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# Glutaraldehyde as a Cold Sterilizing Agent for Endodontic Instruments

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GLUTARALDEHYDE AS A COLD STERILIZING AGENT  
FOR ENDODONTIC INSTRUMENTS

by

Gary M. Ritchie D.D.S.

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

Gary Max Ritchie was born August 24, 1937, the son of Delma Coreen and Max Grady Ritchie, in the city of Dallas, state of Texas.

His grade school and high school education were obtained in Fort Worth, Texas; graduating from Polytechnical High School in 1955. Pre-dental studies were performed at Arlington State College and Texas Wesleyan College.

Following four years at Baylor University School of Dentistry, the degree of Doctor of Dental Surgery was conferred upon him in June of 1962.

His professional and military careers began simultaneously upon entering the U. S. Army, and being sent to Ft. Lewis, Wash. There he performed general dentistry for two years, then was released from active duty.

After two years of private dental practice in Hurst, Texas, he reentered the Army, serving at Ft. Polk, La.; with the 1st Inf. Division in Vietnam; and two years at Brooke General Hospital, San Antonio, Texas, where he performed the specialty of endodontics.

In 1969 he was selected by the U. S. Army and Loyola University to attend the school of dentistry to do graduate study in the field of endodontics.

Dr. Ritchie now holds the rank of Major in the army, and will soon take his new position as Chief of Endodontics, Dental Activities Medical Department, Fort Sill, Oklahoma.

## DEDICATION

This paper is dedicated to my parents, Dell and Max Ritchie, whose love, support and encouragement has enabled me to seek greater knowledge, professional skill, and personal growth. To them goes my love and my gratitude.

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To:

Dr. John V. Madonia --- who strives diligently to teach scientific methods to those lacking in scientificness.

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Dr. Dale C. Birdsell --- who understands, and so gives aid.

Dr. Marshall H. Smulson --- a dedicated teacher, dentist and man, my thanks I give to you.

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## Chapter I

### INTRODUCTION

In the specialty field of endodontics, as in all phases of dentistry and other health professions, the control and prevention of bacterial infection is of major concern. The control of bacterial growth within the root canal of the patient's tooth and finally sterilization of the root canal space are goals of endodontic therapy. Cross contamination between endodontic patients from contaminated instruments must also be considered and prevented.

The endodontic reamers and files, which are used to remove contaminated debris from the root canals of infected and non-infected teeth, by the very nature of their construction, present special problems for sterilization. Corrosion of the stainless steel of the instruments can occur with some methods of sterilization, thereby weakening the instrument. The cutting edges of the reamers and files should not be dulled by the sterilization process. Intricate flutes in the instruments tend to hold tooth debris, possibly infected and bloody,



creating a problem with sterilization. Another problem is produced when small square pieces of rubber band are used on the instruments as tooth length indicators, these pieces can become contaminated from the tooth debris and must be sterilized, and without their deterioration.

The use of chemicals as sterilizing agents has been disappointing in the past, with none being found that met the required standards of sterilization. Development of alkalinized glutaraldehyde brought about the possibility of a true chemical sterilizing agent. Prior studies using glutaraldehyde on medical instruments have indicated that alkaline 2% glutaraldehyde was an adequate sterilizing medium for those particular types of instruments.

Due to the apparent inadequacies of previous chemical agents, their use for sterilizing endodontic instruments has been discouraged. The availability of glutaraldehyde may change this prevalent attitude. The purpose of this study was to test the efficiency of 2% alkalinized glutaraldehyde as a sterilizing agent for endodontic files and reamers, with consideration being given to the special problems involved in the sterilization of these intricate instruments.

## Chapter II

### REVIEW OF THE LITERATURE

#### A. METHODS OF STERILIZING ENDODONTIC INSTRUMENTS

Various methods have been used for sterilizing endodontic instruments, some proved ineffective, others were effective; some methods were simple to use, others were complicated or required special equipment. A review of the literature revealed descriptions of these sterilization techniques.

In 1930, Hall<sup>1</sup> described the following alcohol-flame technique. The endodontic instruments were kept in 95% alcohol, then flamed to sterilize them. Grossman and Appleton,<sup>2</sup> in 1940, tested the sterility of instruments which had been in alcohol and then either flamed or not. They averaged negative cultures 92% of the time using alcohol and flaming and 71% negative cultures when alcohol alone was used. Oliet,<sup>3</sup> in evaluating methods of sterilizing endodontic instruments, found alcohol only partially effective when used for sterilization. Accepted Dental Therapeutics 1969-70<sup>4</sup> lists both the alcohol and/or flaming techniques as ineffective for sterilization.

Baillie<sup>5</sup> used hot liquid petrolatum at 250°C with 2 minutes exposure for instrument sterilization. White mineral oil at 160°C<sup>6</sup> was found to kill spores within 20 minutes, by Knighton. Accepted Dental Therapeutics<sup>4</sup> 1969-70 accepts hot oils or silicones at 160°C for 1 hour as an acceptable sterilization technique.

Using hot air sterilizers and molten metal sterilizers was described in 1950 by Stewart and Williams.<sup>7</sup> Oliet<sup>3</sup> evaluated both the hot air and molten metal sterilizers. He found the hot air sterilizer only partially effective, but the molten metal sterilizer was completely effective. Molten metal is recognized as an effective method by Accepted Dental Therapeutics<sup>4</sup> 1969-70.

Formaldehyde has been used for sterilization: gaseous formaldehyde as used by Schug-Kosters, Wiegandt<sup>8</sup> and Feistl; paraformaldehyde as described by Kantorowicz;<sup>9</sup> and formaldehyde solution USP is acceptable to Accepted Dental Therapeutics<sup>4</sup> 1969-70.

Other chemicals (benzalkonium chloride, metaphen<sup>3</sup> and phenol) have been evaluated (Oliet) and none have proven to be truly sterilizing agents.

For sterilization of endodontic reamers and files at the chairside, the most effective method is a heated container holding sand, salt or glass beads. Hot sand was used by Lavin<sup>10</sup> because it held heat well. Hot salt

was used and described by Grossman;<sup>11</sup> he found that by using table salt heated to 425-475 F. the instruments were sterile after 5 seconds exposure. Nicholls<sup>12</sup> used hot salt, but used an alcohol lamp as the heat source instead of electric heaters as the other researchers did. Glass beads in a heated container was evaluated by Spring<sup>13</sup> and found effective with 10 seconds exposure. Oliet<sup>3</sup> states that the bead sterilizer, with extra fine beads, at 425 F with a 10 second exposure is a completely effective sterilizing technique. The hot salt and hot bead sterilizers are recognized as effective techniques by the Accepted Dental Therapeutics 1969-70.<sup>4</sup>

Dry heat sterilization has been proven an effective sterilizing method. Kantorowicz<sup>9</sup> used dry heat at 320 F with 1 hour exposure. Curson<sup>14</sup> and Brunel et al<sup>15</sup> also described and used dry heat sterilization. Dry heat is also approved as a sterilization method by Accepted Dental Therapeutics 1969-70.<sup>4</sup>

Autoclaving (hot steam under pressure) though frequently used, is described very little in relation to endodontic instruments. Curson<sup>14</sup> and the Accepted Dental Therapeutics 1969-70<sup>4</sup> describe autoclaving as an effective technique. According to Schug-Kosters et al<sup>8</sup> the hot steam fails to reach all the intricate parts of the endodontic instruments.

Mechanically cleaning the endodontic reamers and files before sterilization is an important step in the sterilizing procedure. Hall<sup>1</sup> used brushes to clean the instruments; Grossman<sup>16</sup> used a cotton roll and Curson<sup>14</sup> used a polyurethane foam sponge containing a 1% chlorhexidene solution.

## B. BACTERIAL FLORA OF ROOT CANALS

Since this paper is a microbiological study of alkalinized glutaraldehyde as a sterilizing agent for endodontic instruments, it is necessary to determine what organisms are involved. A review of the literature shows that studies of the bacterial flora of infected root canals have been done. It is primarily this flora which contaminates the endodontic instruments.

The strains of organisms found in infected root canals are many and variable. Yeastlike organisms were isolated in 18 of 100 root canals examined by Bartels and Buchbinder.<sup>17</sup> Lynch and Stuteville,<sup>18</sup> in 100 cases, found that 63% of the organisms they isolated were aerobic and 37% anaerobic. They isolated 216 strains of bacteria, the most prevalent being Staphylococcus aureus, occurring in 42.6% of the cases and alpha hemolytic streptococci in 25.9% of all cases. Leavitt, Naidorf and Shugaevsky<sup>19</sup> demonstrated anaerobes

in 33% of the infected root canals that they studied; their results showed hemolytic streptococci in 35% of the canals, Staphylococcus albus 54%, Lactobacillus acidophilus 30%, Bacillus subtilis 30% and Staphylococcus aureus in 13% of the canals.

In studying infected root canals of unexposed teeth, Brown and Rudolph<sup>20</sup> found streptococci in 32 of 70 cases, Corynebacteria in 28 cases, micrococci in 19 cases and treponema in 14 cases. The researchers did not isolate Micrococcus albus or aureus, nor Lactobacillus or monilia. Brown and Rudolph surmise that these organisms come from the oral cavity after a pulp exposure occurs.

After culturing 709 root canals and obtaining 184<sup>21</sup> positive cultures, Shay reported that 164 were caused by a single organism and 20 were from mixed organisms. He found gamma hemolytic streptococci to be the most frequently occurring organism (82 cases or 44.5%), staphylococci the next most prevalent (35 cases or 19%), beta hemolytic streptococci next (13 cases or 7.1%), then Bacillus subtilis (12 cases or 6.5%).

In examining 46 teeth that had been traumatized and were non-vital, MacDonald, Hare and Wood<sup>22</sup> obtained positive cultures from 38 of the 46 teeth. They isolated aerobic streptococci, micrococci and anaerobic cocci.

Crawford and Shankle<sup>23</sup> studied the relative predomi-

nance of microorganisms in the root canals of closed teeth and open teeth. The bacterial flora in closed teeth was predominantly alpha hemolytic streptococci, anaerobic streptococci, lactobacilli, gamma hemolytic streptococci and actinomyces. In the root canals of open teeth the predominant organisms were anaerobic streptococci, enterobacteria, gamma hemolytic streptococci, alpha hemolytic streptococci, veillonella and diphtheroid bacilli.

Melville and Birch<sup>24</sup> found the bacterial flora of periapical lesions usually identical with the infected root canal flora. The organisms isolated in the root canals and periapical areas were alpha hemolytic streptococci, staphylococci, fusobacterium, gamma hemolytic streptococci, diphtheroids, veillonella and lactobacilli.

Goldberg<sup>25</sup> cultured periapical lesions in 93 patients, using blood agar, he obtained pure cultures of staphylococci in 53 cases, mixed alpha hemolytic streptococci and staphylococci in 15 cases and 10 cases of pure streptococci.

Shindell<sup>26</sup> excised periapical lesions and cultured them on HeLa and amnion tissues to try and obtain viral growth, no viruses were found. Shindell concluded that periapical lesions are not associated with a virus.

Knighton<sup>27</sup> studied the relation of viral hepatitis to dentistry, he concludes that the transmission of both

Virus A and B from patient to patient is by parental injection only.

The incidence of bacteremia in endodontic manipulation was evaluated by Bender, Seltzer and Yermish.<sup>28</sup> They obtained positive blood cultures immediately after overmanipulation beyond the tooth root apex, in 25% of the cases, but obtained no positive blood cultures when the instrumentation was confined within the tooth root canal. All blood cultures taken after 10 minutes were negative.

Also of medical interest is that Kolmer<sup>29</sup> states that no case of subacute bacterial endocarditis has ever been reported following endodontic treatment.

### C. CULTURE MEDIA

To ascertain what organisms were found in root canal infections, culture media is necessary. Various culture media have been tested and used for growing root canal organisms. Morse and Yates,<sup>30</sup> in 1938, advocated using glass tubes for holding the culture media and plugging the tubes with sterile cotton, then incubating the inoculated tubes for 48 to 72 hours. They used 1% dextrose infusion broth for culturing, but recommended brain broth or glucose ascites broth for research procedures.

Five different media were tested by Shay,<sup>21</sup> (1)



trypticase dextrose, (2) brain heart infusion broth (BHIB), (3) serum dextrose, (4) brain agar and (5) Brewer's thioglycollate. Best results were obtained using trypticase dextrose or BHIB (90.6% of the positives occurred in each of these broths). Both media are recommended for clinical use. Shay also concluded from his study that strict anaerobic conditions were not necessary for the culturing of anaerobic organisms from root canals.

<sup>24</sup>  
Melville and Birch used glucose broth and Robertson's cooked meat media in their research. MacDonald,<sup>22</sup> Hare and Wood used penicillinase dextrose broth and thioglycollate broth in their microbiological studies.

<sup>31</sup>  
Ah Moo compared trypticase soy broth with 0.1% agar to thioglycollate and BHIB (brain heart infusion broth). He found trypticase soy with 0.1% agar to be the most sensitive, and thioglycollate the next most sensitive.

<sup>32</sup>  
In 1955, Leavitt, Naidorf and Shugaevsky investigated having one culture medium for both aerobes and anaerobes in the same culture tube. They discovered that the addition of agar to a broth would provide varying degrees of anaerobiosis. Agar prevents oxygen absorbed at the surface from diffusion through to the bottom of the tube. Growth in this type of media shows

pure aerobic growth in upper 1/3 of the media in the tube and pure anaerobic growth in the bottom 1/3 of the media. Recommendations were given for using this type of media: (1) use screw type caps on the tubes, cotton plugs allow oxygenation, (2) culture tubes are not to be shaken, agitation enhances oxygenation, (3) tubes 125 X 16 mm are the most favorable size. Dextrose broth, thioglycollate broth and trypticase soy broth, all with and without 0.1% agar were tested for growing an aerobic organism (Staphylococcus aureus) and an anaerobic organism (Clostridium histolyticum). The most sensitive media for growing both organisms was trypticase soy broth with 0.1% agar.

Leavitt, Naidorf and Shugaevsky,<sup>19</sup> in 1958, tested 4 culture media and rated them by their ability to show growth with small amounts of inoculum. TSA (trypticase soy broth with 0.1% agar) required the largest inoculum, thioglycollate was next, thioglycollate with 0.1% agar was more sensitive, but thioglycollate combined with trypticase soy broth and 0.1% agar showed growth with the greatest dilution of inoculum.

In 1966, Grossman<sup>33</sup> tested 4 media: (1) trypticase soy broth with 0.1% agar, (2) thioglycollate broth, (3) cooked meat, (4) BHIB with 0.1% agar, by using serial dilutions of Streptococcus salivarius, Streptococcus faecalis, Streptococcus mitis and Staphylococcus aureus.

He found BHIB with 0.1% agar and trypticase soy broth with 0.1% agar to be the most sensitive media tested. But the overall study showed no true superiority of one media over the others.

A recent study by Munoz-Noya<sup>34</sup> determined the growth of one aerobe and one anaerobe, Streptococcus faecalis and Actinomyces israeli, in five media: (1) trypticase soy broth with 0.1% agar, (2) BHIB, (3) BHIB with 0.1% agar, (4) fluid thioglycollate broth and (5) glucose ascites broth with 0.1% agar. All of the test media supported growth of Streptococcus faecalis at 10<sup>-11</sup> dilution of a 48 hour culture. Actinomyces israeli, under aerobic environment, grew with the greatest dilution in fluid thioglycollate; in the anaerobic environment it showed equally good growth in fluid thioglycollate and glucose ascites broth with 0.1% agar.

#### D. ALKALINIZED GLUTARALDEHYDE

The qualities that a chemical agent must possess to be an ideal sterilizing agent are: (1) the ability to kill spores rapidly as well as other microorganisms, (2) activity in the presence of organic matter, (3) activity in relatively low concentrations, (4) and a lack of corrosive and toxic properties. Many chemicals have been tested and were found lacking in one or more of these qualities.

Formaldehyde solutions have been found to be sporicidal, but they also possess the properties of irritation and vapor toxicity. Rahn<sup>35</sup> found formaldehyde an effective sterilizing agent in great dilution and to have rapid action.

In 1963, Stonehill, Krop and Borick<sup>36</sup> studied various aldehydes and developed a buffered dialdehyde solution\* which was capable of sterilization and demonstrated low toxicity. The active chemical is glutaraldehyde (a saturated dialdehyde  $\text{CHO}-\text{CH}_2-\text{CH}_2-\text{CHO}$ ) used as an aqueous solution buffered with 0.3% sodium bicarbonate. A micro-biological evaluation of 2% activated glutaraldehyde was performed in this study. Tests against non-spore-forming bacteria using the Millipore Filter Method were conducted, Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Diplococcus pneumoniae, Serratia marcescens, Streptococcus pyogenes, Klebsilla pneumoniae and Micrococcus lysodeikticus were used as test organisms. All of the test organisms were in contact with the glutaraldehyde solution for less than 2 minutes then transferred directly to the culture media and incubated at 37° C for 48 hours, results showed that all the organisms were killed in less than 2 minutes exposure to the glutaraldehyde. Then the 2%

(R)  
\*CIDEX -- aqueous activated dialdehyde solution.

activated glutaraldehyde was tested against spore-forming bacteria, it destroyed Bacillus megaterium in less than 2 hours. Nine parts of 2% activated glutaraldehyde were combined with one part human blood and tested against Bacillus globigii, Bacillus subtilis, Clostridium tetani and Clostridium welchii using the AOAC<sup>37</sup> penicylinder method: all the spore-formers were killed in less than 3 hours. Also using the AOAC procedure, glutaraldehyde killed Trichophyton interdigitale, a fungus, in less than 5 minutes exposure.

In Stonehill's (et al)<sup>36</sup> study the buffered 2% glutaraldehyde solution was shown to be slightly to moderately toxic to animals. It was slightly irritating to the skin and severely irritating to the eye.

The glutaraldehyde was found to be non-corrosive to metal instruments, including dental instruments, after 2 weeks immersion in the solution.

Since the development of buffered glutaraldehyde, several laboratories have published independent evaluations. Klein and De Forest<sup>38</sup> used 2% alkalinized glutaraldehyde against 7 different viruses: Polio type I, Coxsackie B1, ECHO 6, Adeno type 2, Herpes Simplex, Vaccinia and Influenza. They grew Influenza virus in the allantoic cavity of the chick embryo; all others were grown in cultures of HeLa cells, except Herpes

Simplex which was grown in freshly trypsinized rabbit kidney. Viral inactivation was determined by mixing 0.9 ml of glutaraldehyde with 0.1 ml of undiluted virus; after times of 1, 3, 5, and 10 minutes the virus titer was determined and compared with the control viral suspensions. Alkalinized glutaraldehyde inactivated all of the test viruses in 1 minute, thus proving to be a powerful virucidal agent. Sabel, Hillman and Mc Dade<sup>39</sup> found that glutaraldehyde inactivated viral agents in tissue specimens.

In 1964, Borick, Dondershire and Chandler<sup>40</sup> did extensive testing of alkalinized glutaraldehyde. Their study used many organisms: nine types of non-spore-forming bacteria, four sporeforming bacteria, one fungal strain and eight types of viruses. The bactericidal action of glutaraldehyde was determined by using contaminated penicylinders immersed in 10 ml of glutaraldehyde; after predetermined exposures they were transferred to eugenbroth for incubation. All the non-spore-formers were killed in less than 1 minute. The AOAC<sup>37</sup> tests of the spore-forming organisms found all the spores were killed in less than 3 hours. Fungicidal action was assessed by exposing conidial suspensions of the organism to the glutaraldehyde followed by culturing; the fungi were killed in less than 0.5 minutes.

All of the viruses tested were inactivated in less than 10 minutes.

An evaluation of glutaraldehyde was made by Rittenbury and Hench.<sup>41</sup> In their experiments hemostats and cystoscopes were contaminated with mixtures of either Escherichia coli, Staphylococcus aureus, Pseudomonas or Proteus mirabilis in whole blood, then immersed in 2% alkalized glutaraldehyde. The instruments were sterilized in less than 5 minutes even through the dried blood.

A comparison between ethyl alcohol, benzalkonium chloride, phenolic derivatives and alkaline glutaraldehyde was performed by Snyder and Chestle.<sup>42</sup> Spore suspensions, vegetative bacteria and a bacterial virus were tested in this study. Spores were exposed and separated from the disinfectant using sections of catheter tubes which were coated with bacteria and blood, also penicylinders coated with vegetative organisms and whole blood were exposed to the disinfectant, rinsed and placed in test tubes of BHIB. The penicylinder technique was also used in testing the effects of the disinfectants on the bacterial virus. Toxicity tests were performed with all the disinfectants using chick embryos, cell cultures and cells. Alkaline glutaraldehyde was found to be a more effective sterilizing agent than the other tested

chemical agents; it was rapidly efficient as a bactericidal, viricidal and sporicidal agent and demonstrated low tissue toxicity.

<sup>43</sup>  
Borick tested 2% alkalinized glutaraldehyde on various microorganisms (vegetative bacteria: Staphylococcus, Escherichia coli, Diphtheria pneumoniae, Pseudomonas aeruginosa; fungi: Trichophyton interdigitale; and spores: Bacillus subtilis, Bacillus globigii, Clostridium tetani, Clostridium sporogenes) in the presence of 20% blood serum. He found the vegetative bacteria were killed in less than 1 minute, fungi in less than 0.5 minutes and the spores were killed in less than 3 hours. Using tests conducted by the free suspension method, diluted glutaraldehyde was effective against the test organisms Staphylococcus aureus and Escherichia coli. In Borick's paper it was stated that sporicide and chemosterilizer are synonymous, and that glutaraldehyde is a true chemosterilizer because "it destroys all forms of microbiological life including bacterial and fungal spores, tubercle bacilli and viruses." He also states that due to a lack of laboratory methods to evaluate chemical sterilizers against hepatitis viruses, one must assume that if other resistant viruses are killed that the hepatitis viruses are also destroyed.

<sup>44</sup>  
Pepper and Chandler used alcoholic saturated



glutaraldehyde as a test agent against 4 spore-forming organisms: Clostridium tetani, Clostridium sporogenes, Bacillus subtilis and Bacillus punilus. They used the membrane filter method and the penicylinder method of testing. The organisms were all destroyed in less than 2 hours.

Practical evaluations of glutaraldehyde as a sterilizing agent on medical equipment have been performed. O'Brien, Mitchell, Haberman, Rowan, Winford and Pellet<sup>45</sup> used alkalized glutaraldehyde as a cold sterilizing agent for urological instruments, with satisfactory results. The urological instruments were washed then placed in glutaraldehyde for a minimum of 20 minutes; the instruments were rinsed in sterile water prior to use. After the next use of the instruments in a surgical procedure the sterilization was repeated. At the end of the day's surgical procedures, a terminal sterilization, in which the instruments were placed in glutaraldehyde for 2 hours, was performed. The instruments were randomly swabbed before and after sterilization and the swabs cultured to be certain of instrument sterility. These researchers also performed "in use" tests on syringes and needles, as well as laboratory tests similar to those reported on earlier in this paper. The 2% alkalized glutaraldehyde proved effective in

all cases.

Sterilization of anesthesia apparatus was accomplished by Meeks, Pembleton and Hench<sup>46</sup> using glutaraldehyde. Face masks, breathing tubes, rebreathing bags, endotracheal tubes and Y connectors used in anesthesia were washed then soaked in a solution of buffered glutaraldehyde for 10 minutes. Instruments were randomly swabbed prior to sterilization and these swabs were cultured and subcultured for identification. The instruments were swabbed post-sterilization and cultured to test sterilization efficiency. The sterile instruments were then placed in presterilized plastic bags and at intervals of 6, 30, 54, 78 and 102 hours these stored items were exposed and recultured to determine their state of sterility. The glutaraldehyde was an effective sterilizing agent.

Spaulding<sup>47</sup> gave a list of recommendations for the use of glutaraldehyde in disinfection of medical and surgical materials in the hospital. He recommends glutaraldehyde for killing vegetative bacteria, fungi, tubercle bacilli, spores and viruses on rubber tubing, lensed instruments, polyethylene tubing and smooth hard surfaced objects.

Textbooks have now recognized alkalinized glutaraldehyde and its sterilizing capabilities. Rubbo and

<sup>48</sup>  
Gardner recommends it for use with endodontic reamers and files, stating that it has a wide range of activity and is a very active sporicide. Hedgecock<sup>49</sup> lists the formula for glutaraldehyde, gives a summary of its germicidal activity and describes its mechanism of action. Glutaraldehyde is listed as a high level disinfectant by Lawrence and Block,<sup>50</sup> with the capacity to kill large numbers of resistant spores.

Tests for toxicity were conducted by many of the previously reviewed researchers. Rittenbury and Hench<sup>41</sup> reported that glutaraldehyde was non-damaging to equipment and was non-toxic to personnel. Snyder and Cheattle<sup>42</sup> tested the toxicity of glutaraldehyde on an 11 day chick embryo, it retained viability. Hep2 cell cultures were maintained for 3 days in glutaraldehyde with no cell alteration. A dehydrating effect on the hands of operators<sup>45</sup> was noted by O'Brien et al, but after 4,000 patients were treated with instruments sterilized with glutaraldehyde no adverse effects or allergies due to the agent were found. Meeks, Pembleton and Hench<sup>46</sup> using anesthesia masks sterilized by alkalized glutaraldehyde experienced no adverse patient reaction to the masks.

<sup>51</sup>  
Murray notes that buffered glutaraldehyde reacts chemically with carbon steel and recommends the usage of aluminum or stainless steel trays for glutaraldehyde.

## Chapter III

### MATERIALS AND METHODS

#### A. TEST INSTRUMENTS

The endodontic reamers and files used in the study were K type, 25 mm in length, of stainless steel; some had handles of stainless steel\* and others had plastic handles.\*\* To allow correct measurement of the tooth length in the clinical study and to more accurately simulate clinical working conditions in the laboratory study, a 2 mm X 2 mm section of ordinary rubber band was placed on the shaft of each instrument. This is the standard method of establishing how much of the instrument shaft is in the tooth's root canal.

For organization of the instruments during the clinical study while exposing them to the chemical test agent, a  $4\frac{1}{4}$  inch X  $3\frac{1}{2}$  inch stainless steel container was obtained.\*\*\* Two racks of stainless steel mesh, enclosing a sponge, held the endodontic instruments upright in the sterilizing solution. The two racks will fit into

\*Kerr Manuf. Co. -- Detroit, Michigan

\*\*Union Broach Co. -- Elmhurst, New York

\*\*\*Ace Metal Crafts Co. -- Franklin Park, Illinois

the stainless steel container simultaneously. Stainless steel racks and container were used because the manufacturer's warning that carbon steel will be corroded by the test agent; aluminum containers are also satisfactory.

#### B. TEST AGENT

The test agent was alkalized glutaraldehyde,\* a dialdehyde ( $\text{CHO}-\text{CH}_2-\text{CH}_2-\text{CHO}$ ) developed in 1963, which undergoes aldehydic reactions to form acetates, cyanohydrin, oximes and hydrozones. In an aqueous form glutaraldehyde solution is stable up to 2 years, with little change in pH, being mildly acid in reaction. But in an alkaline solution a significant change in pH occurs after 2 weeks and antibacterial activity is reduced.

Additives in the activated glutaraldehyde solution are an alkaline buffer (sodium bicarbonate), a surface tension depressant, an anti-corrosive compound and a non-staining water soluble dye, D & C Green #8. These compounds are blended into a powder which comes packaged in a sealed vial, the addition of this vial's contents to the aldehyde solution brings it to its fully activated form. Antisporicidal action is demonstrated upon the addition of 0.3% sodium bicarbonate to 2% aqueous

(R)  
\* Cidex -- supplied by Johnson & Johnson Co.,  
New Brunswick, New Jersey.

glutaraldehyde solutions. The powdered buffer is added to the 2% glutaraldehyde, the pH becomes alkaline and the solution is then "activated". Alkalinization is necessary to elicit microbial activity. Following the alkalinization, the solution maintains the anti-microbial activity for a minimum of 2 weeks, at room temperature.

The dye indicates the addition of buffer salts to the glutaraldehyde. Before addition of the buffer-dye combination, the inactivated glutaraldehyde solution is colorless, after the addition of the dye the solution turns fluorescent green.

#### C. TEST ORGANISMS

The test organisms all came from stock cultures maintained by the Department of Microbiology, Loyola University School of Dentistry, Maywood, Illinois.

The test organism strains were:

<u>Staphylococcus aureus</u>	24-36 hour broth
<u>Streptococcus fecalis</u>	24-36 hour broth
<u>Streptococcus mitis</u>	24-36 hour broth
<u>Escherichia coli</u>	24-48 hour broth
<u>Candida albicans</u>	24-36 hour broth
<u>Bacillus subtilis</u>	10 day broth (showing evidence of spore formation)

#### D. CULTURE MEDIA

The culture broths of the test organisms were produced by inoculating sterile tubes of BHIB media with the organisms and incubating at 37° C until substantial growth was seen, a minimum of 24 hours. The Bacillus subtilis broth was incubated longer to assure the presence of spores. Hot carbol fuchsin stain was used to confirm the presence of spores in the Bacillus subtilis broth.

Fluid thioglycollate was used as the culture media for testing the sterility of the endodontic instruments.

Both the BHIB and fluid thioglycollate media were prepared, poured into glass tubes, with screw on caps, then sterilized by autoclaving. Approximately 10-13 cc of media were contained in each tube.

#### E. CLINICAL TESTS

To determine the effectiveness of 2% glutaraldehyde in the sterilization of endodontic instruments following contamination by clinical use, patients undergoing endodontic therapy at Loyola University School of Dentistry were selected for study. Forty teeth from 40 randomly selected patients were utilized, with the determining factor for selection being the probable presence of infecting microorganisms in the involved teeth. The 40 teeth were tested for vitality of the pulp tissue

with an electric pulp tester;\* in 31 teeth the readings were non-vital; in 9 teeth vital readings were received. Vital response occurred in 8 teeth that had cariously exposed pulps but no necrosis (chronic ulcerative pulpitis), and in 1 tooth with chronic hyperplastic pulpitis. From the clinical findings diagnoses of the pathology of the 40 teeth were made. (see Table I)

The patient's teeth were isolated by use of a rubber dam, access gained to the root canals with high speed carbide burs, and instrumentation of the root canals performed using the test instruments, 6 reamers or files per tooth. The instruments were then placed in the 2% alkaline glutaraldehyde solution, in a stainless steel instrument box, for timed intervals of exposure.

At intervals of 1, 2, 5, 10, 15 and 20 minutes the instruments were removed from the glutaraldehyde and placed in a sterile tube of fluid thioglycollate media and incubated at 37° C for 48-72 hours.

Three methods of removing any excess Cidex<sup>(R)</sup> from the instruments were used between the glutaraldehyde and the culture media stages: 80 of the instruments were

\*Burton Vitalometer Co., Van Nuys, California.



TABLE I

## TEETH OF CLINICAL STUDY: VITALITY AND DIAGNOSIS

## A. VITALITY:

<u>No. Teeth Studied</u>	<u>No. Non-vital Teeth</u>	<u>No. Vital Teeth</u>
40	31	9

## B. DIAGNOSIS OF TOOTH PATHOLOGY:

40 teeth involved

<u>Diagnosis</u>	<u>No. Teeth</u>
Pulp Necrosis	16
Chronic Ulcerative Pulpitis	9
Chronic Abscess	8
Acute Periapical Abscess	6
Chronic Hyperplastic Pulpitis	1

blotted in a sterile towel, 60 were tapped against the side of the steel container and 23 instruments were rinsed with sterile saline.

One instrument from each tooth was placed directly into the fluid thioglycollate media as a control instrument, to determine the presence or absence of microorganisms on the instruments.

In 2 of the clinical cases, contaminated instruments were placed in sterile saline for time intervals of 2, 5, 10, and 15 minutes, then transferred to tubes of thioglycollate. This test was performed to see if the organisms could be removed by a wash or if a true sterilizing factor was necessary.

Reading of all cultures was done at 48 and 72 hours, any turbidity observed was considered positive growth.

#### F. LABORATORY MICROBIOLOGICAL TESTS

Test organisms were obtained by inoculating tubes of sterile BHIB with samples from stock culture broths, followed by incubation at 37° C. Cultures of Staphylococcus aureus, Streptococcus fecalis, Streptococcus mitis, Escherichia coli and Candida albicans were incubated for 24-36 hours, all demonstrated growth. The Bacillus subtilis culture was incubated longer to assure spore formation. The presence of spores was confirmed by staining with hot carbol fuchsin stain after

10 days of incubation.

Twenty-one endodontic reamers or files were placed into the culture tubes of each organism. For controls, an instrument was removed from each tube of organisms, air dried and placed into separate tubes of sterile fluid thioglycollate media. The remainder of the instruments, 20 for each organism, were air dried and placed into shallow glass dishes containing 2% glutaraldehyde; there was a separate dish for each organism to prevent possible cross contamination between organisms.

Four instruments were removed from each dish at the time intervals of 1, 2, 5, 10 and 15 minutes; each instrument was placed into a separate appropriately labeled tube of fluid thioglycollate culture media and incubated at 37° C. Any growth found in the tubes after 48 hours was identified by direct microscopic examination of gram stains. In all cases the strains of organisms recovered showed the same morphology as the original organisms. Readings of all the culture tubes were made at 48 and 72 hours, with any visible turbidity being considered as positive growth.

#### G. DILUTION TESTS

A series of dilution tests were done to determine how much glutaraldehyde would have to be present in a tube of fluid thioglycollate media to inhibit organism

growth.

The first test was performed using 12 tubes of fluid thioglycollate, containing 10 cc of media per tube. A dilution of 1/10 (glutaraldehyde to thioglycollate) was obtained in 2 tubes by adding 1 cc of glutaraldehyde to each tube. By taking 1 cc of the 1/10 dilution from tube and adding to 2 other tubes of 10 cc thioglycollate, 2 tubes of 1/100 dilution were obtained, likewise 1 cc of the 1/100 dilution was added to another tube of 10 cc thioglycollate giving a dilution of 1/1000. This process was followed until 2 tubes each of 1/10, 1/100, 1/1000, 1/10,000, 1/100,000 and 1/1,000,000 dilutions were obtained. Six tubes, 1 of each dilution, were inoculated with Escherichia coli and 6 with Staphylococcus aureus, by contaminating endodontic files with the desired organisms and dropping them into the culture media. The tubes were then incubated at 37° C for 72 hours.

Growth was inhibited in the tubes with 10% glutaraldehyde, but not in the other dilutions, then more accurate tests were performed.

Twenty tubes of fluid thioglycollate (10 cc each) were prepared; glutaraldehyde was then added to each tube in 0.1 cc progressions from 0.1 cc up to 1 cc. Thus creating 2 tubes each of dilutions of 1%, 2%, 3% etc. up to 10%. Ten endodontic files were contaminated

with Escherichia coli and 10 with Staphylococcus aureus  
and then dropped into the culture tubes, 1 file per  
tube. The tubes were incubated at 37° C and read after  
72 hours.

## Chapter IV

### RESULTS

#### A. CLINICAL TESTS

The results in Table II indicate that alkaline 2% glutaraldehyde was effective in sterilizing the endodontic reamers and files used clinically. It demonstrated rapid effectiveness, killing the microorganisms in 2 minutes or less.

In the first 3 clinical cases studied, the instruments were left in the glutaraldehyde for intervals up to 20 minutes. It became obvious that glutaraldehyde did not require that length of exposure to sterilize the instruments. Fifteen minutes was the maximum exposure in the next 2 cases, the other 35 cases had a maximum exposure time of 10 minutes.

Three of the control instruments showed no growth, indicating that those 3 teeth either had sterile root canals or too few numbers of organisms to show growth in the test media. These teeth were all vital teeth with a diagnosis of chronic ulcerative pulpitis.

The 3 different methods of reducing the amount of glutaraldehyde on the instruments being transferred to the culture, did not affect the results. The amount

TABLE II

## RESULTS: CLINICAL TESTS

## A. Number of Teeth ---- Tooth Vitality:

<u>No. Teeth Cultured</u>	<u>Non-vital Teeth</u>	<u>Vital Teeth</u>
40	31	9

## B. Control Instruments:

<u>No. Instruments</u>	<u>Growth</u>	<u>No Growth</u>
40	37	3

## C. Instruments in Cidex, then cultured:

<u>Time in Cidex</u>	<u>No. Samples</u>	<u>Growth</u>	<u>No Growth</u>
1 min.	35	4	31
2 min.	40	0	40
5 min.	40	0	40
10 min.	40	0	40
15 min.	5	0	5
20 min.	3	0	3

## D. Instruments in Sterile Saline:

<u>No. Samples</u>	<u>Time in Saline</u>	<u>Growth</u>	<u>No Growth</u>
2	2 min.	2	0
2	5 min.	2	0
2	10 min.	2	0
2	15 min.	2	0

of 2% glutaraldehyde carried on the instruments was probably too little to inhibit bacterial growth no matter what method was used, or if no method was used at all.

Instruments soaked in sterile saline showed growth at all time intervals, indicating that a sterilizing action is required and that soaking alone is inadequate for clinical use of the instruments.

#### B. LABORATORY TESTS

The experiments indicate that alkaline glutaraldehyde was bactericidal in less than 2 minutes against Staphylococcus aureus, Streptococcus fecalis, Streptococcus mitis and Candida albicans. Escherichia coli showed some growth at various time intervals, but subsequent tests showed Escherichia coli to be killed in less than 2 minutes. Sporicidal effect against Bacillus subtilis required over 2 hours exposure to the glutaraldehyde. The results of these experiments are shown in Table III.

Staining procedures of all positive growths showed that the organisms showed the same morphology as the original organisms, in all cases.

Control media tubes for all the organisms showed positive growth.



TABLE III

## RESULTS: LABORATORY TESTS

<u>Organism</u>	<u>No. Samples</u>	<u>Time in Cidex</u>	<u>Growth</u>	<u>No Growth</u>
<u>Staphylococcus aureus</u>	4	1 min.	2	2
	4	2 min.	0	4
	4	5 min.	0	4
	4	10 min.	1	3
	4	15 min.	0	4
<u>Streptococcus fecalis</u>	4	1 min.	1	3
	4	2 min.	0	4
	4	5 min.	0	4
	4	10 min.	0	4
	4	15 min.	0	4
<u>Streptococcus mitis</u>	4	1 min.	0	4
	4	2 min.	0	4
	4	5 min.	0	4
	4	10 min.	0	4
	4	15 min.	0	4
<u>Escherichia coli</u>	4	1 min.	0	4
	4	2 min.	2	2
	4	5 min.	2	2
	4	10 min.	1	3
	4	15 min.	1	3
<u>Escherichia coli</u> (same broth)	4	2 min.	0	4
	4	5 min.	0	4
<u>Candida albicans</u>	4	1 min.	0	4
	4	2 min.	0	4
	4	5 min.	0	4
	4	10 min.	0	4
	4	15 min.	0	4
<u>Bacillus subtilis</u>	4	20 min.	4	0
	4	40 min.	4	0
	4	60 min.	4	0
	4	120 min.	1	3
	4	180 min.	0	4

### C. DILUTION TESTS

Initial dilution tests showed that a 10% dilution of 2% glutaraldehyde in fluid thioglycollate will inhibit bacterial growth in the culture tubes. Subsequent testing with dilutions from 1% to 10%, using Staphylococcus aureus and Escherichia coli, indicated that dilutions of 8% or greater will inhibit growth. (See Table IV)

The amount of 2% glutaraldehyde carried to the culture tubes by the endodontic instruments was not measured, but was assumed to be much less than the 8% required to inhibit bacterial growth in the thioglycollate culture media.

### D. TOXICITY

No formal experiment for testing toxicity was used in this study, but personal observations were made. In the clinical studies with patients who had root canals instrumented with reamers and files sterilized with 2% alkaline glutaraldehyde, no increase in patient discomfort during the procedure or post-operatively was noticed. No effect on the operators hands was noticed during the clinical procedures. However, in placing new instruments into the instrument container, the operator's fingers were immersed in the sterilizing agent, a yellow staining of the fingers occurred. It is therefore suggested that contact with a large

concentration of glutaraldehyde may result in a staining of the skin, but exposure to small concentrations will have little or no affect.

Adverse effects to the instruments and rubber band stops were tested by leaving the instruments in the 2% alkaline glutaraldehyde for a 14 day period. No corrosion of the metal portions was seen and no softening of the rubber stops occurred. In general, no undesirable effects were visible. Any dulling of the cutting edges of the files or reamers was not measured technically, however, personal observation noted no apparent changes in the instruments efficiency in cutting tooth dentin.

TABLE IV  
DILUTION TESTS

Effect of Trace Amounts of Alkaline 2% Glutaraldehyde  
in Fluid Thioglycollate Media

A. Experiment I

Growth in Test Organisms

<u>Dilutions</u>	<u>Escherichia coli</u>	<u>Staphylococcus aureus</u>
1/10	neg.	neg.
1/100	pos.	pos.
1/1,000,000	pos.	pos.

B. Experiment II

<u>% Solution</u>	<u>Dilution</u>	<u>Escherichia coli</u>	<u>Staphylococcus aureus</u>
10%	1/10	neg.	neg.
9%	1/11	neg.	neg.
8%	1/12.5	neg.	neg.
7%	1/14.3	pos.	pos.
1%	1/100	pos.	pos.

Growth inhibited at 8% solution or greater.

## Chapter V

### DISCUSSION

The need for a chemical sterilizing agent to be used in endodontics, as well as dentistry in general, has long been evident. Most other sterilization techniques have been either ineffective, as alcohol and flaming;<sup>2</sup> time consuming, as hot mineral oil or dry heat;<sup>9</sup> or deleterious to the instruments, as autoclaving.<sup>8</sup> Prior to the development of alkaline glutaraldehyde, no chemical agent was able to produce sterility,<sup>3</sup> except the formaldehyde solutions which are possibly toxic during use. Alkaline 2% glutaraldehyde is able to produce rapid, efficient sterilization of dental instruments.

Streptococci and staphylococci are the most prevalent organisms found in infected root canals. This prevalence holds true if the root canal is closed or if it is open to oral contamination. Escherichia coli, Candida albicans and Bacillus subtilis are infrequently found in endodontic infections, but were included in this study to give an overall picture of the effectiveness of the test agent on endodontic reamers and files

which were contaminated with microorganisms of various types. This study adequately covered the prevalent organisms and obtained satisfactory results. No investigator has demonstrated the presence of viruses in endodontic infections, therefore none were included in this study.

The results of this study compared favorably with other studies using glutaraldehyde. Stonehill, Krop and Borick<sup>36</sup> found that glutaraldehyde killed Staphylococcus aureus and Escherichia coli in less than 2 minutes and Bacillus subtilis in less than 3 hours. Borick, Donder-<sup>40</sup>shire and Chandler; Rittenbury and Hench;<sup>41</sup> Borick;<sup>43</sup> and Pepper and Chandler<sup>44</sup> all obtained similar results. Previous studies did not use streptococci as a test organism, but since they are the most common organism in endodontic infections 2 strains were tested in this study. The test agent rapidly killed the streptococci.

The reason that the test on Escherichia coli showed growth in some tubes in the laboratory tests is unknown. Perhaps the organism was resistant to the glutaraldehyde, or poor technique resulted in contamination. It possible that microorganisms may have been trapped under the rubber band stops or within holes in the plastic handles of some of the instruments. Later tests showed Escherichia coli, from the same stock culture,

to be rapidly killed.

Results of this study favor the use of alkaline 2% glutaraldehyde as a sterilizing agent, effectively killing the organisms of greatest concern to endodontics. The rubber stops on the files and reamers did not cause any difficulty in sterilization. Although it was thought that microorganisms might harbor between the rubber and the shafts of the files thereby escaping the sterilizing agent. No special effort was made to clean the endodontic instruments prior to sterilization, but in actual clinical practice mechanical cleansing by wiping the shafts of the instruments with a dry 2 X 2 gauze is recommended.

It is possible that the reason that a larger number of positive cultures was not obtained would be due to the small number of organisms present in the culture media. If this is true, it becomes fairly negligible when the triad necessary for the disease state is considered: (1) resistance of the host, (2) virulence of the organism and (3) the number of organisms present. Even if the number of microorganisms is only reduced in a short period of exposure to the glutaraldehyde (2 minutes or less) this reduced number of organisms would probably be insufficient to produce an infection in a new host, especially if root canal instrumentation is

performed in the presence of sodium hypochloride, as is the normal recommended endodontic procedure.

The clinical study evolved a suggested technique for the chemical sterilization of endodontic files and reamers. An aluminum or stainless steel instrument box is recommended, similar to the one used in this study (stainless steel with inside dimensions of  $3\frac{1}{2}$ " wide,  $4\frac{1}{2}$ " long and  $2\frac{1}{4}$ " deep). A removable lid covered the box. Two mesh racks of stainless steel, plastic or aluminum are selected to fit the container. These should be approximately  $1\frac{1}{2}$ " high, 4" long and  $1\frac{1}{4}$ " wide. Sponges inserted between the mesh holds the endodontic instruments upright. The files and reamers are inserted through the mesh into the sponge, in the desired order. The design of the container and racks are such that the instruments are completely submerged in the chemical agent when the racks are in the box.

At the time of patient treatment one of the racks of instruments is removed from the container and placed on a tray in the dentist's work area. This technique allows the use of one set of reamers and files while the other set is in the container being sterilized.

Root canal instrumentation is performed in the desired manner. A small "bankers" sponge in a glass dish is used to hold the instruments following their use.



This sponge can contain glutaraldehyde, or perhaps some other chemical disinfectant. (A commercial brand of quarternary ammonia compound was used in this study due to its lower cost.) If the instruments are needed again for that patient, chairside sterilization can be performed by placing the instruments into a hot bead sterilizer for 10 seconds. The hot bead sterilizer is useful for resterilizing any instruments that become contaminated before their use is completed. However, repeated sterilization in the hot bead sterilizer may cause accumulative detempering of the metal of the instrument, allowing their fracture during use.

Upon completion of the endodontic procedure, the instruments are cleaned and placed back into the rack. This rack is then returned to the container of 2% glutaraldehyde for sterilization. The instrument rack which was previously in the glutaraldehyde is removed for use during the next endodontic treatment, allowing time for the resterilization of the first set of instruments.

## Chapter VI

### SUMMARY

In this study the effectiveness of alkaline 2% glutaraldehyde as a chemical sterilizing agent for endodontic reamers and files was tested. In a clinical test endodontic instruments were used for mechanical instrumentation of the root canals of 40 teeth and then exposed to the test agent for varied time intervals. Following the contamination and disinfection the instruments were placed into tubes of fluid thioglycollate media and incubated at 37° C for 72 hours. The 2% glutaraldehyde adequately sterilized the instruments after 2 minutes exposure.

In the laboratory tests, cultures of Staphylococcus aureus, Streptococcus fecalis, Streptococcus mitis, Escherichia coli, Candida albicans and Bacillus subtilis were used as test organisms to contaminate the endodontic instruments. The instruments were then exposed to alkaline 2% glutaraldehyde for timed intervals and incubated in fluid thioglycollate media. Results showed that activated glutaraldehyde was effectively bactericidal

and fungicidal within 2 minutes. It was also sporicidal with 2-3 hours of exposure.

Based upon the results of this study, 2% alkalized glutaraldehyde appears to be an adequate sterilizing agent for use with endodontic files and reamers. It also demonstrated ease of utilization with no apparent toxicity.

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The thesis submitted by Dr. Gary M. Ritchie has been read and approved by members of the Department of Oral Biology.

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

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

May 10, 1971  
Date

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