed. After six hours, sections of the kidney, liver, lung and heart tissues were removed and prepared for histological evaluation. The investigators found that in a single dog that received 16 formocresol pulpotomies there was evidence of early cellular injury in the kidney and liver. Tissue recovery was expected since there was no evidence of inflammation (Myers et al. 1983).
Ranly (1985b) carried out single five minute pulpotomies on rats using 19% $^{14}$C-formaldehyde. Metabolic and radioisotope studies were used to determine that approximately 30 percent of the formaldehyde placed onto the pulp chamber was distributed systemically within five minutes, permitting more rapid metabolism of formaldehyde and expiration of labeled carbon dioxide.

The higher level of absorption and detoxification was attributed to the higher metabolic rate of rodents versus dogs. The relative level of absorption was dependent on the animal model used accounting for difference in basal metabolic rate.

Using rats, Ranly & Horn (1987) administered several doses of formaldehyde into the jugular vein until systemic morbidity was achieved. Histologic examination did not reveal tissue pathology in the liver or kidney for up to 24 hours after the administration of formaldehyde. The investigators believed that the histologic and biochemical changes associated with formocresol toxicity developed only after a chronic insult. Biochemical changes including elevated urinary lactate dehydrogenase, protein and reduced level of liver respiration were coincident with toxicity. The elevated lactate dehydrogenase indicated nephron damage and the presence of proteinuria was suggestive of altered glomerular filtration. These changes were seen with formocresol doses that were 125 times greater than that of a single pulpotomy.

In a recent study Cortés et al. (2007) evaluated the presence of systemic toxicity at therapeutic doses following formocresol pulpotomies through histologic and biochemical changes in 32 rats. Intravenous injection of physiological serum was delivered to the control group. The experimental groups received intravenous injections of formaldehyde that was equivalent to ten, twenty and one-hundred pulpotomies. Blood samples were taken for biochemical analysis 12 and 24 hours following the experiment. Samples of hepatic tissue were taken for histologic analysis. The histologic examination of hepatic tissue showed no evidence of a liver cell lesion. The results were similar in all groups with no differences between the control and experimental
groups. In addition, biochemical analysis did not reveal any significant difference between the control and experimental groups (Cortés et al. 2007).

Investigators at The Children’s Hospital in Colorado determined the plasma concentration of formocresol in 30 children undergoing 85 pulpotomies. Hemostasis was achieved with a sterile dry cotton pellet and radicular pulp stumps treated with five-minute contact with full-strength formocresol. Preoperative and postoperative blood samples were taken immediately, five, 15, 30, 60, 90 and 120 minutes. 312 blood samples collected. The authors concluded that formaldehyde was undetectable above baseline physiologic concentration (Kahl et al. 2008).
13. Allergenic Effects of Formocresol

Rølling & Thulin (1976) attempted to determine the prevalence of sensitivity to formaldehyde, cresol and eugenol following formocresol pulpotomy in 128 children. The interval from the pulpotomy to the patch test varied from two months to eight years. The investigators reported that none of the children showed a positive result against formaldehyde, cresol or eugenol. This indicated that the components of formocresol do not induce cutaneous delayed hypersensitivity.

Dilley & Courts (1981) mixed rabbit serum albumin with formaldehyde and injected it into the animal. Low antibody titers were present in the serum, and skin tests failed to show an immediate hypersensitivity response. Although weak humoral and cell-mediated responses were noted these reactions were limited and considered clinically insignificant.

The plausibility of inducing an immune response from the clinical use of formocresol in humans was studied by Longwill et al. (1982). Children with a history of two or more pulpotomies were compared with a control group by exposing peripheral blood extracts of the pulp and evaluating lymphocyte transformation. The results suggested that formocresol pulpotomy in children does not cause significant sensitization when used in moderation.

Doi et al. (2003) evaluated 155 asthmatic Japanese children for prevalence of formaldehyde-specific IgE. The relationship of IgE sensitization to formaldehyde exposure and severity of asthma were evaluated. The investigators found that the prevalence of formaldehyde specific IgE was very low and independent of asthmatic status. Additionally, the presence of formaldehyde-specific IgE was deemed to be of limited clinical relevance.
14. Mutagenic Effects of Formocresol

Mutagenicity refers to the ability to cause change in the genetic material within a cell. Mutagenic effects are associated with most chemicals known to cause cancer. Tests for mutagenicity can therefore, help in determining carcinogenic potential. The types of DNA damage induced by formaldehyde include sister chromatid exchanges, micronuclei, chromosomal aberrations and deletions (Merk & Speit, 1998, Crosby et al. 1988). In this regard formaldehyde has been demonstrated to being mutagenic in laboratory experiments with *Drosophila*, flowering plants, fungi and bacteria (Fishbein 1978, Auerbach 1976, Auerbach et al. 1977).

Casanova et al. (1989) reported the occurrence of DNA-protein cross-links (DPX) at sites of initial contact in the nasal mucosa of rats and in upper respiratory tract of monkeys exposed to formaldehyde (Casanova et al. 1991). Quievryn & Zhitkovich (2000) reported that DPX are present in tissues for no more than a few hours and undergo spontaneous hydrolysis or active repair by proteolytic degradation.

Goldmacher (1982) reported that formaldehyde at a level of 4 ppm was found to be mutagenic in diploid human lymphoblasts in culture. In another study no chromosome abnormalities were found in a group of 15 workers exposed to formaldehyde over an average of 28 years (Fleig et al. 1982).

Zarzar et al. (2003), in an *in vivo* study investigated whether formocresol is mutagenic in lymphocyte cultures obtained from the peripheral blood of twenty children aged five to ten years old who underwent vital pulpotomies. Peripheral venous blood samples were collected and lymphocytes were assessed for chromosomal aberrations. The investigators observed that formocresol did not alter the number of cells in division among 2000 randomly scanned lymphocytes and it was concluded that formocresol is not mutagenic in humans. These findings are in agreement with other *in vitro* and *in vivo* studies in mammals that have shown that
formaldehyde in low concentrations (0.1-5 ug/ml and 0.003-0.024 ul/ml in vitro; 0.4 ml injected peritoneally and 0.7-6 ppm by inhalation in vivo) does not demonstrate mutagenic activity (Kreiger & Garry 1983, Natarajan et al. 1983, Heck & Casanova 1999).

Ribeiro et al. (2005) investigated the ability of formocresol to induce genetic damage such as gene mutations, chromosomal breakage, altered DNA capacity and cellular transformation. Chinese hamster ovary cells were evaluated under exposure to formocresol by single cell gel assay in vitro. Formocresol did not induce strand breaks in DNA or any DNA lesions (Ribeiro et al. 2005).

Ramos et al. (2008) evaluated the genotoxic potential of formocresol on healthy human donors by exposing different dilutions of formocresol for 45 minutes at 37°C to peripheral blood lymphocytes. Formocresol did not produce detectable DNA damage.
15. Carcinogenic Effects of Formocresol

Swenberg et al. (1983) demonstrated that formaldehyde caused an increase in the rate of cell turnover in the respiratory mucosa of rats. As cell replication increased the stability of the DNA double helix is decreased and consequently, an increased number of DNA sites were available for reaction with formaldehyde. The formaldehyde-DNA damage could result in mutations and initiate neoplastic transformation (Swenberg et al. 1980). These authors reported that exposure to high concentrations (15 ppm) of formaldehyde vapour, five days a week for 18 months induced 36 squamous cell carcinomas in the nasal cavities of 200 rats examined (Swenberg et al. 1980).

Berke (1987) evaluated the changes related to formaldehyde exposure through clinical examination of the nose and throat and cytologic examination of exfoliated nasal cells. The investigator reported a significant prevalence of mucosal irritation in formaldehyde-exposed workers but no relationship was found between formaldehyde exposure and atypical squamous metaplasia.

Formaldehyde was classified as a “probable human carcinogen” by Health Canada (1987), the International Agency for Research on Cancer (IARC, 1987), the Agency for Toxic Substances and Disease Registry (ATSDR, 1999) in the U.S. Department of Health and Human Services, and the U.S. Environmental Protection Agency (USEPA). A recent press release (IARC, 2004) reclassified formaldehyde from a ‘probable’ to a ‘known’ human carcinogen based on exposure levels to laboratory animals. In these studies the exposure levels are substantially higher than common human exposures. Dose response analysis was not undertaken. The cancer risk was predicted by extrapolating from laboratory animal data. Various researchers have noted the anatomic and physiologic differences between humans and other animal models (Nilsson et al. 1998, Schlosser et al. 2003). Kimbell et al. (2001) and Conolly et al. (2004) developed dynamic three-dimensional airflow models that accurately exemplified airflow and regional deposition of
formaldehyde on mucosal surfaces of rodents, monkeys and humans. On the basis of these investigations Conolly et al. (2004), the Chemical Industry Institute for Toxicology Centers for Health Research (CIIT) reported that cancer risk is negligible until formaldehyde exposure reaches levels in the range of 600 to 1,000 parts per billion. A major component of the epidemiologic evidence evaluated by IARC to categorize formaldehyde as a human carcinogen (Group 1) was the analysis published by Hauptmann et al. (2004) of the National Cancer Institute (NCI) historical cohort which comprised industrial workers exposed to formaldehyde in 10 U.S. plants. The NCI authors emphasized the relationship found between highest formaldehyde peak exposure and death from nasopharyngeal carcinoma (NPC). Marsh & Youk (2005) showed that NCI’s suggestion of a causal association with formaldehyde and NPC was driven entirely by anomalous findings for one of the 10 study plants. Six of 10 NPC deaths observed in the NCI study occurred in one plant and the remaining four cases occurred individually in four of the other nine plants investigated. In 2007 Marsh et al. performed additional re-analyses of the NCI cohort data to further investigate whether the interaction observed by Hauptmann et al. (2004) was appropriate and to explore the degree of instability of the risk estimates for NPC in relation to highest peak exposure. Marsh et al. (2007) demonstrated that NCI authors failed to account for ‘an important interaction structure between plant group and the exposure variable’ which would prohibit a generalization of formaldehyde effects within the NCI cohort. Their (Marsh et al. 2007) sensitivity analysis demonstrated considerable uncertainties in risk estimates and in fact, pointed to instability problems related to one plant. The authors concluded that the re-analysis of the NCI study did not support the NCI’s suggestion of a causal association with formaldehyde exposure and nasopharyngeal carcinoma.

Sofritti et al. (1989) reported that leukemia was not observed in any of seven long-term inhalation bioassays in rodents nor was it observed in three drinking water studies in which rodents were exposed to doses as high as 1.9 to 5 g/L. A study of British chemical workers exposed to high
chronic formaldehyde levels and high peak exposures demonstrated no causal relationship between formaldehyde and leukemia (Coggon et al. 2003).

In summary, no correlation has been demonstrated between formocresol pulpotomies and cancer in humans.
16. Comparison of Alternative Pulpotomy Agents to Formocresol

a. Calcium Hydroxide.

Calcium hydroxide has not compared favourably to formocresol as a pulpotomy agent in vital pulp therapy. Calcium hydroxide is thought to stimulate pulp healing by the formation of a dentin bridge (Holland et al. 1979).

i. Histologic studies.

The success rates of calcium hydroxide vital pulpotomies have been found to be approximately half of the success rates reported using formocresol. Magnusson (1970) reported a 2.5% success rate in a histologic study of 130 pulpotomized primary mandibular molars. Internal resorption below the amputation site has been reported as the most common cause of failure associated with calcium hydroxide (Via 1955, Magnusson 1970, Schroder 1978). Schroder (1978) reported a 38 percent success after two years when using calcium hydroxide following amputation of pulpal tissue of primary molars.

Spedding et al. (1965) reported a 60 percent histologic success rate in 25 non-carious teeth treated with calcium hydroxide compared to 70 percent in 21 formocresol treated teeth.

Fadavi & Anderson (1996) assessed the response of the pulp to calcium hydroxide in primary teeth after a period of six months. The investigators reported pulpal necrosis and moderate to severe inflammation in all teeth treated with calcium hydroxide.

ii. Histologic, clinical and radiographic study.

Doyle et al. (1962) used a two-visit procedure in their comparison of formocresol and calcium hydroxide. The investigators compared the use of calcium hydroxide and formocresol pulpotomies on mechanically exposed, healthy, primary dental pulps. After coronal pulp
amputation one-half of the teeth in the study were capped with calcium hydroxide and the other half were treated using formocresol. A formocresol pellet was sealed in place for four to seven days, and the histologic study was conducted from four to 388 days. Of the 18 teeth in the calcium hydroxide group, only 50% were judged histologically successful. Of the 14 teeth treated with formocresol, 92% were histologically successful. Radiographically, the success rates were 64% and 93%, whereas the clinical success rates were 71% and 100% for calcium hydroxide and formocresol, respectively.

iii. Clinical and radiographic studies.

Acceptable clinical outcomes in human investigations have ranged between 31-59 percent and radiographic success rates seldom exceeded 60 percent with the use of calcium hydroxide (Via 1955, Law 1956, Doyle et al. 1962, Schroder 1978).

b. Glutaraldehyde.

Wemes & s’Gravenmade (1973) proposed that a 2% glutaraldehyde solution, a mild fixative, be used as a pulpal fixative and a substitute for formaldehyde. In comparison to formocresol, 2% glutaraldehyde was noted to demonstrate a more active fixation (Russell 1976), limited tissue penetration (Tagger et al. 1986), and a more restricted zone of infiltration after application to exposed pulps (Davis et al. 1982).

i. Histologic studies

Kopel et al. (1980) performed pulpotomies on carious human primary teeth using 2% glutaraldehyde and studied its effects on the pulp over one year following treatment. Initially they showed that there was a zone of fixation adjacent to the glutaraldehyde dressing. Under the zone of fixation they found that the cellular detail was consistent with that found in normal pulp tissue. After a period of one year, however, they found that the fixed zone was replaced with
dense collagenous tissue. The investigators speculated this was due to a high degree of molecular cross-linking with minimal diffusion of the glutaraldehyde into subjacent tissues.

In an eight-week study, Davis et al. (1982) compared the effects of pulpotomies on mechanically exposed rat teeth using a one-fifth dilution of Buckley’s formocresol and 5% buffered glutaraldehyde. Their findings showed that the coronal third of the glutaraldehyde treated pulpal tissue was fixed and that there was a mild and minimal inflammation in the middle and apical thirds respectively. The depth of penetration of the glutaraldehyde was significantly less than that of formocresol. Compared to pulp treated with formocresol, glutaraldehyde treated pulps displayed repair of fixed coronal tissues with less apical damage and necrosis.

**ii. Clinical and radiographic studies**

Clinical studies using 2% glutaraldehyde have also produced favourable results. García-Godoy (1983a) evaluated the effects of 2% unbuffered glutaraldehyde on the pulps of 55 cariously exposed primary human teeth for one to three minutes. Six to twelve months later 96.4% of the treated teeth showed no clinical or radiographic signs of failure. Prakash et al. (1989) and García-Godoy (1986) reported 100% success after 6 months and 98% clinical and radiographic success after 19-42 months.

Fuks et al. (1986) in a clinical and radiographic investigation of 53 primary molars reported an initial clinical and radiographic success of 94% after six months, which decreased to 90.4% at one year and 82% after two years. In a later study Fuks et al. (1990) investigated the use of two percent glutaraldehyde in vital pulp therapy in primary molars. Nine out of 50 treated teeth showed internal and external resorption as well as inter-radicular pathology. The authors concluded that there were no benefits of using gluteraldehyde as a substitute for formocresol in vital molar pulp therapy.
In 1988, Lloyd et al. examined the histologic response of dental pulp to various concentrations of glutaraldehyde over various time intervals in pulpotomies performed on 160 monkey teeth. The investigators observed that the depth of tissue fixation increased with the concentration and application time and therefore suggested that the reaction of the pulp tissue to glutaraldehyde was directly related to the concentration and application time. The investigators also observed aggressive internal resorption in teeth treated with low concentrations of glutaraldehyde and for lesser application times (Lloyd et al. 1988).

iii. **Systemic distribution**

Myers et al. (1986) estimated that 3-5% of labeled glutaraldehyde placed in pulpotomy sites of dogs could be detected systemically. Ranly et al. (1989) estimated that 25% of the pulpotomy dose of glutaraldehyde used in rats was found to be systemically distributed.

iv. **Cytotoxicity**

Jeng et al. (1987) compared the cytotoxicity of formocresol and its constituents, and that of glutaraldehyde using human pulp fibroblasts as test cells. They demonstrated that 2.5% glutaraldehyde was 15 to 20 times less toxic and damaging to the test cells than formocresol. The glutaraldehyde seemed to diffuse more slowly and needed a longer time to produce its maximum toxic effects.

v. **Concentration and time studies**

Ranly & Horn (1987) investigated the effects of time, concentration, and pH on glutaraldehyde fixation using a medium of collagen- bovine serum albumin gels to simulate cell cytoplasm. Their results led the investigators to conclude that the degree of fixation was increased by buffering the glutaraldehyde, increasing its concentration and prolonging the application time on pulpal tissue.
The authors suggested that for clinical use a buffered four or eight percent glutaraldehyde solution be applied for four or two minutes respectively.

Ranly & Horn (1987) investigated the purity and efficacy of several glutaraldehyde solutions before and after six months of storage. Buffered and unbuffered 2% and 5% solutions and a 25% stock solution were tested after storage for six months. Ranly & Horn (1987) reported that buffered, unrefrigerated preparations developed organic impurities and resulted in a diminished fixation of the pulp. Their findings appear to indicate that fresh solutions should be prepared if the successful outcomes shown by other studies are to be reproducible (García-Godoy 1983a, Prakash et al. 1989). For fixation to be equivalent to that obtained through formocresol, Ranly & Horn (1987) and Feigal & Messer (1990) suggested that a higher concentration (4-8%) of glutaraldehyde be applied. Systemic distribution and toxicity may be increased as a result of the application of a greater concentration of the agent.

c. Electrosurgery.

Electrosurgery has been advocated as a method for pulp therapy for many years (Oringer 1975, Anderman 1982, Harris 1976). After removal of the coronal pulp a layer of coagulation necrosis is produced by electrosurgery and this was postulated to provide a barrier between healthy radicular pulp and the base material thus sealing the pulp chamber. It was thought that odontoblasts would then be stimulated to form a dentin bridge and the tooth be maintained until it was ready to exfoliate (Sheller & Morton, 1987). This investigation did not incorporate a control group.

i. Histologic studies

Results reported in an eight week study by Ruemping et al. (1983) indicated that an electrosurgical pulpotomy was comparable to a formocresol pulpotomy in non-caries primary
and permanent primate teeth. In contrast, Shulman et al. (1987) in an in vivo study using 80 non-carious primary teeth of four macaca fascicularis monkeys histologically compared the results of electrosurgery to formocresol from three to 65 days post-treatment. Three groups of 20 teeth received pulpotomies using either electrosurgery, $^{14}$C-labeled formocresol in a zinc oxide and eugenol base or electrosurgery followed by the $^{14}$C-labeled formocresol-zinc oxide and eugenol base. The experimental groups were compared to a control group that received no treatment. The investigators demonstrated pathologic root resorption, periapical and inter-radicular pathology in those teeth treated with electrosurgery. By 41 days the pulps of teeth treated with electrosurgery showed signs of irreversible degeneration (Shulman et al. 1987). In contrast, little periapical or pulpal pathology was noted in the teeth treated with formocresol. Oztas et al. (1994) later replicated these findings in a study that compared the formocresol technique to the electrosurgical pulpotomy technique. They reported an intense inflammatory cell infiltration with periodontal abscesses in the latter group. These observations may have been due to prolonged exposure times of the electrical currents to the pulp and lateral heat production during the electrosurgical technique.

A pilot study by Sheller & Morton (1987) evaluated the effects of electrosurgery on the pulp in pulpotomies carried out on 11 non-carious deciduous teeth in children over a time interval of one hour to 100 days post-treatment. At 100 days their results showed clinical and radiographic success in ten teeth, while only seven teeth showed histologic success. The authors reported that the use of electrosurgical pulpotomy could not be recommended as a method that produced superior results to formocresol pulpotomy.

In a study with a larger number of teeth and a longer post-treatment period, Shaw et al. (1987) histologically evaluated the primary teeth of five Macaca monkeys treated using either a formocresol or electrosurgical technique up to six months post-operatively. The authors concluded that the electrosurgery pulpotomy technique produced a tissue response comparable to
the formocresol technique. Statistical analysis was not performed in this investigation and therefore any potential differences in outcome may not have been detectable.

**ii. Clinical and radiographic studies**

Mack & Dean (1993), in a retrospective study, evaluated 164 electrosurgical pulpotomies clinically and radiographically over an observation period of up to five years and ten months. They reported a clinical and radiographic success rate of 99.4 percent.

Electrosurgery has not gained widespread acceptance for pulpotomy of vital primary molars in North America (Primosch et al. 1997). The investigations have had relatively short follow-up periods and the results are inconsistent. The outcomes of inflammatory root resorption and pulp necrosis following electrosurgical pulpotomy have limited its further investigation.

In a randomized clinical trial (Bahrololoomi et al. 2008) 70 pulpotomies were performed using dilute formocresol (1:5) for five minutes or an electrosurgical technique by passing an electrical arc for one second followed by a cooling period (10-15 seconds). In both groups a reinforced ZOE dressing was placed directly over the radicular pulpal stumps. The teeth were subsequently restored with amalgam. At three, six and nine months the teeth were evaluated for the presence of pain, abscess, fistula, mobility, internal and external resorption, and radiolucency. After nine months of follow-up, the clinical and radiographic success rates were 96 percent and 84 percent respectively in the electrosurgical group. In the formocresol group, the clinical and radiographic success reates were 100 percent and 96.8 percent. The differences between the two groups were not statistically significant (p>0.05). No significant conclusions can be drawn from these results since the study was followed up for a short period of time.
d. Ferric Sulfate.

Ferric sulfate is used for its hemostatic properties prior to impression taking and during endodontic surgery. Ferric sulfate forms a ferric ion-protein complex when in contact with blood and seals the cut blood vessels mechanically producing hemostasis (Fischer 1981).

i. Histologic studies

Landau & Johnsen (1988) used ferric sulfate before applying calcium hydroxide to pulpotomized monkey teeth. Their results showed that after 60 days vital pulpal tissue was found at the apical third of teeth treated with ferric sulfate and that the ferric sulfate group had a greater amount of secondary dentin and partial bridging compared with the calcium hydroxide group.

In a histologic study, Fuks et al. (1997) investigated the pulp response to a 15.5% ferric sulfate solution and a 1:5 dilution of formocresol in pulpotomized non-carious primary teeth of baboons, after four and eight weeks. Following treatment with either ferric sulfate or dilute formocresol, the teeth were restored with Intermediate Restorative Material (IRM). The investigators reported that 60 percent of teeth treated with ferric sulfate presented with normal pulps and they concluded that these responses were similar to those of the dilute formocresol.

Cotes et al. (1997) histologically assessed the pulpal reaction after use of formocresol and ferric sulfate in maxillary first molars of 120 Sprague-Dawley rats at weekly intervals for four weeks. The authors concluded that the teeth treated with formocresol showed the least pulpal inflammatory response and the use of ferric sulfate did not improve the pulpal response.

ii. Clinical and radiographic studies

Fei et al. (1991) compared the clinical and radiographic performance of ferric sulfate and formocresol in 56 primary molars. At twelve month follow-up, 28 of the 29 teeth treated with ferric sulfate were considered to be clinically and radiographically successful. Over the same time
period 21 of the 27 teeth treated with formocresol were judged to be clinically and radiographically successful.

Fuks et al. (1997) compared the effects of a 15.5% ferric sulfate solution to dilute formocresol in 96 carious human primary molars. Three pediatric dentists performed pulpotomies under local anesthetic under rubber dams. The investigators reported 92.7 percent and 83.8 percent success rates of ferric sulfate and formocresol respectively after a 24 to 35 month follow-up period. Radiographically, 74.5 percent of the ferric sulfate treated teeth and 73 percent of the formocresol treated teeth showed an absence of radicular pulp pathology. This success rate included ten (18.2 percent) teeth in the ferric sulfate group and four (10.8 percent) in the formocresol group that presented with pulp canal obliteration. Molars with pulp canal obliteration were not considered failures. In the same study the investigators reported that six (15.4 percent) ferric sulfate treated teeth and four (14.4 percent) of the formocresol group showed faster root resorption than non-pulpotomized controls. The difference between the success rates of 92.7 percent for the ferric sulfate and 83.8 percent for the formocresol were not statistically significant (Fuks et al. 1997).

In a retrospective study, Smith et al. (2000) assessed clinical and radiographic data from 242 primary molars pulpotomized with ferric sulfate. Follow-up times ranged from four to 57 months. At four to twelve months, they reported a success rate of 80 percent (n=12). At periods longer than 36 months, a success rate of 74 percent (n=31) was observed.

Ibricevic et al. (2000) compared 35 pulpotomies treated with full-strength formocresol to 35 pulpotomies treated with ferric sulfate. Over a 20 month period, the investigators reported a 100 percent clinical success and 97.2 percent radiographic success in both treatment groups based on panoramic radiograph examinations.

Papagiannoulis (2002) compared ferric sulfate with formocresol in 133 molars in 90 children. The pulpotomies were performed by three pediatric dentists. Most of the molars were restored with
stainless steel crowns and some were restored with composite restorations. Clinical success was 97 percent for formocresol and 90 percent for ferric sulfate. Molars with pulp canal obliteration or small, non-progressive internal resorption were not considered failures. Radiographic success was 78 percent for formocresol and 74 percent for ferric sulfate.

Burnett & Walker (2002), in a retrospective study, reported a 98.2 percent clinical success in teeth treated with formocresol (N = 285) compared with a 93 percent clinical success in teeth treated with ferric sulfate (N = 357). Beyond 36 months, formocresol showed a 75 percent radiographic success rate whereas that of ferric sulfate was 50 percent.

Casas et al. (2003) compared ferric sulfate pulpotomy and primary root canal therapy on cariously exposed vital pulps of primary molars. One-hundred and eighty-two molars received ferric sulfate pulpotomies and 109 molars received primary root canal therapy obturated with zinc oxide and eugenol. At two years 116 molars (73 pulpotomized with ferric sulfate and 43 rootcanal treated) were available for clinical and radiographic evaluation. Ninety-six percent of ferric sulfate treated teeth and 98 percent of root canal treated molars demonstrated no clinical pathology. Ferric sulfate treated molars had 61 percent acceptable radiographic outcomes while root canal treated molars showed 91 percent acceptable radiographic outcomes. Casas et al. (2004) reported on a three-year evaluation of their 2003 study. Twenty-nine molars from a total of 291 (Casas et al. 2003) were available for assessment. The probability of a three-year survival was 0.62 for ferric sulfate treated teeth versus 0.92 for root canal treated molars. In light of this, the authors concluded that ferric sulfate pulpotomy should be avoided on primary teeth that need to be retained for a period of three years or more. Internal resorption and radiographic pathology without clinical signs and symptoms were considered success which artificially inflates the success rates.
Huth et al. (2005), in a randomized control trial evaluated the relative effectiveness of Erbium:Yttrium-Aluminium Garnet (Er:YAG) laser, calcium hydroxide, ferric sulfate (15.5%) and dilute formocresol in 200 primary molars randomly allocated to one of the techniques. At 12 months, their results showed clinical and radiographic success rates of 96 percent for formocresol, 93.4 percent for Er:YAG laser, and 86 percent for calcium hydroxide and ferric sulfate. At 24 months, the success rates were reported as 85.4 percent for formocresol, 77.5 percent for Er:YAG laser, 52.6 percent for calcium hydroxide and 85.7 percent for ferric sulfate. The authors recommended that an increased sample size and power would be required to indicate if there was a statistically significant difference between treatments with Er:YAG laser or ferric sulfate as compared to formocresol.

Markovic et al. (2005) compared formocresol (34 teeth), ferric sulfate (37 teeth) and calcium hydroxide (33 teeth) in one-hundred and four molars in 104 children. The treatment was carried out by three pediatric dentists and outcomes assessed by a separate examiner. At eighteen months the clinical success rate was 90.9 percent for formocresol, 89.2 percent for ferric sulfate and 82.3 percent for calcium hydroxide. The radiographic success rate was 84.8 percent for formocresol, 81.1 percent for ferric sulfate and 76.5 percent for the calcium hydroxide group.

Vargas et al. (2005) investigated the radiographic findings of teeth treated with ferric sulfate (n=35), formocresol (n=41), or a combination of ferric sulfate and formocresol (n=9) in 85 primary molars. Forty-three percent of the teeth treated with ferric sulfate, 56 percent of the teeth treated with formocresol and 55 percent of the teeth treated with a combination of both were normal radiographically. Internal resorption was present in 24 percent of teeth treated with formocresol and 40 percent of the teeth treated with ferric sulfate. The authors regarded presence of calcific metamorphosis, internal resorption, furcation, and external resorption as radiographic failures (Vargas et al. 2005).
In a randomized control trial, Neamatollahi & Tajik (2006) clinically and radiographically evaluated primary second molars treated with formocresol or ferric sulfate as pulpotomy agents. The subjects were monitored at three and twelve months. After three months, none of the patients in the formocresol or ferric sulfate groups showed any sign of clinical failure. Radiographic evaluation demonstrated a 100 percent success for both groups. After one year, the clinical success for both groups remained at 100 percent. Radiographically, the formocresol group had the highest (92.5 percent) success rate whereas the ferric sulfate group displayed success rates of 80.5 percent.

Loh et al. (2004) published a meta-analysis of formocresol and ferric sulfate. A total of 13 studies (three randomized clinical trials and 10 clinical trials) contributed to the meta-analysis. They concluded that formocresol and ferric sulfate produced similar clinical and radiographic success rates. The authors agreed that “there is no reliable evidence supporting the superiority of one particular treatment method for pulpally involved primary molars” (Loh et al. 2004). In their meta-analysis, Loh et al. (2004) included a trial (Ibrecevic & Al-Jame, 2000) that evaluated radiographic observations using panoramic views. Non-uniformity among trials included in the meta-analysis detracts from the reliabilities of the conclusion made by the authors that pulpotomies performed with either formocresol or ferric sulfate result in similar clinical and radiographic success (Loh et al. 2004).

e. Mineral Trioxide Aggregate.

In 1998, mineral trioxide aggregate (MTA) was approved as a therapeutic endodontic material for humans (Schwartz et al. 1999). Torabinejad et al. (1995) demonstrated that MTA had a pH of 10.2 initially, which increased to 12.5 three hours after mixing. Variations in the pH value of host tissues due to pathological conditions at the time of MTA placement could affect its physical and chemical properties (Lee et al. 2004). The cement’s setting time is three to four hours and its
compressive strength is 70 megapascals and this is comparable to that of Intermediate Restorative Material (IRM) (Torabinejad & Chivian, 1999).

i. Clinical and radiographic studies

In primary molars with carious pulp exposures, Eidelman et al. (2001) compared MTA to formocresol as pulp dressing agents. Follow-up evaluation from six to 30 months revealed one failure in a molar treated with formocresol and no failures in MTA treated teeth. Pulp canal obliteration was observed in two out of 15 teeth treated with formocresol (13%) compared to seven out of 17 teeth treated with MTA (41%). The limitations of the study, as noted by the authors, were the small sample size and short follow-up period.

Agamy et al. (2004) compared the clinical and radiographic outcomes of pulpotomies on 60 vital human primary molars using gray MTA, white MTA and formocresol. At 12 months, radiographic evaluation demonstrated that 42 percent of the gray MTA presented with normal pulp anatomy compared with 75 percent treated with white MTA and 90 percent treated with formocresol. Calcific metamorphosis was noted in 58 percent and five percent of teeth treated with gray and white MTA respectively. Clinical evaluation at the twelve month period showed a 100 percent success in the group treated with gray MTA, 80 percent success in the group treated with the white MTA and 90 percent success in the formocresol group. This study found a high percentage of pulp canal obliteration in the MTA group (58 percent with the gray MTA and 5 percent with the white MTA) and no obliteration in the formocresol group. Pulp canal obliteration was categorized as a normal response of the pulp and not regarded as a radiographic failure. Histologically, both types of MTA induced a thick dentine bridge at the amputation sites, whereas formocresol induced thin, poorly calcified dentine.

In a randomized clinical trial comparing MTA with formocresol in 120 primary molars, Farsi et al. (2005) reported that 38 percent of the teeth at 24 months were lost to follow up and 74 molars
were left for evaluation. The MTA clinical and radiographic success rates were each 100 percent. The clinical and radiographic success rate for the formocresol treated teeth were 97 percent and 86 percent, respectively (Farsi et al. 2005).

Holan et al. (2005) compared the effect of MTA as a pulp dressing material following pulpotomy in 33 primary molars with carious pulp exposure and compared them to 29 formocresol treated teeth. Clinical and radiographic follow-up ranged between four and 74 months. The success rate reported with MTA use was 97 percent and with formocresol was 83 percent, at 74 months. Radiographically, normal pulp anatomy was seen in 39 percent of teeth treated with MTA and 31 percent of teeth treated with formocresol. Pulp canal obliteration was present in 58 percent teeth treated with MTA and in 52 percent teeth treated with formocresol. Internal resorption was considered a failure only when it reached the bone. Arrested internal resorption, calcific metamorphosis, and pulp canal obliteration were not considered failures.

Neamatollahi & Tajik (2006) compared clinical and radiographic performance in randomly assigned MTA and formocresol treated groups. The investigators reported a clinical success rate of 82.1 percent for MTA after one year and this was significantly less than the 100 percent observed in the formocresol group. The formocresol group also demonstrated a 92.5 percent radiographic success as opposed to MTA (69.2 percent). The authors concluded that MTA should not be recommended as a pulpotomy medicament in primary teeth.

In a meta-analysis, Peng et al. (2006) compared the clinical and radiographic effects of MTA with formocresol. The authors reported that MTA was superior to formocresol.

Maroto et al. (2007) evaluated the results of MTA pulpotomies carried out on 69 primary molars. The clinical success rate of this study was 100 percent. The authors reported a radiographic success rate of 98.5 percent. Internal resorption was observed 42 months after treatment in one of
the 69 treated molars. At 42 months, 84 percent of the treated teeth illustrated pulp canal stenosis.

Aeinehchi et al. (2007) investigated clinical and radiographic outcomes after six months in 43 MTA- and 57 formocresol treated teeth. Formocresol pulpotomized teeth were restored with amalgam or glass ionomer. MTA treated teeth were restored with amalgam. At six months all treated teeth were asymptomatic. The authors concluded that the MTA treated teeth had fewer cases of root resorption or intra-radicular infection compared with the formocresol pulpotomy treatment group (Aeinehchi et al. 2007).

Inherent disadvantages of MTA include its prolonged final setting time of approximately three hours (Torabinejad et al. 1995), its short working time to initial set, its low compressive strength, its single use packaging, its high cost and lack of evidence to support its use. Furthermore, the rationale for recommending MTA as an alternate medicament is the reported carcinogenicity of formocresol (IARC 2004). However, MTA contains trace amounts of free crystalline silica. Prolonged exposure to free crystalline silica may aggravate certain lung conditions (Merget et al. 2002). It may also cause delayed lung injury including silicosis. The IARC has determined that silica is a known human carcinogen (IARC 2004) (Appendix V). Therefore, the benefit of substituting MTA for formocresol is of questionable value.

f. Laser.

Carbon dioxide (CO₂) lasers have been used in procedures involving soft tissue (Miller & Truhe 1993, Partovi et al. 1987). The CO₂ lasers emit an infra-red beam at a wavelength of 10.6 μm and cause coagulative necrosis of soft tissue through conversion of the laser beam to heat (Elliot et al. 1999).

The erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser has been used for procedures involving hard and soft tissues and for its coagulative properties (Marx 2002). Other
suggested uses include caries removal (Kinoshita et al. 2003) and cavity preparation (Hadley et al. 2000). In an in vitro study, Wang et al. (2002) suggested that the Er,Cr:YSGG laser produces precise cutting and ablation with minimal thermal damage to adjacent tissue. Eversole et al. (1997) suggested the Er,Cr:YSGG laser does not cause a pulpal inflammatory response. The use of lasers in pulpotomies was first published by Shoji et al. (1985).

i. Histologic studies

Conflicting results regarding pulp healing following laser pulpotomy have been published.

Shoji et al. (1985) investigated the effects of a CO₂ laser on amputated dental pulps in dogs. They observed no detectable damage in the radicular pulp in teeth that were treated. Wilder-Smith et al. (1997) and Dang et al. (1998) found that secondary dentine was formed and a regular odontoblast layer was present.

Jukic et al. (1997) compared the effects of CO₂ and Nd:YAG lasers on amputated vital dental pulps in molars and premolars of dogs at 30 and 45 days. Laser irradiation caused carbonization, necrosis, inflammatory infiltration, edema and hemorrhage in pulpal tissues, and no new dentine formation as was found by previous investigators (Wilder-Smith et al. 1997, Dang et al. 1998).

Toomarian et al. (2008) histologically evaluated pulpotomies performed using the Er,Cr:YSGG laser in 48 caries-free primary canines versus pulpotomies using formocresol (mixture of 50% formocresol and 50% formaldehyde) in twelve dogs. The animals were sacrificed at seven and 60 days after the treatment. The teeth and tissue were placed in formalin 10% solution, decalcified in formic acid 10%, dehydrated, placed in methyl salisilate solutions, embedded in paraffin and transverse cross-sections were made. The samples were stained with hematoxylin and eosin (H&E) and viewed under a microscope. The investigators found that samples treated with laser showed favourable histological features. Two months after treatment with formocresol or laser, the apical portion of the dental pulp remained vital. The authors concluded that the Er,Cr:YSGG
laser system was an acceptable alternative for formocresol pulpotomy in pulpotomy of deciduous teeth based on six carious-free primary cuspids.

**ii. Clinical, radiographic and histologic studies**

Elliott *et al.* (1999) investigated clinical and histological responses to the carbon dioxide laser in 15 caries-free primary canines. The outcomes were compared to 15 caries-free primary canines treated with five-minute application of formocresol. The teeth were extracted at either 28 days or 90 days after treatment. Soft tissue and radiographic examination took place prior to extractions. The extracted teeth were placed in 10% formalin solution, decalcified in 5% formic acid, embedded in paraffin, sectioned and stained with hematoxylin and eosin. No teeth demonstrated signs of pathologic mobility, history of pain, or presence of fistula. Radiographically, one formocresol treated cuspid and two laser treated cuspids showed internal root resorption. Histologic sections of formocresol treated teeth at 28 days showed moderate to severe inflammatory cell infiltrate in the coronal portion. In 90 day sections, the formocresol treated teeth showed coronal pulp necrosis and infiltrates of inflammatory cells. The 28 day laser specimens demonstrated edema and inflammatory cell infiltrates below a zone of fixation and necrosis. Ninety day laser specimens showed a moderate inflammatory cell infiltrate. The authors concluded that the carbon dioxide laser for pulpotomy was a favourable alternative to formocresol treatment based on fifteen non-carious teeth after 28 and 90 day treatments.

Odabaş *et al.* (2007) compared the effects of Nd:YAG laser pulpotomy to formocresol pulpotomy in children with a mean age of 7.9 years in 42 canines and molars, followed up clinically and radiographically at one, three, six, nine and 12 months. The investigators found 85.71% clinical success rate for teeth treated with laser and 90.47% for formocresol treated teeth at nine and 12 months following treatment. Radiographically, the laser treated teeth showed 71.42% success rate at 9 and twelve months while formocresol treated teeth showed a 90.47% success rate in the same
observation period. The investigators reported no statistically significant differences between laser and formocresol groups with regard to both clinical and radiographic success rates. In the same investigation, six primary canines and 12 first primary molars were extracted at 7 and sixty days after treatment. The teeth were preserved in 10% buffered formalin, decalcified in 10% formic acid solution, embedded in paraffin blocks and serial sections made. The slides were stained and evaluations performed under light microscopy. The dentin bridge was found to be absent in all samples at both observation periods. In the laser group four samples at 60 days presented with mild inflammation whereas the formocresol treated samples demonstrated moderate inflammation in the coronal pulp in all samples. Based on these findings and sample size the investigators concluded that the Nd:YAG laser may be considered an alternative to formocresol for pulpotomies in primary teeth (Odaş et al. 2007).

iii. Clinical and radiographic studies

Saltzman et al. (2005) utilized a randomized single-blind, split-mouth clinical trial to compare a diode laser pulpotomy with MTA to formocresol pulpotomy. Twenty-six pairs of teeth were followed clinically and radiographically for 15 months. At 15.7 months, seven of the laser treated and three of the formocresol treated teeth were radiographic failures.

Liu (2006) evaluated the effects of Nd:YAG laser pulpotomy (n=68) with formocresol pulpotomy (n=69) on human primary teeth. The teeth were restored with composite resin or stainless steel crown. The follow-up time ranged from six months to 64 months. Thirty-five teeth in the laser group and 55 teeth in the formocresol group presented for evaluation in the one to two year observation period. The investigators found a clinical success rate of 97% and a radiographic success rate of 94% in the laser treated group. The formocresol treated group had an 85% clinical success rate and 78% radiographic success rate. These success rates were based on eleven teeth in
the laser group that presented for evaluation at the four to five year observation period and six teeth in the formocresol group.

In spite of the numerous alternative agents investigated no studies have demonstrated higher clinical, radiographic and histologic success rates over a longer period of time than formocresol. Most of the studies on alternate agents have had relatively small sample size with short follow-up periods as compared to literature on formocresol.
C. EXPECTED OUTCOMES

1. A pulpotomy technique using a one minute application of full strength Buckley’s formocresol and concurrent hemostasis with the medicated cotton pledget will have a better or equal outcome compared to the current gold standard using full strength Buckley’s formocresol pulpotomy technique in human primary teeth.

2. The modified formocresol pulpotomy treatment under investigation will have little or no effect on permanent successors or on exfoliation times of treated primary teeth when compared to contralateral untreated teeth.
D. AIMS AND OBJECTIVES

1. To assess the clinical and radiographic outcomes of a one minute application of full strength Buckley’s formocresol and concurrent hemostasis with the medicated cotton pledget, in pulpotomized human primary teeth.

2. To examine the effect of this formocresol pulpotomy technique on the timing of exfoliation of the treated teeth.

3. To evaluate the effect of this formocresol pulpotomy technique on enamel defects of permanent successors.
E. MATERIALS AND METHODS

This is a retrospective investigation approved by the University of Toronto Health Sciences Research Ethics Board.

1. Sample

The subjects selected for this investigation were treated at a pediatric private practice between 1997 and 2008. Informed consent for treatment and academic use was obtained from guardians prior to treatment (Appendix I).

Inclusion criteria:

1) ASA I or ASA II Patients

2) At least one vital pulpotomy

3) Minimum of 6 months follow-up (teeth with less than six month follow-up were excluded whether they would have been a success or a failure).

The clinical indications for the vital pulpotomy included:

1) Pulp is cariously or traumatically exposed during the operative procedure

2) No history of spontaneous or severe pain

3) Possible history of thermal sensitivity to hot or cold

4) No evidence of draining fistula

5) Tooth is restorable
6) Tooth is not mobile

7) Hemostasis of amputated pulp following placement of dried formocresol pellet after one minute

The radiographic indications for the vital pulpotomy included:

1) Absence of furcal or peri-apical radiolucencies

2) No internal or pathologic external root resorption

3) No more than one-third physiologic root resorption

4) No pathology of the succedaneous follicle

2. Operative Procedure

Radiographic assessments were made by examining bitewing and or periapical radiographs. A standardized bisecting angle technique was performed using bitewing tabs and a Rinn XCP holder for periapical films. The film used over the duration of the study was either E speed (Kodak, Ektaspeed) or F speed (Kodak, Insight) film depending upon availability. The films were processed using an Air Techniques AT 2000 automatic developer operated with Kodak solutions.

Inferior alveolar nerve block or infiltration of local anesthetic using 2% lidocaine 1:100 000 epinephrine was administered. The tooth was then isolated with rubber dam, and all coronal caries was removed leaving the last carious dentin over-lying the pulp. This ensured a clean operating field. Sterilized and disinfected instruments and burs were used for pulp removal. The
roof of the pulp chamber was outlined and removed using a No. 558 straight crosscut fissure bur. The exposed coronal pulp was subsequently amputated with a #6 slow-speed round bur and debris was removed with copious amounts of water. A #4 pledget of cotton wool dampened with full strength Buckley’s formocresol was dried by squeezing on a cotton gauze and then placed in contact with the pulp stumps for one minute. The pledget of cotton wool was removed and if no excessive bleeding was noted, a putty-like paste consisting of one drop eugenol and pure zinc oxide powder was placed in contact with the pulp stumps to a thickness of approximately 2 mm. The preparation for the stainless steel crown was completed. A stainless steel crown was fitted, occlusion checked and cemented in place using glass ionomer cement (KETAC-CEM). The contact points and margins were checked for proper adaptation, excess cement was removed and appropriate occlusion verified.

3. Sample-Size Calculation

The sample size required to achieve 90% power and 0.05 alpha level using a Fisher Exact test was determined to be 124 teeth (Appendix 2).

4. Data Collection

Each patient was designated a numerical code for purposes of maintaining anonymity. Data collected for each numerical entry included date of birth (age), gender, the tooth treated, the treatment date, follow-up time in months, clinical notes regarding the treated tooth at follow-up or recall intervals, and condition of the contralateral tooth.

The condition of the contralateral tooth served as a control for the treated tooth, in evaluating exfoliation times. The contralateral tooth was designated as: absent (A), untouched (U), restored with amalgam or stainless steel crown (R), pulpotomy with stainless steel crown (P), or root canal
treatment (E). In the event that the condition of the tooth changed the final condition of the tooth was recorded.

The presence and the condition or orientation of the succedaneous teeth on both the treated side and the contra-lateral side were noted. Normal (N) was noted for lack of positional abnormality (P) and uniform or expected radio-density of the succedaneous tooth (I).

At recall dates of at least six months, clinical and radiographic observations were noted as per the criteria outlined for success and failure. These observations were noted until the treated and contralateral teeth exfoliated or were extracted. All teeth that demonstrated radiographic failures were followed to exfoliation or extraction. Additional radiographic failures were noted until the teeth were extracted or exfoliated. The dates of extraction and indications for extractions were noted. When the precise date of the two events (extraction or exfoliation) were unavailable the first recall date that indicated absence of the teeth was noted as the endpoint of the treated and contralateral teeth. Clinical and radiographic observations of succedaneous teeth were followed to the last available recall date. The teeth were polished and dried prior to clinical examination. Clinical and radiographic observations were recorded as intact (I), hypoplastic or pitted (Hp), hypomineralized (Hm), positionally altered (P), demonstrating abnormal root morphology (Rm) and or caries (C).

All data was entered into an Excel software format and SAS format for statistical analysis.

5. **Statistical Methods**

Data for continuous variables such as patient age and recall interval were presented as means and standard deviations. Categorical data such as clinical and radiographic observations were summarized as percentages.
The principal investigator was standardized to determine inter-rater reliability by independently reading radiographs of 30 patients (Appendix III). Inter-rater agreement was measured using the Kappa statistic. The observations of the principal investigator were further evaluated to determine the degree of intra-rater reliability. All radiographs were read using a standard view box illuminator. Measures of inter-rater and intra-rater reliability were categorized as poor, slight, fair, moderate, substantial, and almost perfect (Landis and Koch 1977)(Appendix III).

Survival analysis was used to more accurately estimate the probability of success by taking into account the varying observation time for each tooth. The survival probability was calculated as the number of teeth surviving divided by the number of teeth at risk. The teeth that “failed” due to radiographic or clinical failure, “dropped out” or exfoliated were not counted as “at risk”. Teeth that were lost were considered “censored” and not counted in the denominator. Probability of surviving to any point was estimated from cumulative probability of surviving each of the preceding time intervals. This was done using the SAS procedure, Proc Lifereg (SAS release 8.0 SAS Institute, Cary, NC, 1999).

To test the relationship of the failures of the modified technique to different variables such as age, gender, first versus second molars, maxillary versus mandibular molars, a regression analysis (Cox proportional hazards model) was performed. A likelihood ratio chi-square test from the Cox proportional hazards model is reported. The analysis was carried out using Statistical Product and Service Solution (SPSS) software (8.0 Window, SPSS International, Chicago, Ill).

6. Outcome Assessment

The therapy was considered successful when (a) the clinical findings specified below were fulfilled, and (b) the radiographic findings were normal and (c) the tooth exfoliated naturally.
Teeth scored as clinical success if they had:

1) No symptoms of pain

2) No swelling, fistula, or pathologic tooth mobility

Teeth scored as radiographic success if they had:

1) Absence of pathologic internal or external resorption

2) Absence of interradicular or periapical radiolucency

3) Absence of pulp canal obliteration

4) Crypt of the surrounding succedaneous tooth is intact

Clinical and radiographic assessments were made by the principal investigator (ZK). Clinical assessments were made by reviewing chart entries pertaining to each tooth up to the period that the treated tooth exfoliated or until the last date that an entry was made (Appendix IV). Radiographic assessments were made by reviewing radiographs pertaining to the treated tooth. Prior to radiographic assessments, the principal investigator (ZK) was calibrated against two experienced pediatric dentists to obtain inter-rater and intra-rater agreement using Cohen’s Kappa Test. Intra-rater and inter-rater reliability was carried out using randomly selected patients. The data was entered into Microsoft X-cel Spreadsheet (Excel 2007, Microsoft Corporation, Redmond, WA, USA) by the principal investigator.

In order to determine the effect on permanent successors, any abnormality in the surface morphology or color between the treated side and the contralateral untreated side was noted (coronal and radicular morphology, caries, restorations, coronal position, fluorosis) by evaluating chart entries and radiographs. In addition, any areas of hypoplasia (pitting, furrowing) or
hypomineralization (round or oval lesions differentiated from normal enamel as creamy yellow or brown in color on normally contoured enamel surfaces) were noted by evaluating chart entries.

The life-span of formocresol-pulpotomized teeth was determined by recording the time of exfoliation or extraction. In the event that a tooth under question exfoliated between recall dates, the first recall date that indicated the absence of the tooth was noted as terminal survival date.
F. RESULTS

The mean age of the 323 patients (males = 186, females = 137) included in the study was 73.2 months (6.1 years) ± 21.8 months with a range of 24.7 months to 172.6 months. These patients comprised 557 teeth treated (first primary molars = 378, second primary molars = 179, maxillary molars = 262, mandibular molars = 295). A full description of the teeth can be found in Table 2.

Table 2. Distribution of Teeth.

<table>
<thead>
<tr>
<th></th>
<th>Primary First Molar</th>
<th>Primary Second Molar</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxillary Right</td>
<td>87</td>
<td>55</td>
<td>142</td>
</tr>
<tr>
<td>Maxillary Left</td>
<td>87</td>
<td>33</td>
<td>120</td>
</tr>
<tr>
<td>Mandibular Right</td>
<td>101</td>
<td>46</td>
<td>147</td>
</tr>
<tr>
<td>Mandibular Left</td>
<td>103</td>
<td>45</td>
<td>148</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>378</strong></td>
<td><strong>179</strong></td>
<td><strong>557</strong></td>
</tr>
</tbody>
</table>

The mean follow-up period was 44.97 months ± 23.2 months with a range from 6 months to 118.3 months. Observation times were grouped into 12-month increments for purpose of reporting findings. Five-hundred and fifty-seven teeth were available for clinical and radiographic evaluation after one year. Five-hundred and twenty-two teeth were available for evaluation after two years. Four-hundred and thirty-three teeth were available for evaluation after three years. Three-hundred and forty-seven teeth were available for evaluation after four years. Two-hundred and twenty-nine teeth were available for evaluation after five years.
1. Clinical Findings

All teeth were assessed at each follow-up visit as clinically sound according to the previously discussed outcome assessment criteria. There were no significant differences in clinical success rates between first and second molars or between maxillary and mandibular molars (Table 3). As a result of these findings the results for maxillary and mandibular molars were combined and the success rates reported as one entity.

Table 3. Clinical Success Rates for Primary Molars Over Time by Molar Type and Arch.

<table>
<thead>
<tr>
<th>Time</th>
<th>1st molars</th>
<th>2nd molars</th>
<th>Maxillary molars</th>
<th>Mandibular molars</th>
<th>All molars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=34</td>
<td>N=90</td>
<td>N=86</td>
<td>N=118</td>
<td>N=158</td>
</tr>
<tr>
<td>6-12 months</td>
<td>23/24 (96%)</td>
<td>64/65 (99%)</td>
<td>62/62 (100%)</td>
<td>73/74 (99%)</td>
<td>50/51 (98%)</td>
</tr>
<tr>
<td>&gt;12-24 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;24-36 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;36-48 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;48-60 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>p=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p=0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Distribution of Type of Clinical Failures versus Time*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Clinical Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-12 months</td>
</tr>
<tr>
<td>Pain</td>
<td>-</td>
</tr>
<tr>
<td>Swelling</td>
<td>-</td>
</tr>
<tr>
<td>Abscess</td>
<td>1</td>
</tr>
<tr>
<td>Mobility</td>
<td>1</td>
</tr>
</tbody>
</table>

* More than one type of clinical failure may have occurred for each tooth

Four teeth from a total of 557 failed accounting for a clinical success rate of 99.3%. Within the first year one tooth (1/557) presented with an abscess and mobility. In the second follow-up year, one tooth presented with pain, abscess, and mobility. Failures also occurred in the fourth and fifth years of follow-up as tabulated in Table 4.

Figure 2. Clinical Success Rate versus Time.
As illustrated in Figure 2, the clinical success rate remained over 99% at all stages of follow-up. Five-hundred and fifty-seven teeth were available for clinical evaluation at the end of one year. At one year the clinical success rate was 99.3%. The reason for a declining number of teeth with increased follow-up is primarily a result of exfoliation. Two-hundred and twenty-nine teeth were available for evaluation at five years and clinical success at five year follow-up was 99.6%.

Table 5. Estimated Survival Time for Clinical Failure.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Probability of Survival</th>
<th>Standard Error</th>
<th>Time (months)</th>
<th>Probability of Survival</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>0.000</td>
<td>60</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>6</td>
<td>0.998</td>
<td>0.002</td>
<td>66</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>12</td>
<td>0.998</td>
<td>0.002</td>
<td>72</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>18</td>
<td>0.996</td>
<td>0.003</td>
<td>78</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>24</td>
<td>0.996</td>
<td>0.003</td>
<td>84</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>30</td>
<td>0.996</td>
<td>0.003</td>
<td>90</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>36</td>
<td>0.993</td>
<td>0.004</td>
<td>96</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>42</td>
<td>0.993</td>
<td>0.004</td>
<td>102</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>48</td>
<td>0.993</td>
<td>0.004</td>
<td>108</td>
<td>0.987</td>
<td>0.007</td>
</tr>
<tr>
<td>54</td>
<td>0.987</td>
<td>0.007</td>
<td>114</td>
<td>0.987</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Data from all treated molars contributed to survival analysis (Table 5). The cumulative probability that any one tooth survived to one year was 0.998. This probability declined slightly (0.996) at two years and further declined to 0.993 at four years. At fifty-four months the probability of survival was 0.987. The probability of survival at 60 months was 0.987. This probability remained the same throughout the remaining periods of follow-up. The clinical survival analysis described has been plotted as a survival curve in Figure 3.
Figure 3. Clinical Survival Curve.
2. Radiographic Findings

All available radiographs were assessed according to radiographic assessment criteria. Inter-rater reliability was 0.62 (substantial) with 70% agreement and intra-rater reliability was 0.70 (substantial) with 86% agreement (Appendix IV).

Table 6. Radiographic Success Rates for Primary Molars over Time by Molar Type and Arch.

<table>
<thead>
<tr>
<th>Molar Type</th>
<th>Time</th>
<th>0-12 months</th>
<th>12-24 months</th>
<th>24-36 months</th>
<th>36-48 months</th>
<th>48-60 months</th>
<th>&gt;60 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st molars</td>
<td>N=34</td>
<td>20/24 (83%)</td>
<td>55/65 (85%)</td>
<td>51/62 (82%)</td>
<td>69/74 (93%)</td>
<td>49/51 (96%)</td>
<td>100/102 (98%)</td>
<td>344/378 (91%)</td>
</tr>
<tr>
<td>2nd molars</td>
<td>N=90</td>
<td>10/10 (100%)</td>
<td>19/25 (76%)</td>
<td>19/24 (79%)</td>
<td>36/44 (82%)</td>
<td>19/20 (95%)</td>
<td>53/56 (95%)</td>
<td>156/179 (87%)</td>
</tr>
<tr>
<td>Max. molars</td>
<td>N=86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.3</td>
<td>p=0.4</td>
<td>p=0.8</td>
<td>p=0.7</td>
<td>p=1.0</td>
<td>p=0.3</td>
<td>p=0.2</td>
</tr>
<tr>
<td>Mand. molars</td>
<td>N=118</td>
<td>20/22 (91%)</td>
<td>40/41 (98%)</td>
<td>40/42 (95%)</td>
<td>60/61 (98%)</td>
<td>26/26 (100%)</td>
<td>70/70 (100%)</td>
<td>256/262 (98%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.6</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
<td>p=0.3</td>
<td>p=0.1</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>All molars</td>
<td>N=71</td>
<td>88%</td>
<td>82%</td>
<td>81%</td>
<td>89%</td>
<td>96%</td>
<td>97%</td>
<td>90%</td>
</tr>
</tbody>
</table>

The radiographic success rate in the 12 to 24 month period was 98% for maxillary molars and 69% for mandibular molars (p<0.05). In the 24 to 36 month observation period, the radiographic success rates were 95% and 68% respectively (p<0.05). The radiographic success rate for
maxillary molars in the 36 to 48 month observation period was 98% and for mandibular molars in the same time period was 80% (p<0.05).

The types of radiographic failures (n=57/557) that occurred through the follow-up periods are shown in Figure 4. The specific types of radiographic findings presented in Figure 4 are expressed as a percentage of the total number of radiographic findings and are classified according to the first radiographic finding observed. The most frequently observed pulpal responses were internal root resorption (n=27/557) and pulp canal obliteration (n=11/557). Less common radiographic findings included periapical radiolucencies (8/557), inter-radicular radiolucencies (7/557), external root resorption (3/557) and disruption of the follicle of succedaneous tooth (1/557).

**Figure 4.** Distribution of Radiographic Failures.
Some teeth progressed to display more than one kind of radiographic finding. The distribution of radiographic observations which also include multiple findings in single teeth are presented in Table 7.

**Table 7.** Distribution of Type of Radiographic Failures versus Time*.

<table>
<thead>
<tr>
<th>Failure type</th>
<th>Time</th>
<th>0-12 months</th>
<th>12-24 months</th>
<th>24-36 months</th>
<th>36-48 months</th>
<th>48-60 months</th>
<th>&gt;60 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal resorption</td>
<td></td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>External resorption</td>
<td></td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Inter-radicular radiolucency</td>
<td></td>
<td>1</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Periapical radiolucency</td>
<td></td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Pulp canal obliteration</td>
<td></td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Non-intact follicle</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>5</td>
<td>18</td>
<td>24</td>
<td>17</td>
<td>4</td>
<td>6</td>
<td>74</td>
</tr>
</tbody>
</table>

* More than one radiographic failure may have occurred for each tooth over time

Table 7 illustrates the greatest number of failures (24) occurred between 24-36 months following treatment. The most common radiographic finding overall was internal resorption, whether it occurred singularly or in combination with other findings. At 12-24 months the teeth showed a slightly higher number of radiographic failures (18/74) compared to 36-48 month follow-up period (17/74). The least amount of failures occurred in the 48-60 months following treatment.
Figure 5. Distribution of Radiographic Failures (n=57) over Time.

Figure 5 shows the highest incidence of failures occurred in the 24-36 months follow-up period. Slightly lower failures occurred in the 12-24 month and the 36-48 month period. The incidence of failures was lowest in the 48-60 month period.
Radiographic success rate increased after the first year following treatment (89.8%). The number of teeth decreased at successive follow-up periods (Figure 6) primarily due to exfoliation. After the first year the radiographic success rate remained over 90%. At two years 522 teeth available for radiographic evaluation and a success rate of 90% was observed. The highest radiographic success rate (96.5%) was obtained at five years in 229 teeth.
Using all treated teeth a survival analysis (Table 8) and a survival curve (Figure 7) was plotted. Overall, the probability of survival decreased throughout the follow-up period. Thirty-six months following treatment, the probability of survival remained over 0.9. The probability of survival decreased most dramatically between 72 and 96 months, but remained stable at 0.679 thereafter to 114 months of followup.

**Table 8.** Estimated Survival Time for Radiographic Failure.

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Probability of Survival</th>
<th>Standard Error</th>
<th>Time (months)</th>
<th>Probability of Survival</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.000</td>
<td>0.000</td>
<td>60</td>
<td>0.859</td>
<td>0.019</td>
</tr>
<tr>
<td>6</td>
<td>0.993</td>
<td>0.004</td>
<td>66</td>
<td>0.859</td>
<td>0.019</td>
</tr>
<tr>
<td>12</td>
<td>0.979</td>
<td>0.006</td>
<td>72</td>
<td>0.859</td>
<td>0.019</td>
</tr>
<tr>
<td>18</td>
<td>0.960</td>
<td>0.009</td>
<td>78</td>
<td>0.839</td>
<td>0.027</td>
</tr>
<tr>
<td>24</td>
<td>0.942</td>
<td>0.011</td>
<td>84</td>
<td>0.776</td>
<td>0.050</td>
</tr>
<tr>
<td>30</td>
<td>0.922</td>
<td>0.013</td>
<td>90</td>
<td>0.776</td>
<td>0.050</td>
</tr>
<tr>
<td>36</td>
<td>0.904</td>
<td>0.014</td>
<td>96</td>
<td>0.679</td>
<td>0.101</td>
</tr>
<tr>
<td>42</td>
<td>0.880</td>
<td>0.017</td>
<td>102</td>
<td>0.679</td>
<td>0.101</td>
</tr>
<tr>
<td>48</td>
<td>0.876</td>
<td>0.017</td>
<td>108</td>
<td>0.679</td>
<td>0.101</td>
</tr>
<tr>
<td>54</td>
<td>0.866</td>
<td>0.018</td>
<td>114</td>
<td>0.679</td>
<td>0.101</td>
</tr>
</tbody>
</table>
Figure 8 shows that maxillary teeth demonstrate higher probability of survival than mandibular teeth at all periods of follow-up. Maxillary teeth have a survival probability of above 0.9 at all periods of follow-up. In comparison, the survival probabilities of mandibular teeth continue to decrease with time.

**Cox Proportional-Hazards Regression Analysis**

The Cox proportional-hazards regression analysis was used to determine the association between age at treatment, gender, mandibular versus maxillary teeth, first versus second molars and survival of teeth. The analysis showed that age, gender and type of molar had no effect on survival. However, there was a significant effect ($p<0.0001$) of arch type on survival, with mandibular teeth at much higher risk of radiographic failure when compared to maxillary teeth with a likelihood ratio of 6.695 (Table 9).
Table 9. Survival Analysis of Treatment Related Failures.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Likelihood ratio</th>
<th>Confidence limits</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male vs female)</td>
<td>1.22</td>
<td>0.71-2.10</td>
<td>0.4682</td>
</tr>
<tr>
<td>Age</td>
<td>0.994</td>
<td>0.98-1.00</td>
<td>0.4225</td>
</tr>
<tr>
<td>Molar type (first vs second)</td>
<td>0.701</td>
<td>0.41-1.19</td>
<td>0.1907</td>
</tr>
<tr>
<td>Molar type (mand. vs max.)</td>
<td>6.695</td>
<td>2.86-15.63</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Figure 8. Survival Analysis of Mandibular and Maxillary Teeth.
Table 10. Radiographic Success by Tooth Type over Time.

<table>
<thead>
<tr>
<th>Time</th>
<th>0-12 months</th>
<th>12-24 months</th>
<th>24-36 months</th>
<th>36-48 months</th>
<th>48-60 months</th>
<th>&gt;60 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=34</td>
<td>N=90</td>
<td>N=86</td>
<td>N=118</td>
<td>N=71</td>
<td>N=158</td>
<td></td>
</tr>
<tr>
<td>Max. 1st molars</td>
<td>14/16 (88%)</td>
<td>31/32 (97%)</td>
<td>26/27 (96%)</td>
<td>35/36 (97%)</td>
<td>20/20 (100%)</td>
<td>43/43 (100%)</td>
<td>169/174</td>
</tr>
<tr>
<td>Max. 2nd molars</td>
<td>6/6 (100%)</td>
<td>9/9 (100%)</td>
<td>14/15 (93%)</td>
<td>25/25 (100%)</td>
<td>6/6 (100%)</td>
<td>27/27 (100%)</td>
<td>87/88</td>
</tr>
<tr>
<td>Mand. 1st molars</td>
<td>6/8 (75%)</td>
<td>24/33 (73%)</td>
<td>25/35 (71%)</td>
<td>34/38 (89%)</td>
<td>29/31 (94%)</td>
<td>57/59 (97%)</td>
<td>175/204</td>
</tr>
<tr>
<td>Mand. 2nd molars</td>
<td>4/4 (100%)</td>
<td>10/16 (63%)</td>
<td>5/9 (56%)</td>
<td>11/19 (58%)</td>
<td>13/14 (93%)</td>
<td>26/29 (90%)</td>
<td>69/91</td>
</tr>
<tr>
<td>All molars</td>
<td>88%</td>
<td>82%</td>
<td>81%</td>
<td>89%</td>
<td>96%</td>
<td>97%</td>
<td>90%</td>
</tr>
</tbody>
</table>

As illustrated in Table 10 there is a statistically significant difference in radiographic success rates between the mandibular first and second molars. This effect is primarily due to radiographic failures occurring in the mandibular second molars at the 36-48 month interval.
3. Outcomes of Treated Teeth

The mean age at which the teeth were lost was 128.9 months (10.7 years). Figure 9 illustrates the proportion of teeth exfoliated (n=308), extracted (n=39) and teeth that have not yet exfoliated (n=210).

![Graph showing outcomes of treated teeth]

**Figure 9.** Outcomes of Treated Teeth.

The mean length of time between the date of treatment and time at which a tooth was extracted was 32.5 months (2.7 years) ±13.7 months with a range of 7.8 months (0.7 years) to 60.5 months (5 years) after treatment.

4. Exfoliation of Treated Teeth

Of the 308 teeth that exfoliated, 191 teeth were compared to contralateral teeth that were non-pulpotomized. Fifty-five (28.8%) exfoliated earlier than the contralateral teeth, 125 (65.5%)
exfoliated in the same time period as the contralateral teeth and 11 (5.8%) treated teeth exfoliated later than the non-pulpotomized contralateral teeth (Figure 10).

**Figure 10.** Exfoliation of Treated Teeth versus Contra-lateral Non-pulpotomized Teeth.

Early exfoliation occurred at a mean of 7.4 months ±4.7 months (p<0.001) prior to exfoliation of the contralateral tooth.
5. Condition of Succedaneous Teeth

The condition of the 191 succedaneous teeth that erupted was evaluated based on the previously cited criteria. Five premolars (2.6%) that succeeded pulpotomized primary molars presented with surface enamel defects and or positional alterations. There was no statistically significant difference in the incidence of defects on the treated versus the control side (Table 11).

Table 11. Number of Teeth with Defects in Test versus Control Group

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Defects</td>
<td>185</td>
<td>188</td>
</tr>
<tr>
<td>Defects</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

P=0.4 (> than 0.05)
G. DISCUSSION

The purpose of this retrospective investigation was two-fold. Firstly, to compare the success rates of a modified one-minute formocresol pulpotomy technique with the standard five minute formocresol pulpotomy technique reported in the literature and secondly, to examine the effects of the modified one minute technique on succedaneous teeth.

The five minute application of formocresol has been arbitrarily assigned (García-Godoy, 1981). In a recent survey, Dunston & Coll (2008) noted more diplomates (22%) used application times less than the traditional five minute placement compared to 2005 directors (8%) and 1997 directors (0%). Few investigations have evaluated the outcomes of a decreased application time of formocresol to the pulps of primary teeth. Venham (1967) used a 15 second application time and García-Godoy et al.(1982) used one and three minute application times and evaluated their histologic effects. In a clinical investigation, Hyland (1969) assessed a two minute formocresol application in 34 teeth.

In this study a retrospective chart review was used to evaluate the outcomes of a one minute formocresol pulpotomy technique. It was pre-determined that a sample size of 124 teeth was required to achieve a 90% power and a 0.05 alpha level. The sample size in the present investigation exceeded the pre-determined sample size and included 557 teeth from 323 healthy patients requiring at least one pulpotomy with a minimum of six month follow-up period.

The teeth in the present investigation were treated under rubber dam isolation. The caries were removed and a one-minute application of full strength formocresol was performed with concurrent hemostasis obtained with the medicated cotton pledget. Prefabricated stainless steel crowns were used to restore the teeth in this investigation so as to reduce leakage and pulpotomy
failure due to restoration failure. Stainless steel crown restorations have consistently been reported to be more durable than other restorations in the primary dentition (Dawson *et al.* 1981, Holland *et al.* 1986, Messer & Levering 1988, Roberts & Sheriff 1990). The modified technique in this investigation was carried out on all the teeth by a single operator. Using a single operator offers the advantage of a strictly consistent and reproducible technique. A potential disadvantage is that the outcomes may be related to a superior operator rather than a superior technique.

One of the limitations of the present investigation includes the retrospective design relying on the accuracy of written records and that the study sample cannot be manipulated to include a control group. The Evidence-Based Medicine Levels of Evidence (Levels of Evidence and Grades of Recommendation, 2001) indicate that the highest level of evidence (level 1a) is represented by a systematic review of randomized controlled trials (RCTs). The second highest level of evidence (level 1b) is a RCT with narrow confidence intervals, that is, RCTs with large sample sizes. Systematic reviews restricted to RCTs include a small number of RCTs and the main conclusion from these reviews is a call for high-quality RCTs to be conducted. RCTs rank highest due to the fact that they are not susceptible to biases. However, RCTs have disadvantages of non-compliance and high drop-out rates (Needleman *et al.* 2005). In fact, RCTs with more than 20% dropout are considered “low quality” RCTs (Levels of Evidence and Grades of Recommendation, 2001). These disadvantages result in investigations with small sample sizes and short follow-up times. The present study design allows for a larger sample size to be studied and therefore small differences to be detected. The present investigation far exceeded the predetermined sample size at a 90% power and 0.05 % alpha level. Using one operator in the investigation allows for consistency in the technique being evaluated and allows for treatment outcomes to be examined over long periods of time. In pediatric dentistry vital pulpotomies are carried out on a routine basis and long-term outcome evaluation becomes necessary.
In assessing the effect of the modified technique on succedaneous teeth the present investigation compared the treated tooth to contralateral non-pulpotomized tooth. Using a paired-tooth design eliminated possible confounders regarding the types of teeth involved (that is, maxillary versus mandibular, first molar versus second molar) and inter-patient confounders.

1. Clinical Outcomes

In the present investigation, the criteria for clinical success included absence of pain, swelling, fistula or pathologic tooth mobility. Clinical assessment of success at six months was 97% in this investigation and no significant differences between first and second molars and maxillary or mandibular teeth were evident (p>0.05). The overall clinical success rate was 99.3% as measured at six month intervals over the course of the study. Previous investigations that have studied decreased application times and clinical outcomes for shorter periods of time have demonstrated similar results. Hyland (1969) reported a similar clinical outcome (97%) after six months using full strength formocresol over pulp stumps for two minutes. The clinical success rate in this study at six months is higher than that obtained by Aktören (1998) who reported a 90% success rate while assessing a one-minute full-strength formocresol application. A two year follow up of the same technique by Aktören & Gençay (2000) reported an 88% clinical success rate while the present investigation, with a larger sample size showed a 99% success rate over the same time period.

The clinical success rate of the present study is comparable to previous investigations that use a five minute formocresol application technique (Thompson et al. 2001, Verco & Allen 1984, Sweet 1953 as reported by Emmerson et al. (1959), Berger 1965, Magnusson 1978).

Modifications to the formocresol pulpotomy technique have included variations in the concentration of formocresol, inclusion of formocresol in the sub-base cement and decreased application times. All of these techniques in previous literature reports include obtaining
hemostasis of the radicular pulp stumps with cotton pellets prior to placement of the medicament. In this investigation and an investigation carried out by Thompson et al. (2001) the use of non-medicated cotton pellets prior to the use of formocresol was omitted. Thompson et al.(2001), in a retrospective investigation, used the same medicated cotton pledget technique to obtain hemostasis and obtained an overall clinical success rate of 98%. The present study obtained a similar success rate (99.3%) with a one minute application time and the clinical success rate at all stages of follow-up was greater than 99%.

2. Radiographic Outcomes

The criteria for radiographic success in the current investigation included the absence of pathologic internal or external resorption, inter-radicular or periapical radiolucency, absence of pulp canal obliteration and the presence of an intact crypt of the succedaneous tooth. The radiographic success rate of 89.8% found in the present investigation is comparable to the radiographic success rates that have been previously reported. Earlier investigations have reported radiographic success rates ranging from 85% to 98% (Sweet 1953 as reported by Emmerson et al.1959, Redig 1968, Berger 1965, Morawa et al. 1975, Beaver et al. 1966, Verco & Allen 1984, Thompson et al. 2001).

The overall radiographic success rate in the present study was lower than that reported by Berger (1965) who observed a 97% success rate using substantially fewer teeth with a shorter follow-up period than the present study. The radiographic criteria in assessing success and failure employed by Berger (1965), Waterhouse et al. (2000) were similar to those used in the present investigation. Verco & Allen (1984) also reported a slightly higher (92%) radiographic success rate in 1006 primary teeth in children with a mean age of 7.0 years. However, the investigators did not state the length of the follow-up period over which this success rate was obtained.
The present investigation demonstrated higher radiographic success rates than previous investigations that employed a one minute formocresol application technique. At six months Aktören (1998) demonstrated a lower (85%) radiographic success rate while the present investigation demonstrated an 88% radiographic success rate in the same time period. In the 12-24 month follow-up period the present investigation showed a slightly higher (82%) radiographic success rate compared to Aktören & Gençay (2000) who reported an 80% success rate. The present study followed the treatment outcomes for longer periods with larger sample sizes compared to previous investigations and still demonstrated more favourable success rates.

In the present study, the rates of radiographic success varied between teeth. Although the probability of radiographic success for first molars was equal to the probability of radiographic success for second molars, there was a statistically significant difference (p<0.05) between maxillary and mandibular molars in the 12-24 month observation period. Similar differences were noted in the 24-36 month and the 36-48 month follow-up periods. Overall, the radiographic success rate in the present investigation was 98% for maxillary molars and 83% for mandibular molars and this difference was statistically significant (p<0.05). Similar differences in success rates over time between maxillary and mandibular molars were reported by Strange et al. (2001). They reported a statistically significant difference (p<0.001) between the radiographic success rate in maxillary (89%) and mandibular molars (74%). The investigators (Strange et al. 2001) attributed this statistical difference to difficulty in interpreting maxillary radiographs since maxillary sinuses obscured radiographic changes. This explanation may also hold true for the present investigation. In fact, Magnusson (1977) used only mandibular primary molars in evaluating the presence or absence of internal root resorption and predicated this on the fact that radiographic changes are more readily evident in mandibular rather than in maxillary molars.

Age, gender, first or second molars had no significant effect on success in the current study. However, the present investigation demonstrated that mandibular teeth were 6.7 times (p<0.0001)
more likely to fail when compared to maxillary teeth. This increased risk of failure in mandibular molars may be attributed to a similar pattern of failure observed in restorations. Restorations are more likely to fail in mandibular teeth than maxillary teeth. McDaniel et al. (2000) reported lower restoration survival rates in the mandibular arch. Also, despite using rubber dam isolation technique while restoring teeth, mandibular teeth are more likely to be subject to moisture contamination as saliva pools and collects in the posterior lingual region. Tooth morphology and the fact that the number of mandibular teeth in the study was larger than maxillary teeth may have contributed to this difference. Mandibular teeth are subject to more forces from various vectors. Increased masticatory forces and stresses may lead to a more pronounced inflammatory response and contribute to a higher failure rate in mandibular teeth. Conversely, Thompson et al. (2001) reported no statistical difference in radiographic outcomes between maxillary and mandibular molars.

It was observed in the present study that the frequency of a normal appearing pulp chamber and root canal configuration remained constant over time. This finding is similar to those of Thompson et al. (2001) and Burnett & Walker (2002) but differs from many other published reports that demonstrate decreasing radiographic success rates over time (Redig 1968, Berger 1965, Morawa et al. 1975, Rølling & Thylstrup 1975, Beaver et al. 1966, Fuks et al. 1997, Smith et al. 2000). Rølling & Thylstrup (1975) reported a three month radiographic success rate of 91% which decreased to 70% at 36 months. Fei et al. (1991) reported a success rate of 85% at three months that decreased to 71% at 12 months in a study comprising 27 human molars. Farooq et al. (2000) reported that formocresol radiographic success rates decreased with time and that the largest number of failures occurred within the first two years. In his investigation, however, Redig (1968) reported that most radiographic failures occurred in the first six months. The present investigation demonstrated that the greatest number of failures occurred between 24 to 36
months and similar to the observations noted by Thompson et al. (2001), an increase in radiographic failures with time was not illustrated.

In the present study 39 treated teeth were extracted due to clinical and or radiographic failure. The average duration of time the teeth were extracted due to failure following treatment was 2.7 years. This was longer than that reported by Verco & Allen (1984) where teeth were extracted at 1.3 years after treatment. The present investigation demonstrated that with the modified technique, teeth were retained for a longer period prior to failure.

The two most common radiographic failures in the current study were internal root resorption and obliteration of pulp canals. These findings are consistent with other investigations (Fuks et al. 1997, Smith et al. 2000, Holan et al. 2005).

i. Internal Root Resorption.

Internal root resorption accounted for the majority (27/557=4.9%) of the radiographic failures in this investigation. This is similar to other investigations (Hicks et al. 1986, Thompson et al. 2001, Verco & Allen 1984, Doyle et al. 1962, Papagiannoulis 2002) that showed lower rates of internal resorption. Hicks et al. (1986) and Thompson et al. (2001) found internal resorption in 10.9% of primary molars treated. This is lower than reported in Magnusson’s (1977) investigation where internal root resorption accounted for 37% of the radiographic findings and Vargas et al. (2005) where it was 24%.

Histological investigations have revealed that the occurrence of internal root resorption after pulpal treatment has been attributed to inflammation of the residual pulp (Magnusson 1970b, 1971, 1978). Internal resorption is preceded by chronic pulpal inflammation, a disappearance of odontoblasts and predentin and pulpal invasion by macrophage-like resorbing cells (Pindborg 1970, Wedenberg & Lindskoj, 1985). Internal root resorption is considered a failure as it is an indication of chronic inflammation of the residual pulp (Kalaskar & Damle, 2004 and Magnusson
et al. 1980). Resorption of dentin occurs as a result of a chronic inflammatory process in the pulp tissue combined with loss of the protective layer of odontoblasts and predentin (Trope 1994, Tronstad 1988). The present study defined success of pulpotomy treatment as the absence of clinical symptoms or the absence of radiographic changes at follow-up appointments. An overwhelming majority of published literature considers internal resorption to be an indication of failure (Schröder et al. 1994, Papagiannoulis 2002, Kalaskar & Damle 2004, Magnusson 1980, Markovic et al. 2005, Law 1956, Waterhouse et al. 2000, Magnusson 1978). As a result, internal resorption was considered a sign of treatment failure in the present investigation.

Some investigators do not consider radiographic evidence of internal resorption to be a sign of failure (Smith et al. 2000, Holan et al. 2005, Maroto et al. 2005, Fuks et al. 1997). The etiology of internal resorption is thought to result from chronic pulpitis (Law 1956, Waterhouse et al. 2000) and that for internal resorption to be progressive there must be presence of necrotic tissue (Tronstad 1988). As a result it is postulated that the presence of radiographic internal resorption indicates an unsuccessful treatment outcome. In spite of categorizing internal resorption as a radiographic failure in the present investigation, the teeth were not treated immediately if they were asymptomatic and did not present with additional clinical or radiographic failures at later recall dates. In the present study 41% (11/27) of cases with internal resorption progressed to osseous changes or clinical signs and symptoms and were extracted. The very low rate of internal resorption in the current investigation is likely due to the one minute application of formocresol. This short duration of application would result in less inflammation in the radicular pulp and therefore less internal resorption.

Smith et al. (2000) attributed the presence of internal root resorption with pulpal response to the procedure and or the medicament. As a result, Smith et al. (2000) proposed that internal root resorption was not clinically significant since the teeth remained asymptomatic and they did not regard them as as radiographic failures over a mean follow-up period of 19 months. They based
this on the fact that none of the teeth in their sample presenting with internal resorption progressed to inter-radicular bone destruction, pathologic external root resorption or periapical bone destruction. This is in contrast to the current investigation where 41% of the teeth that presented with internal root resorption progressed to external root resorption, inter-radicular radiolucency or periapical radiolucency over an average period of 17.5 months. A similar progression was reported by Hicks et al. (1986) who reported that 2.4% of all pulpotomized teeth or 22% of teeth that presented with internal resorption showed internal resorption ‘that compromised the integrity of the root structure’. Maroto et al. (2007), Eidelman et al. (2001) and the present investigation demonstrated that even if internal resorption does not need treatment other than follow-up observations it cannot be considered a successful outcome because it is a sign of pulpal inflammation and may progress to radiographic failures as was the case in the present investigation.

**ii. Pulp Canal Obliteration.**

In the present study it was found that pulp canal obliteration (also known as calcific metamorphosis) accounted for 19.3% (11/57) of the radiographic failures or 2% (11/557) of all treated teeth. This is lower than the 22% calcific metamorphosis reported by Vargas et al. (2005). Rølling & Lambjerg-Hansen (1978) noted that ‘root canal calcification’ [sic] was a typical histological response following formocresol pulpotomy. Holan et al. (2005) observed that 52% of the teeth treated with formocresol showed pulp canal obliteration. Thompson et al. (2001) reported that calcific metamorphosis (34%) was the most common radiographic observation in their investigation and that it was a normal finding. Hicks et al. (1986) found 62.2% of 164 primary molars with calcific metamorphosis and also reported this finding to be normal and attributed it to the prolonged length of time following treatment (43 months). In contrast, the current investigation with a mean follow-up time of 45 months demonstrated a lower percentage (2%) of pulp canal obliteration. Willard (1976) using a four-minute application of formocresol
found postoperative calcification in 24 of the 30 formocresol treated teeth over a three year period. Willard (1976) explained that the calcification was a result of odontoblastic activity following treatment and that the pulp retained some degree of vitality. Fuks et al. (1997) found that 10.8% (n=4) of teeth treated with dilute formocresol and 18.2% (n=10) treated with ferric sulfate showed pulp canal obliteration.

The present investigation categorized pulp canal obliteration (PCO) as a radiographic failure since it demonstrated a deviation from normal appearing pulp. PCO is the result of extensive activity of odontoblast-like cells (Willard 1976). Reactionary dentine is formed when teeth experience an injury as an attempted repair process within the pulpal tissue. Waterhouse et al. (2000) postulated that after an initial attempt by the pulp tissue to ‘wall off’ the bacterial and inflammatory insult, the protective processes fail and result in clinical failures. This has been histologically verified by Waterhouse et al. (2000) who showed that reactionary dentin apposition occurred in all teeth that failed clinically or radiographically. In the present investigation, 1.75% of the treated teeth that showed pulp canal obliteration progressed to inter-radicular radiolucency. Advanced pulp canal obliteration also prevents the possibility of root canal treatment in the event that the tooth needs to be re-treated.

Previous investigations (Verco & Allen, 1984, Thompson et al. 2001) that report a high radiographic success rate and those that report comparable radiographic success rates (Aktören 1998 and Aktören & Gençay, 2000) use radiographic criteria that do not include pulp canal obliteration as a criterion for failure. This is in contrast to the current investigation and previous investigations (Berger et al. 1965, Vargas et al. 2005) which have included increased radiopacity as one of the radiographic criteria for evaluating radiographic failure following pulpotomies. In the present study if pulp canal obliteration had been included as a normal response the radiographic success rates would have been higher (92%) but pulp canal obliteration was designated as a deviation from the normal radiographic appearance of the pulp. The inclusion of
pulp canal obliteration as a failure enables comparisons to be made with previously described literature that includes pulp canal obliteration as one of the criteriae for evaluating radiographic outcomes of formocresol pulpotomies.

3. Effect on Exfoliation Times.

A survey of the literature indicates there are no previous studies that evaluate the effect of a decreased formocresol application time on succedaneous teeth and exfoliation times. Previous investigations have assessed the effects of a five minute formocresol application time (Vargas et al. 2005, Roberts 1996, van Amerongen et al. 1986, Thompson et al. 2001). The present investigation has the largest sample size compared with other published reports (Hicks et al. 1964, Vargas et al. 2005, Roberts 1996, van Amerongen et al. 1986, Fuks & Bimstein, 1981) that evaluate the effects of formocresol pulpotomy.

Even though exfoliation accounted for the majority of teeth treated (308/557), only those teeth with non-pulpotomized counterparts were selected to evaluate the effects on succedaneous teeth (190/308). In doing so the treated teeth are compared to non-pulpotomized contralateral teeth in the same patient. The majority of the teeth in the current investigation exfoliated in the same time period as the contra-lateral non-pulpotomized teeth (controls). There was, however, a group of treated teeth that exfoliated earlier (28.8%) and this effect was found to be statistically significant (p<0.001). The pulpotomized teeth that exfoliated earlier than the contralateral teeth exfoliated at a mean age of 10.7 years that is, 7.4 months earlier than the control teeth. This result was comparable to that of Hicks et al. (1986) where exfoliation of pulpotomized molars was found to occur approximately 6 months prior to exfoliation of their antimeres. However, Hicks et al. (1986) also found that a higher proportion exfoliated earlier than their contralateral counterparts and this is different from the findings of the present investigation. Despite being statistically significant, the clinical relevance of the finding in the current investigation remains uncertain as
the teeth exfoliated within the normal range of physiologic exfoliation defined by Lunt & Law (1974). Although the early exfoliation time was statistically significant as compared to the contralateral tooth, the premolar erupted soon after the exfoliation of the pulpotomized tooth. Hicks et al. (1986) used a non-medicated cotton pellet followed by a paste of zinc oxide and equal parts of eugenol and formocresol. The authors postulated that the high rate of earlier exfoliation was due to the leaching out of formocresol from the zoe paste into the surrounding periodontal tissue resulting in an accelerated root resorption due to the presence of a chronic inflammatory reaction. These findings are higher than those reported by Vargas et al. (2005) where 10% of teeth treated with formcresol exfoliated prematurely. However, the pulpotomy technique employed in the present investigation eliminated the incorporation of formocresol from the zoe paste and the application time of formocresol was shorter. The early exfoliation seen in the present investigation may be a result of progressive inflammation that had extended to the radicular pulp. In contrast, other investigations (van Amerongen et al. 1986, Roberts 1996, Thompson et al. 2001) using full strength formocresol for five minutes reported no significant difference in life-spans.

A small portion (5.8%) of the teeth in the present investigation exfoliated later than the contralateral non-pulpotomized teeth. This portion was lower than reported by Hicks et al. (1986).

In the present investigation exfoliation times were estimated based on the first recall date that the treated teeth and or the contralateral teeth were noted as missing. This is similar to the methodology employed by Roberts (1996) and Thompson et al. (2001) in that the date of exfoliation was that of the next recall visit when loss of the tooth was recorded. This method of estimating exfoliation times will systematically produce a bias towards late exfoliation times. In doing so, this would also produce a similar bias towards the exfoliation times of controls. Therefore this still represents a valid method in evaluating the effect of the treatment on
exfoliation times relative to the contralateral controls. In contrast, van Amerongen et al. (1986) assessed the effect on exfoliation by asking patients to report the time of exfoliation but this method can be subject to recall bias particularly if more than one tooth is involved.

4. Effect on Succedaneous Teeth.

The relationship between treated primary molars and presence of defects on succedaneous teeth in the present investigation was explored using the same sample used to evaluate the effect on exfoliation times (n=190 pairs). A minority (2.6%) of the succedaneous teeth in the experimental group showed defects. The control group comprising of succedaneous teeth from non-pulpotomized primary molars also demonstrated defects (1.0%). This observation was not found to be statistically significant (p>0.05). The present study showed that there was no relationship between opacities and hypoplasia of the enamel on permanent teeth and formocresol pulpotomy of its primary predecessor. This is consistent with the findings of other investigations (Rolling & Poulsen 1978, Mulder et al. 1987). Similar to the findings in the present investigation, Messer et al. (1980) found that there were no alterations in the coronal or radicular morphology of succedaneous teeth, although 40% of the bicuspsids that succeeded treated primary molars had positional alterations. These authors proposed that the developing tooth bud alters its position in order to avoid the pathologic processes associated with the primary tooth. Messer et al. (1980) did not collect data regarding the extent and distribution of crowding in the dentition which could have been a potentially significant confounding variable with regards to positional alterations reported in the succedaneous teeth.

The findings of the present study are in contrast to those of Pruhs et al. (1977) where the investigators concluded that there was a definite relationship between formocresol pulpotomies in primary teeth and enamel defects on their permanent successors. However, the authors (Pruhs et al. 1977) did not describe how many of the primary molars were diagnosed as vital or non-vital
when treated. This becomes relevant since it has been shown that non-vital teeth are more susceptible to causing disturbances in the formation of succedaneous teeth. Alternatively, this could be due to differences in the examination procedure. The findings from Pruh et al. (1977) were based on 25 pairs of teeth. Their (Pruh et al. 1977) procedure involved teeth being dried for a minimum of 1.5 minutes prior to clinical examination. This method of drying the tooth results in the dessication of enamel surface that may result in an opaque discoloration. The opaque discoloration could thus have been noted as an abnormality. In the present investigation a larger sample size is assessed and prior to clinical examination the teeth were prophied and dried for only a brief period. This method of examining the teeth may be less prone to create these pseudodefects. Messer et al. (1980) air-dried and examined each bicuspid using a mirror and explorer. The authors did not mention how long the teeth were dried prior to clinical examination.

It was also demonstrated in the present study that the one minute application of formocresol has no effect on the the surface or root morphology of the succedaneous teeth. This is in agreement with the histological evaluation of pulp tissue where formocresol was applied for less than five minutes (Venham 1967, Garcia-Godoy et al. 1982) and producing the least inflammatory and tissue response. The apical portion of the root canal was noted to be free of inflammation and comprises vital tissue in investigations that utilize the five minute formocresol application (Rølling et al. 1976, Rølling & Lambjerg-Hansen, 1978) and therefore not likely to cause disturbance in the surface or disturbance in the morphology of the root.

The present study was limited in the fact that the estimation of exfoliation times was based on the absence of the tooth at a future recall date. The exfoliation date could have been more accurate if the patient was asked to note the exact date that each tooth exfoliated. In addition, the comparison of the treated tooth to the contralateral side of exfoliation times could have been accompanied with radiographic root resorption rates at recall appointments.
Concern over the use of formocresol as a pulpotomy agent has been questioned. Formaldehyde, a component of formocresol has been classified as a ‘known’ human carcinogen (IARC 2004). This classification was not based on a dose-response analysis. Daily exposure to formaldehyde from food, water and air has been documented (Owen *et al*. 1990). Normal cellular metabolism in humans produces endogenous levels of formaldehyde which is incorporated into biosynthetic pathways or converted to carbon dioxide and water and thereafter, excreted. Exogenous formaldehyde is rapidly metabolized (Heck & Casanova 1999, Casanova *et al*. 1988). Recently, Kahl *et al*. (2008) demonstrated that formaldehyde was not detected in any blood sample of children who underwent primary pulpotomies using full strength formocresol. The authors concluded that it is unlikely that formocresol when used in doses employed in the vital pulpotomy technique poses any risk to children. To date, no correlation has been demonstrated between formocresol pulpotomies and cancer in humans.

5. Future Direction

In the current investigation, the modified technique was carried out by a single operator. Further studies in the form of prospective multi-centre clinical trial to assess the outcomes of this technique by different operators should be evaluated. Additional clinical studies that evaluate the modified technique against the standard five minute formocresol technique in a split-mouth design should be studied. The effect on exfoliation times and on succedaneous teeth using these study designs should be assessed.
Summary

In summary, the present investigation has the largest reported sample size in studying the outcomes of modified formocresol pulpotomy technique and also appears to be the only one that evaluates the outcomes of a decreased application time of full strength formocresol coupled with concurrent hemostasis using the medicated cotton pellet technique in vital pulpotomy of primary molar teeth. Studies that have comparatively large sample sizes (Mulder et al. 1987, Rølling & Poulsen, 1978) also report similar conclusions.

Conclusions

1. The modified formocresol pulpotomy technique demonstrated a high clinical (99%) success rate over a follow-up period exceeding five years.

2. The modified formocresol pulpotomy technique demonstrated a high radiographic (89.8%) success rate over a follow-up period exceeding five years.

3. The two most common types of radiographic failure were internal root resorption (4.9%) and pulp canal obliteration (2%).

4. Although the modified technique resulted in slightly earlier exfoliation times this was not found to be clinically significant.

5. The modified technique showed no detrimental effect on succedaneous teeth.

6. The modified technique represents an effective and efficient alternative to the standard five-minute formocresol pulpotomy technique based on a large sample size and a long follow-up period.
APPENDIX I: INFORMED CONSENT

FORM A

PATIENT CONSENT FORM: FOR COLLECTION, USE AND DISCLOSURE OF PERSONAL INFORMATION

Privacy of your personal information is an important part of our office providing you with quality dental care. We understand the importance of protecting your personal information. We are committed to collecting, using and disclosing your personal information responsibly. We also try to be as open and transparent as possible about the way we handle your personal information. It is important to us to provide this service to our patients.

In this office, Dr. acts as the Privacy Information Officer. All staff members who come in contact with your personal information are aware of the sensitive nature of the information that you have disclosed to us. They are all trained in the appropriate uses and protection of your information. Do not hesitate to discuss our policies with me or any member of our office staff.

Attached to this consent form, we have outlined what our office is doing to ensure that:
- only necessary information is collected about you;
- we only share your information with your consent;
- storage, retention and destruction of your personal information complies with existing legislation, and privacy protection protocols;
- our privacy protocols comply with privacy legislation, standards of our regulatory body, the Royal College of Dental Surgeons of Ontario, and the law.

Please be assured that every staff person in our office is committed to ensuring that you receive the best quality dental care.

How Our Office Collects, Uses and Discloses Patients’ Personal Information

Our office understands the importance of protecting your personal information. To help you understand how we are doing that, we have outlined here how our office is using and disclosing your information.

This office will collect, use and disclose information about you for the following purposes:
- to deliver safe and efficient patient care
- to identify and to ensure continuous high quality service
to advise you of treatment options

to enable us to contact you and maintain communication with you

to offer and provide treatment, care and services in relationship to the oral and maxillofacial complex and dental care generally

to communicate with other treating health-care providers, including specialists and general dentists who are the referring dentists and/or peripheral dentists

to allow us to efficiently follow-up for treatment care, billing and collect unpaid accounts

for teaching and demonstrating purposes on an anonymous basis

to complete and submit dental claims for third party adjudication and payment

to deliver your charts and records to the dentist's insurance carrier to enable the insurance company to assess liability and quantify damages, if any

to comply with legal and regulatory requirements, including the delivery of patients' charts and records to the Royal College of Dental Surgeons of Ontario in a timely fashion, when required, according to the provisions of the Regulated Health Professions Act

to comply with agreements/undertakings entered into voluntarily by the member with the Royal College of Dental Surgeons of Ontario, including the delivery and/or review of patients' charts and records to the College in a timely fashion for regulatory and monitoring purposes

to prepare materials for the Health Professions Appeal and Review Board (HPARB)

to permit potential purchasers, practice brokers or advisors to evaluate the dental practice

to invoice for goods and services

to process bank card and credit card payments

to assist this office to comply with all regulatory requirements

to comply generally with the law

By signing the consent section of this Patient Consent Form, you have agreed that you have given your informed consent to the collection, use and/or disclosure of your personal information for the purposes that are listed. If a new purpose arises for the use and/or disclosure of your personal information, we will seek your approval in advance.

Your information may be accessed by regulatory authorities under the terms of the Regulated Health Professions Act (RHPA) for the purposes of the Royal College of Dental Surgeons of Ontario fulfilling its mandate under the RHPA, and for the defence of a legal issue.

Our office will not under any conditions supply your insurer with your confidential medical history. In the event this kind of a request is made, we will forward the information directly to you for review, and for your specific consent.

When unusual requests are received, we will contact you for permission to release such information. We may also advise you if such a release is inappropriate.

You may withdraw your consent for use or disclosure of your personal information, and we will explain the ramifications of that decision, and the process.
Patient Consent

For Collection Use and Disclosure of Personal Information

I have reviewed the above information that explains how your office will use my personal information, and the steps your office is taking to protect my information.

I know that your office has a Privacy Code, and I can ask to see the Code at any time.

I agree that Dr [patient’s name] can collect, use and disclose personal information about [patient’s name] as set out above in the information about the office’s privacy policies.

__________________________________________
signature

__________________________________________
print name

__________________________________________
date

__________________________________________
signature of witness
APPENDIX II: SAMPLE SIZE CALCULATION

1. The primary comparison between treatment groups is a proportion (or a percentage).

2. The research question is “Does the modified technique work better than the existing one?”

3. With the standard five minute application of full-strength Buckley’s formocresol technique, the success rate is 70% (Rolling et al. 1975, Vij et al. 2004) - 99% (Roberts et al. 1996). The average of these success rates: \((70 + 99)/2 = 84.5\%\).

4. It is anticipated that the modified technique will increase the success rate to 100%.

5. What sample size would be required in order to detect such an effect with 90% power at a 5% level of statistical significance?

i. Calculating \(\Delta\) the standardized difference.

   • In the case of two proportions, \(p_1\) and \(p_2\), \(p_1 = 1.0\) (100%), \(p_2 = 0.845\) (84.5%)
   
   the standardized difference \(\Delta = (p_1 - p_2)/\sqrt{p \times (1-p)}\) \(\text{where p} = (p_1 + p_2)/2 = 0.9225\)

   • \(\Delta = 1 - 0.845/\sqrt{0.9225 \times (1-0.9225 \times (1-0.9225)} = 0.58\)

ii. Using the values from from the table for a significance level of 5%, \(z_{(1-\alpha/2)}= 1.96\), and a power of 90%, \(z_{(1-\beta)} = 1.2816\) (Machin et al. 1997).

iii. Using the formula \(m= 2 \times [z_{(1-\alpha/2)}+ z_{(1-\beta)}]^2 / \Delta^2\)

iv. \(2 \times [1.96 + 1.2816]^2/058^2 = 62\) teeth in each group

v. \(62 \times 2 = 124\) teeth

The predetermined sample size calculation to obtain a 90% power at a 5% level of statistical significance is 124 teeth.
Adapted from: ‘Medline Statistics Online Help: Sample size & power for clinical trials’

APPENDIX III: INTER- AND INTRA-OPERATOR RELIABILITY

Radiographs available for evaluation were mounted in plastic frame holders. Each radiograph was identified by a code number and raters were blinded to the subject’s name, age and gender. All radiographs were viewed using a viewing box.

Evaluation Form for Review of Radiographs

Name of Rater: _________________________

Radiograph #: __________________________

Tooth #: ___________

A. Tooth 74

Identify any of the following radiographic changes:

Pathologic Internal Root Resorption: □ Present ■ Absent

Pathologic External Root Resorption: □ Present ■ Absent

Inter-radicular Radiolucency: ■ Present □ Absent
Area:

Periapical Radiolucency: □ Present □ Absent

Area:

Invasion of crypt of surrounding succedaneous follicle: □ Present □ Absent

Area:

None of the above:
B. Tooth 75

Identify any of the following radiographic changes:

Pathologic Internal Root Resorption: □ Present ■ Absent
Area:
Pathologic External Root Resorption: ■Present □ Absent
Area:
Inter-radicular Radiolucency: ■Present □ Absent
Area:
Periapical Radiolucency: ■Present □ Absent
Area:
Invasion of crypt of surrounding succedaneous follicle: ■Present □ Absent
Area:
None of the above:

Comments:
B. Tooth 54

**Identify any of the following radiographic changes:**

Pathologic Internal Root Resorption: □ Present ■ Absent

Area:

Pathologic External Root Resorption: □ Present ■ Absent

Area:

Inter-radicular Radiolucency: □ Present ■ Absent

Area:

Periapical Radiolucency: ■ Present □ Absent

Area:

Invasion of crypt of surrounding succedaneous follicle: □ Present ■ Absent

Area:

None of the above:

**Comments:**
### Data Entry Template (1):

<table>
<thead>
<tr>
<th>Patient</th>
<th>Vulg=528</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOB (m-d-y)</td>
<td>4/2/1989</td>
</tr>
<tr>
<td>Gender</td>
<td>m</td>
</tr>
<tr>
<td>Tooth treated</td>
<td>74</td>
</tr>
<tr>
<td>Initial r/g (m-d-y)</td>
<td>11/13/1995</td>
</tr>
<tr>
<td>Initial r/g (notes)</td>
<td>2/3 root</td>
</tr>
<tr>
<td>Treatment date (m-d-y)</td>
<td>11/13/1995</td>
</tr>
<tr>
<td>Age at Treatment (days)</td>
<td>2410.00</td>
</tr>
<tr>
<td>Age at Treatment (months)</td>
<td>79.23</td>
</tr>
<tr>
<td>Age at Treatment (years)</td>
<td>6.60</td>
</tr>
<tr>
<td>Contra-lateral tooth</td>
<td>84</td>
</tr>
<tr>
<td>Condition (contral.)</td>
<td>R(SSC)</td>
</tr>
<tr>
<td>Source</td>
<td>C</td>
</tr>
<tr>
<td>Succedaneous(tx. Side)</td>
<td>y</td>
</tr>
<tr>
<td>Condition(succ. Tx.side)</td>
<td>N</td>
</tr>
<tr>
<td>Condition(succ.contra.)</td>
<td>N</td>
</tr>
<tr>
<td>Evid. of presence/condition of succed. tx. side</td>
<td>R</td>
</tr>
</tbody>
</table>

Absent (A)/Untouched (U), Restored (R), Pulpotomy (P), Root Canal treatment (E)
<table>
<thead>
<tr>
<th>Recall</th>
<th>Date</th>
<th>Clinical Obs. (tx. tooth)</th>
<th>R/g Obs.</th>
<th>Clinical Stat.(contr-l)</th>
<th>R/g Obs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st recall</td>
<td>6/11/1996</td>
<td>NF</td>
<td>NF: 2/3 root</td>
<td>NF</td>
<td>NF: 2/3 root</td>
</tr>
<tr>
<td>2nd recall</td>
<td>1/10/1997</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>3rd recall</td>
<td>7/10/1997</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
<tr>
<td>4th recall</td>
<td>1/12/1998</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
<td>NF</td>
</tr>
</tbody>
</table>
### 6th recall (m-d-y) 1/28/1999
<table>
<thead>
<tr>
<th>Clinical Obs. (tx. tooth)</th>
<th>NF</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
<tr>
<td>Clinical Stat.(contr-l)</td>
<td>NF</td>
</tr>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
</tbody>
</table>

### 7th recall 7/25/1999
| Clinical Obs. (tx. tooth) | 34 P |
| R/g Obs.                  | NF  |
| Clinical Stat.(contr-l)   | NF  |
| R/g Obs.                  | NF: <1/2 root |

Intact (I), Hypoplastic or pitted (P), Hypomineralized (Hm), Positionally altered (P), Abnormal root (Rm), Caries (C).

### 8th recall 1/28/2000
| Clinical Obs. (tx. tooth) | 34 P |
| R/g Obs.                  | NF  |
| Clinical Stat.(contr-l)   | NF  |
| R/g Obs.                  | NF: crown |

### 9th recall 7/22/2000
| Clinical Obs. (tx. tooth) | 34 P |
| R/g Obs.                  | NF  |
| Clinical Stat.(contr-l)   | 44 P |
| R/g Obs.                  | NF  |

### 10th recall 12/19/2000
<p>| Clinical Obs. (tx. tooth) | 34 P |
| R/g Obs.                  | NF  |</p>
<table>
<thead>
<tr>
<th>10th recall</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Obs. (tx. tooth)</td>
<td>34 P</td>
</tr>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
<tr>
<td>Clinical Stat. (contr-l)</td>
<td>44 P</td>
</tr>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>11th recall</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Obs. (tx. tooth)</td>
<td>34 P</td>
</tr>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
<tr>
<td>Clinical Stat. (contr-l)</td>
<td>44 P</td>
</tr>
<tr>
<td>R/g Obs.</td>
<td>NF</td>
</tr>
<tr>
<td><strong>Additional recalls</strong></td>
<td><strong>9/28/2001</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>First date assoc. with absence of treated tooth</strong></td>
<td><strong>7/22/1999</strong></td>
</tr>
<tr>
<td><strong>Source of this data</strong></td>
<td><strong>C</strong></td>
</tr>
<tr>
<td></td>
<td><strong>84 NYE</strong></td>
</tr>
<tr>
<td>First date assoc. with absence of contralateral tooth</td>
<td>6/22/2000</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Source of this data</td>
<td>C (44)</td>
</tr>
<tr>
<td>DIFFERENCE IN EXFOLIATION (months)</td>
<td>330 (months) 27.50</td>
</tr>
<tr>
<td>Notes:</td>
<td>Extra Notes:</td>
</tr>
<tr>
<td>NYE: not yet exfoliated</td>
<td></td>
</tr>
<tr>
<td>Observations of Succ. Teeth</td>
<td>N</td>
</tr>
<tr>
<td>Last date with this observation</td>
<td>5/2/2007</td>
</tr>
<tr>
<td>Source of information</td>
<td>C, R</td>
</tr>
<tr>
<td>Extraction (tooth #)</td>
<td></td>
</tr>
<tr>
<td>Source of information</td>
<td></td>
</tr>
<tr>
<td>Date (m-d-y)</td>
<td>Indication</td>
</tr>
<tr>
<td>Additional recalls</td>
<td>9/22/2004</td>
</tr>
<tr>
<td></td>
<td>10/3/2005</td>
</tr>
<tr>
<td></td>
<td>4/7/2006</td>
</tr>
<tr>
<td></td>
<td>10/26/2006</td>
</tr>
</tbody>
</table>
## APPENDIX V: SUBSTANCES THAT HAVE BEEN EVALUATED BY IARC AS HUMAN CARCINOGENS


Group 1: Carcinogenic to humans (105) Agents and groups of agents

<table>
<thead>
<tr>
<th>Substance or mixture</th>
<th>IARC Monograph</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Aminobiphenyl</td>
<td>1</td>
</tr>
<tr>
<td>Arsenic</td>
<td>14</td>
</tr>
<tr>
<td>Asbestos</td>
<td>14</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>26</td>
</tr>
<tr>
<td>Benzene</td>
<td>29</td>
</tr>
<tr>
<td>Benzidine</td>
<td>29</td>
</tr>
<tr>
<td>Benzopyrene</td>
<td>32</td>
</tr>
<tr>
<td>Beryllium and beryllium compounds</td>
<td>58</td>
</tr>
<tr>
<td>N, N-Bis(2-chloroethyl)-ether and chloromethyl ether</td>
<td>4</td>
</tr>
<tr>
<td>Bis(chloromethyl) ether and chloromethyl methyl ether</td>
<td>4</td>
</tr>
<tr>
<td>1, 3-Butadiene</td>
<td>71</td>
</tr>
<tr>
<td>1,4 – Butanediol dimethanesulfonate</td>
<td>4</td>
</tr>
<tr>
<td>Cadmium and cadmium compounds</td>
<td>58</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>26</td>
</tr>
<tr>
<td>1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea</td>
<td>4</td>
</tr>
<tr>
<td>Chromium</td>
<td>49</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>50</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>26</td>
</tr>
<tr>
<td>Diethylstilboestrol</td>
<td>21</td>
</tr>
<tr>
<td>Dyes metabolized to benzidine</td>
<td>99</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>70</td>
</tr>
<tr>
<td>Erionite</td>
<td>42</td>
</tr>
<tr>
<td>Estrogen-progestogen menopausal therapy</td>
<td>72</td>
</tr>
<tr>
<td>Estrogens, nonsteroidal</td>
<td>7</td>
</tr>
<tr>
<td>Estrogens, steroidal</td>
<td>7</td>
</tr>
<tr>
<td>Estrogen therapy, postmenopausal</td>
<td>72</td>
</tr>
<tr>
<td>Ethanol in alcoholic beverages</td>
<td>96</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>60</td>
</tr>
<tr>
<td>Etoposide in combination with cisplatin and bleomycin</td>
<td>76</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>88</td>
</tr>
<tr>
<td>Gallium arsenide</td>
<td>86</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> (infection with)</td>
<td>61</td>
</tr>
<tr>
<td>Hepatitis B virus (chronic infection with)</td>
<td>59</td>
</tr>
<tr>
<td>Hepatitis C virus (chronic infection with)</td>
<td>59</td>
</tr>
</tbody>
</table>
Appendix V (2): Substances that have been evaluated by IARC as human carcinogens.

Group 1: Carcinogenic to humans (105)

Agents and groups of agents

<table>
<thead>
<tr>
<th>Substance or mixture</th>
<th>IARC Monograph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immunodeficiency virus type 1 (infection with)</td>
<td>67</td>
</tr>
<tr>
<td>Human papillomavirus types 16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59 and 66</td>
<td>64</td>
</tr>
<tr>
<td>Human T-cell lymphotropic virus type I</td>
<td>67</td>
</tr>
<tr>
<td>Melphalan</td>
<td>9</td>
</tr>
<tr>
<td>8-Methoxypsoralen plus ultraviolet A radiation</td>
<td>24</td>
</tr>
<tr>
<td>Methylenebis</td>
<td>57</td>
</tr>
<tr>
<td>MOPP and other combined chemotherapy including alkylating agents</td>
<td>7</td>
</tr>
<tr>
<td>Mustard gas</td>
<td>9</td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>4, 99</td>
</tr>
<tr>
<td>Neutrons</td>
<td>75</td>
</tr>
<tr>
<td>Nickel compounds</td>
<td>49</td>
</tr>
<tr>
<td>N-Nitrosonornicotine (NNN)</td>
<td>37, 89</td>
</tr>
<tr>
<td><em>Opisthorchis viverrini</em> (infection with)</td>
<td>61</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>7</td>
</tr>
<tr>
<td>Phosphorous-32, as phosphate</td>
<td>78</td>
</tr>
<tr>
<td>Plutonium-239 and its decay products</td>
<td>78</td>
</tr>
<tr>
<td>Radioiodines, short lived isotopes, including iodine-131, from atomic reactor accidents and nuclear weapons detonation (exposure during childhood)</td>
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