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GRADUATE STUDIES

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THE EFFECTS OF CHLORHEXIDINE GLUCONATE ON ORTHODONTIC PATIENTS AGED ELEVEN THROUGH SEVENTEEN WITH ESTABLISHED GINGIVITIS

A THESIS

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by
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The Effects of Chlorhexidine Gluconate on
Orthodontic Patients Aged Eleven Through Seventeen
With Established Gingivitis

Abstract

by

Laurie Joan Brightman, D.D.S.

The purpose of this investigation was to determine the efficacy of 0.12% chlorhexidine gluconate mouthrinse on full-banded orthodontic patients aged eleven through seventeen with established gingivitis. Most patients undergoing active orthodontic therapy for the treatment of a malocclusion have an increased number of bacterial retention sites. These retention sites cause unfavorable gingival conditions because of increased bacterial plaque. This problem, in addition to contributing to periodontal disease, also becomes an esthetic concern for the patient.

Thirty-four subjects were chosen for this study and evaluated at baseline, six-weeks and at 12 weeks. They were divided into two groups based on gender. Within each group, some patients used a placebo mouthrinse, and others used a 0.12% chlorhexidine gluconate mouthrinse determined in a double-blinded design. At the three intervals, the Gingival Index (Loe and Silness), the Plaque Index (Silness and Loe), the Eastman Interdental Bleeding Index and the Case Western Reserve University Staining Index were recorded for each subject. Two samples of plaque were taken from each patient, one at the mandibular right central incisor, and the other, at

the mandibular right first molar. The samples were quantitatively cultured on Streptococcus mutans selective media which was incubated anaerobically for 48 hours and aerobically for 24 to 48 hours, and Actinomyces selective media which was incubated in 10% CO_2 for four days. Colony forming units of Actinomyces and Streptococcus mutans were used to determine the effectiveness of 0.12% chlorhexidine gluconate in reducing the number of bacteria that may contribute to gingivitis and dental caries.

The results showed that a significant reduction of plaque accumulation, gingival inflammation, gingival bleeding, and Actinomyces and Streptococcus mutans levels, could be attained while using the chlorhexidine gluconate mouthrinse. The reduction of plaque was associated with a reduction of gingival inflammation.

The staining seen with the use of chlorhexidine was more significant than the stain seen in the placebo group. There was more mandibular stain observed overall, and it was concentrated on the lingual surfaces of the teeth, making it less of an esthetic concern.

Therefore, it can be concluded that 0.12% chlorhexidine gluconate in combination with mechanical plaque removal, proved to be an important therapeutic agent in controlling gingival inflammation, gingival bleeding, plaque accumulation, and in reducing selected bacterial levels in orthodontic patients with established gingivitis.

DEDICATION

I dedicate this Thesis to my family members who have been supportive throughout my education. My mother, Gloria, who was my first teacher. She taught me the importance of an education, and that I should always strive to reach my ultimate goals in life. I thank her for her emotional support and her love. My grandfather, Joseph H. Goodman, D.D.S., who shall always remain in my thoughts. He was my first dentist, and even at a very young age, he inspired me to become a doctor, as did my grandmother, Henrietta M. Goodman. My great-uncle William J. Goodman, D.D.S., who was like a second grandfather to me because he reminded me so much of my own grandfather. I will always remember him for his support and encouragement to pursue dentistry. And, to my sister, Brenda B. Brightman, D.D.S., the newest dentist in our family, who always helped me with my constant learning throughout my years of school. I finally got to return the favor, and help her to become the last dentist, of our generation, in our family.

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I. INTRODUCTION

Mechanical tooth cleaning is a skill that many individuals are unable to perfect. The addition of full banded, fixed orthodontic appliances increases the difficulty of this task, and thereby, produces more unfavorable periodontal problems in most orthodontic patients (Balenseifen and Madonia, 1970, Corbett et.al., 1981).

Periodontal problems following the placement of orthodontic bands, brackets, and arch wires are the result of inherent irregularities on fixed orthodontic appliances. These irregularities provide additional opportunities for the collection and retention of food and debris. The increased supply of substrate permits luxuriant bacterial growth, and accounts for the increased concentration of bacteria in the plaque. Orthodontic appliances also protect the plaque from the actions of brushing, mastication and salivary flow. It would therefore, be of great clinical benefit if a chemical agent could be used during the active phase of orthodontic treatment to reduce bacterial plaque accumulation, thereby, improving the gingival conditions and possibly reduce the incidence of caries and periodontal disease in these patients.

Chlorhexidine gluconate mouthrinse has been found to effectively reduce bacterial growth. Although every drug used may have some side effects, the clinical benefits of 0.12% chlorhexidine gluconate seem to far outway the undersirable disadvantages of this drug.

Although the antiplaque abilities of chlorhexidine have been well documented, its use in teen-age orthodontic patients has not been extensively supported. This study was designed to include both of these parameters, and relate microbiological findings to clinical observations over a three month period.

II. LITERATURE REVIEW

Role of Gingivitis

Most patients who undergo orthodontic therapy for the treatment of a malocclusion receive fixed orthodontic appliances which increase the number of bacterial retention sites. Increased retention of bacterial plaque contributes to unfavorable gingival conditions (Friedman et.al., 1985; Balenseifen and Madonia, 1970; Zachrisson, 1972). Clear evidence that plaque is responsible for gingivitis was presented by Loe, et.al. who studied the induction of experimental gingivitis in man (Loe, et.al., 1965; Theilade, et.al., 1966; Holm-Peterson, et.al., 1975), and implicated specific groups of bacteria in the initiation of inflammation.

Gingivitis is the most common form of periodontal disease (Carranzo, 1979). Inflammation may be:

1) primary and the only pathologic change (most common),
2) secondary, superimposed upon systemically caused gingival disease, or
3) the precipitating factor responsible for clinical changes in patients with systemic disease, which of themselves, do not produce clinically detectable gingival disease.

The development of gingivitis depends on the accumulation of supragingival plaque, and the regular and thorough removal of plaque will prevent gingivitis (Lang et.al., 1973). The three basic approaches to the prevention of gingivitis are 1) the elimination of all clinically detectable plaque, 2) the reduction of plaque below the individual's threshold for disease, and 3) the alteration of the microbial composition of plaque such that periodontitis

will not develop (Kornman, 1986).

The first approach has shown that frequent prophylaxis and good oral hygiene will inhibit plaque formation and gingivitis, and it will prevent loss of the periodontal attachment (Axelsson and Linde, 1978). It has also been shown that chemical agents such as chlorhexidine prevent plaque accumulation, and therefore, prevent gingivitis (Segreto et.al., 1986; Grossman et.al., 1986; Briner et.al., 1986a; Briner et.al., 1986b). Both chemical and mechanical approaches work to essentially eliminate plaque.

The second approach involves the reduction of plaque below the individual's threshold for disease. Most individuals do not efficiently remove plaque. Therefore, some level of plaque is present, but the levels of pathogens usually are below the host's threshold for disease. It must be realized that the threshold may undergo changes that may cause a decrease in the host's ability to deal with the microbial challenge, or the microbial challenge may change.

The third approach is to alter the microbial composition of the plaque such that disease does not develop. There must be a more selective way in which the clinical quality of supragingival plaque may be altered. This result may not be attainable simply by mechanical means except for the fact that repeated disruption of plaque maturation should result in a plaque with lower levels of certain bacterial groups which require a specific ecological succession (Kornman, 1986). There are preliminary studies in animals that suggest, however, that it is possible for chemical agents to prevent or retard the progression of periodontitis without reducing

plaque or gingivitis (Kinder, Kornman and Holt, 1984).

Actinomyces and Streptococcus mutans

Dental infections such as tooth decay and periodontal disease are perhaps the most common bacterial infections in humans. The accumulation of bacterial colonies on tooth surfaces, in aggregates known as dental plaque, causes both decay and periodontal disease (Loesche, 1986). A variety of bacterial types are indigenous to the mouth. This may be due to the diversity of surfaces available for colonization, as well as, to the comparatively unselective conditions that prevail (Holmberg et.al., 1973; Socransky and Manganiello, 1959). Moore (1982) reported that 166 bacterial species and subspecies were detected in periodontally healthy patients. Of these bacteria, Actinomyces naeslundii, Actinomyces odontolyticus, Fusobacterium nucleatum, Lactobacillus species D-3, Streptococcus anginosus, Veillonella parvula and Trepomona species A appeared to be the most likely etiological agents of gingivitis. There have been other studies that indicate 200 to 300 species are indigenous to human dental plaque, but only a finite number may be considered odontopathogens (Socransky, 1982).

Members of the genus Actinomyces are gram positive and appear to be strict parasites. A. israeli is found in the human mouth and in salivary calculi. A. odontolyticus occurs in human saliva and in carious teeth. A. naeslundii, also an inhabitant of the human mouth, is thought be be non-pathogenic, and is closely related to A. viscosus. This organism has been isolated from the oral cavity of man, in which it produces gingival plaque and periodontal

disease (Wilson, Miles and Parker, 1983).

The largest biomass of bacteria is found on the surfaces of the teeth and on the dorsum of the tongue (Gibbons and van Houte, 1975). Bacterial plaque accumulations on teeth contain in the order of 10^{11} organisms per gram wet weight (Gibbons et.al., 1964).

The physical association of certain Streptococcus and Actinomyces species with the tooth surface may be necessary prerequisite to colonization by Veillonella, Fusobacteria and Treponemes (Ritz, 1967) that produce stronger irritants such as propionic and butyric acids or antigens or both (Moore et.al., 1982). Physical associations between Actinomyces or Streptococci and other bacteria, including Veillonella and Fusobacteria, have been shown by Bladen et.al., (1970) and Peros et.al., (1982). In addition to their dual association with the tooth surface and with other bacteria, Streptococci, Actinomyces, and Lactobacillus produce lactic acid, which is a preferred substrate for Veillonella, and can convert lactic acid into propionic acid, which may be a potent gingival irritant.

The essential role of Streptococci and Actinomyces is indicated by established knowledge that strict control of the initial flora (primarily Streptococci and Actinomyces) prevents gingivitis. However, the presence of relatively high numbers of Actinomyces and Streptococci in healthy sites indicate that these species alone usually do not produce gingivitis. Rather, an increase in certain associated species is probably required.

Although these species of bacteria in dental plaque contribute to periodontal disease, they may only represent a minor percentage of the total number of bacteria that cause periodontal disease.

Because of this, the non-specific plaque hypothesis (Loesche, 1976) is generally accepted.

Syed and Loesche (1978) postulated that either the length of exposure of the gingival tissues to the plaque accumulation or the development of more virulent bacteria in the flora, not plaque size or bacterial numbers per se, is responsible for the development of gingivitis. Initially, in the development of gingivitis, streptococcal (gram positive) species dominate. Actinomyces species dominate in the older plaques. There was a definite shift from a Streptococcus-dominated plaque to an Actinomyces-dominated plaque observed as plaque ages. Plaque formation on teeth is the prerequisite for the development of dental caries. Streptococcus mutans, Streptococcus sanguis, Streptococcus mitior, and Streptococcus milleri comprise a significant percentage of dental plaque, and contribute to its development (Hamada and Slade, 1980).

Streptococcus mutans, a gram positive bacteria, is strongly associated with the onset of dental caries (Hamada and Slade 1980; Emilson and Krasse, 1985; Gibbons et.al., 1966; Littleton et.al., 1970), and it colonizes predominantly on tooth surfaces which are usually referred to as retentive (Ikeda et.al., 1973; Gibbons et.al., 1974; Suanberg and Loesche, 1978).

Initially, Streptococcus mutans adsorbs to the pellicle-coated tooth surface in a sucrose-dependent fashion which is facilitated by divalent cations and is dependent upon salivary glycoproteins deposited on the tooth surface. Once Streptococcus mutans is attached to the pellicle-coated tooth surface, a sucrose-dependent adherence phase ensues. This is mediated by the synthesis of

water-insoluble glucans, that not only allow for the firm attachment to the tooth surface, but also, because of the presence of glucan-binding proteins on the Streptococcus mutans cell surface, leads to the aggregation or agglutination of Streptococcus mutans cells. The result of this is the formation of dental plaque, which provides a suitable microenvironment for invasion by other microorganisms that do not attach too well to the tooth surface but are capable of acid production. When there are many sugars present, Streptococcus mutans not only metabolizes these to produce lactic acid, but also stores large quantities of extracellular and intracellular polysaccharide reserves which can then be metabolized during non-meal times, when free sugars are not available. All of these metabolic activities yield lactic acid, which leads to enamel demineralization and the onset of dental decay (Loesche, 1986).

Studies have shown that regardless of dental caries status, banded orthodontic patients had significantly greater Streptococcus mutans plaque populations than did non-banded patients (Corbett et.al., 1981). Since fixed orthodontic treatment increases the number of retention sites, an increase in the number of Streptococcus mutans may result during active orthodontic treatment (Scheie et.al., 1984; Zachrisson and Zachrisson, 1971).

Although some orthodontic patients may have good or even excellent plaque control, they still may develop an unexpectedly high number of new carious lesions (Lundstrom et.al., 1980). This may be due to the presence of Streptococcus mutans that colonizes in retention sites, and the increased difficulty of totally removing the bacteria simply through mechanical methods.

Criteria for the Chemical Control of Plaque

The utilization of antibacterial agents for the prevention and control of periodontal disease and caries was not generally accepted in the past. This was most likely due to the fact that the pathogenesis and etiology of these diseases were not well understood.

Systemic influences play a minor role in the actual development of these diseases, and the deposition of bacterial plaque on tooth surfaces and on the gingiva and its products, represent the most important link in the chain of events that lead to both periodontal destruction and tooth decay (Loe, 1973).

Many scientific experiments have consistently and conclusively shown that accumulation of dental plaque on the healthy gingiva produces gingivitis, and this is resolved with the reinstitution of oral hygiene (Theilade et.al., 1966). No other factor has ever been shown to be able to produce and maintain chronic gingivitis in man (Loe, 1973).

The prevention and control of periodontal diseases and caries must be based on the regular and complete removal of bacterial plaque (Kornman, 1986). To assist patients in the mechanical control of bacterial plaque, Loe (1973) strongly suggested the use of chemical plaque control. Chemical control includes (1) the inhibition of plaque development (2) the inhibition of early microbial colonization on tooth surfaces, (3) the elimination of all existing plaque and (4) the alteration of "pathogenic" plaque into "non-pathogenic" plaque (Lang and Brecx, 1986). The threshold levels for the development of disease varies greatly among individuals. The successful reduction of the plaque biomass

is not sufficient evidence of a more favorable clinical condition (Kornman, 1985). To achieve effective chemical plaque control, there must be either total inhibition or elimination of plaque development. If this is not possible, then the development of plaque must be controlled to the point that the clinical parameters used to monitor the host response are substantially improved.

Many chemical agents have been studied for their influence on the development of plaque with or without concomitant monitoring of the host response (Lang, 1980; Kornman, 1985). The criteria that need to be discussed when reviewing chemical plaque control agents include (Loesche, 1976) (1) Specificity, (2) Efficacy, (3) Substantivity, (4) Safety, and (5) Stability.

Specificity. The use of systemic agents such as antibiotics should be used for specific systemic conditions and not for daily chemical plaque control.

Efficacy. An antimicrobial agent used in treating dental infection should be effective against organisms that are known to be pathogens in gingivitis and periodontitis. Substances of choice for chemical plaque control are directed at plaque non-specifically (Loesche, 1976) because the non-specific nature of dental plaque is generally accepted, and the prime model is the experimental gingivitis model in man (Loe, 1965; Theilade, 1966).

A chemical plaque control agent chosen for this non-specific hypothesis should eliminate the plaque biomass, prevent its formation, or reduce its amount below the threshold level for pathogenicity. Therefore, this agent should either completely inhibit or significantly delay the development of gingivitis.

Substantivity. This is a measure of the adsorption of a material to a substrate in a given medium. The slow release of this material is of utmost importance, in order to provide an antimicrobial benefit, over time (Bonesvoll, 1974a).

Safety. This is of utmost importance with an antimicrobial agent. These agents must be tested in animal studies prior to its clinical use and all side effects carefully investigated in human studies. The effects of antimicrobial agents and their metabolic products on the environment have been studied (Greenstein et.al., 1985).

Stability is an important characteristic of antimicrobial agents. They should be stable at room temperature for a long period of time.

Chlorhexidine

Chlorhexidine is an antimicrobial agent which meets all of the above basic criteria. It was developed with other polybiguanides in the late 1940's. It's structural formula consists of two symmetric 4-chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain (Fig. 1).

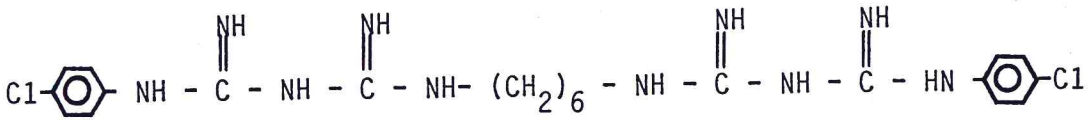


Fig. 1 Structural formula of chlorhexidine

1, 6 - bis - 4 - chlor - phenyldiguanidohexane

Chlorhexidine is a base and is stable as a salt. It is prepared as a 0.12% chlorhexidine gluconate oral rinse which is water soluble,

and at physiologic pH, it readily dissociates releasing the positively charged chlorhexidine component. This drug has a bacteriocidal effect which is due to the cationic molecule binding to extra microbial complexes and negatively charged microbial cell walls. The surface structures of these microbial cell walls are changed, and the osmotic equilibrium is lost. As a consequence of this, cytoplasmic membrane is extruded, vesicles are formed, and the cytoplasm precipitates which inhibits the repair of the cell wall, and the bacteria are no longer able to recover (Hugo and Longworth, 1964; Hugo and Longworth, 1968; Brecx and Theilade, 1984; Davies, 1973). Chlorhexidine also functions to inhibit the formation of plaque (1) by binding to anionic acid groups on salivary glycoproteins, thereby, reducing the pellicle formation and plaque colonization and (2) by binding to salivary bacteria and interfering with their adsorption to teeth (Rolla and Melson, 1975).

Chlorhexidine is both bacteriocidal and bacteriostatic, and it is effective against Gram-positive organisms, Gram-negative organisms and yeasts (Loe and Schlott, 1870). It derives its antiplaque efficacy from its ability to adhere to anionic substrates that are found in the oral cavity, such as hydroxylapatite, pellicle, salivary glycoproteins and mucous membranes. After it adsorbs on oral surfaces, it is slowly released in its active form. The kinetic mechanisms of chlorhexidine adsorption from mouthrinses and its slow release in the saliva have been tested with radioactively labeled chlorhexidine (Gjermo, 1974). It has also been shown that approximately 30% of this drug was retained in the oral cavity after a patient rinsed with 10 ml. of 0.2% chlorhexidine solution

for one minute (Bonesvoll et.al., 1974a).

The bound chlorhexidine was subsequently released over an 8 to 12 - hour period, and weak concentrations could be found in saliva for 24 hours. This prolonged antimicrobial effect is an important complement to chlorhexidine's high initial bacteriocidal activity. If a chlorhexidine rinse is followed by a rinse with distilled water, 25% of the bound chlorhexidine is lost (Bonesvoll et.al., 1974b). These experiments have clearly shown that the success of chlorhexidine is, in part, due to its substantivity. The slow release of chlorhexidine from retention sites provides a prolonged bacteriocidal effect which should make it ideal for use with patients who are undergoing fixed orthodontic therapy.

The mucosal and gingival penetration of chlorhexidine has been shown to be minimal in experiments conducted with radiolabeled chlorhexidine rinses (Magnuson and Heyden, 1973; Haugen and Johansen, 1975), and this is a factor in its low toxicity. Chlorhexidine has also been shown to be poorly absorbed from the gastrointestinal tract (Case, 1977). It has been reported that when this drug is used as an oral rinse, 4% of the solution and all of the adsorbed drug was eventually swallowed. Ninety percent of the retained drug was excreted in the feces and the remainder was eliminated through the urinary tract (Winrow, 1973). Studies that have monitored chlorhexidine have shown that none accumulated in the body or was metabolically altered into potentially harmful by-products (Winrow, 1973).

The most common side effect of chlorhexidine is the development of a yellowish-brown stain that usually appears in the gingival

third and the interproximal areas of affected teeth. This can occur on approximately 50% of the individuals using this drug (Loe et.al., 1976).

It has been postulated that there is a dietary etiology to the staining that occurs (Rolla et.al., 1981, Ellingsen, 1982). It was suggested that stain was formed by the precipitation of iron sulfide (Rolla et.al., 1981). Sulfur may originate from exposed thiol groups from denatured proteins and iron found in the diet. This would help to explain why smoking, drinking fluids containing tannic acid, and antibacterial therapeutics that contain denaturing agents could result in dark staining.

Other side effects include the occurrence of occasional dulling of taste sensation for several hours (Case, 1977; Gjermo, 1974), and desquamative lesions associated with chlorhexidine application (Flotra et.al., 1971).

Bacterial resistance to certain drugs appears to result from the selection of mutants that develop due to chromosomal alterations or through transfer of genetic information by conjugation (Greenstein et.al., 1986). Antimicrobial agents usually do not cause mutations, but instead, they assist in the selection by providing an environment conducive to growth of the less susceptible microbes. Chlorhexidine has been reported to cause mutations, but this does not occur frequently (Hennesey, 1973).

Several researchers have studied the possibility that chlorhexidine application resulted in the development of resistant bacterial strains (Hamp et.al., 1973). In vitro tests have been performed on plaque samples, and they have shown that microorganisms that

were exposed to 2% chlorhexidine were found to be less sensitive to chlorhexidine (Hamp et.al., 1973). A possible explanation was that microorganisms were developing resistance to chlorhexidine application. Researchers have also reported that after the discontinuation of chlorhexidine application, bacteria returned to pretherapy sensitivity (Hennessey, 1973; Schiott et.al., 1976).

Many studies have shown that chlorhexidine is a very effective antimicrobial agent in the treatment of gingivitis and in inhibiting recolonization of plaque bacteria (Loe et.al., 1976; Schiott, Briner and Loe, 1976; Grossman et.al., 1986; Briner et.al., 1986a). Segreto et.al. (1986) published an article comparing two concentrations, 0.20% and 0.12%, of chlorhexidine gluconate at six weeks and at twelve weeks. Both groups showed signficantly less plaque and gingivitis than the control group. The 0.12% chlorhexidine gluconate resulted in 27% to 31% less gingivitis, 28% to 33% less severe gingivitis, 48% to 59% less gingival bleeding, 36% less plaque accumulation, and there were no siginficant advantages for using a 0.20% over a 0.12% mouthrinse. Other studies, which have been longer in duration, have shown very similar percentage reductions (Grossman et.al., 1986).

In addition to these clinical indices, other studies have emphasized the importance of reducing the amounts of certain bacterial populations (Briner et.al., 1986a, Briner et.al., 1986b, Lundstrom and Krasse, 1987, Schiott et.al., 1976a, Schiott et.al., 1976b). Briner (1986a) reported that using a 0.12% chlorhexidine gluconate mouthrinse resulted in a 85% to 97% decrease in the number of Actinomyces, and a 65% to 75% reduction in the number of Streptococci,

after a 3 month period. After 6 months, there was significant clinical improvement in plaque and gingival indices, and significant reductions in four bacterial populations frequently identified in human plaque: 1) total aerobes, 2) total anaerobes, 3) total Streptococci and 4) total Actinomyces. Also, there was no alteration in the composition of the oral microflora toward organisms that are less sensitive to chlorhexidine (Briner, 1986b), and there was no detectable shift in microbial populations.

The introduction of full-banded orthodontic appliances into the mouth has been shown to cause an increase of the oral microbial flora (Bloom et.al., 1964, Lundstrom et.al., 1980, Balenseifen and Madonia, 1970, Corbett et.al., 1981). Bloom and Brown (1964) showed that there was a numerical increase in seven microbial populations. The degree of microbial increase correspond to the number of orthodontic bands in the mouth. Of these seven microbial categories, the total anaerobic population was found to be the most numerous. The aerobic population was found to be about one half the anaerobic, about 80 percent of which was accounted for by Streptococci.

In the most recently published study on the effects of chlorhexidine treatments on the frequency of Streptococcus mutans and Lactobacilli in orthodontically treated adolescents (Lundstrom and Krasse, 1987), it was found that in spite of an introductory period of oral hygiene instruction and training combined with dietary advice, the levels of Streptococcus mutans in saliva increased significantly during the first six months of fixed appliance therapy in the control group not using chlorhexidine. This result corresponded

with the findings of Corbett et.al. (1981), which also showed significant increases in the levels of Streptococcus mutans in microbial plaque in orthodontic patients. In the former study, it was found that the number of Streptococcus mutans could be significantly suppressed through the use of chlorhexidine. The mean value for the chlorhexidine group was significanlty lower than in the control group during the entire period of orthodontic treatment. This study also demonstrated that chlorhexidine treatment had no effect on the incidence of Lactobacilli, bacteria which are associated with dental caries (Balenseifen and Madonia, 1970). This is probably due to the fact that Lactobacilli, in contrast to Streptococcus mutans, show low sensitivity to chlorhexidine (Emilson, 1977).

Studies, such as these all indicate a greater caries risk for orthodontic patients compared to non-orthodontic patients due to the increase in the levels of Streptococcus mutans caused by an increased number of retention sites. Also, the increase in the levels of bacteria causing gingivitis has been demonstrated.

Objectives of the Study

The purpose of this study is to examine the effects of a 0.12% chlorhexidine gluconate mouthrinse on orthodontic patients aged eleven through seventeen with established gingivitis.

Four indices are used for the evaluation of gingival, plaque, bleeding and staining conditions. Actinomyces levels and Streptococcus mutans levels are measured as indicators of the gingival and dental caries diseases, respectively.

This study will test whether a statistically significant difference exists between subjects in the placebo group versus subjects in the experimental chlorhexidine group as measured by the four indices and the bacterial culturing data. It will also attempt to establish guidelines for the use of chlorhexidine in pediatric orthodontic patients.

Null Hypothesis

The following null hypotheses will be tested:

1) There will be no statistically significant differences between the control group and the 0.12% chlorhexidine gluconate group in the baseline values.
2) There will be no statistically significant differences between the control group and the 0.12% chlorhexidine gluconate group in the six week values.
3) There will be no statistically significant differences between the control group and the 0.12% chlorhexidine gluconate group in the twelve week values.
4) There will be no statistically significant differences in the experimental group or in the control group from the baseline values to the six-week values.
5) There will be no statistically significant differences in the experimental group or in the control group from the baseline values to the twelve-week values.
6) There will be no statistically significant difference between stain in the maxillary arch and stain in the mandibular arch.

III. MATERIALS AND METHODS

1. Introduction

Chlorhexidine is an antibacterial agent with substantivity. It is effective due to its retention and release kinetics. It is capable of reducing plaque and gingivitis by 50% to 90% when used once or twice daily (Briner et.al., 1986a; Siegrist, 1986; Lang and Brecx, 1986). Years of documented research have established that chlorhexidine is safe, stable and effective in preventing and controlling plaque formation, breaking up existing plaque, and inhibiting and reducing the development of gingivitis.

The randomized control trial (RCT) has become the standard experimental tool for evaluation of medical therapies. Randomly assigning subjects to different groups is the best method available for ensuring comparability across treatments (Antczak et.al., 1986). The validity of a clinical trial depends on several aspects of the study design, including the method of randomization, the criteria for subject selection, treatment descriptions, blinding procedures, and the use of appropriate statistical analyses (Chilton and Barbano, 1974).

2. Subject Selection

Subjects for this study were selected from the Department of Orthodontics at Case Western Reserve University School of Dentistry, Cleveland, Ohio. Forty-four subjects qualified for this study because they all met the following criteria: 1) they were undergoing full-banded edgewise extraction treatment with brackets on their anterior teeth and bands on their molars, 2) four premolars had been extracted, 3) they were at least eleven years of age and

no more than seventeen years of age, 4) evidence of gingivitis was present, but no evidence of periodontitis, 5) there was no evidence of decalcification on their teeth, 6) they could have no known hypersensitivity to chlorhexidine and 7) they were excluded if they had an unfavorable medial history, or if they used antibiotics regularly.

Of these forty-four subjects, thirty-six minor patients and their parents consented to participate in this study. There were 21 female subjects and 15 male subjects. These subjects were randomly divided by gender into two groups of eighteen subjects each. One group used a placebo mouthrinse (control group), and the other group used a 0.12% chlorhexidine gluconate mouthrinse (test group).

3. Experimental Design

This was a randomized, double-blinded, controlled study utilizing a 0.12% chlorhexidine gluconate mouthrinse and a placebo mouthrinse. The placebo mouthrinse was identical to the experimental mouthrinse, except that the placebo mouthrinse did not contain chlorhexidine gluconate. The appearance and the taste of both mouthrinses were similar. They were both bluish in color and minted in flavor, and they were both contained in amber colored bottles.

Blinding of the randomization process (Chalmers et.al., 1983) ensured that there was no way to predict or influence which treatment any of the patients received. The allocation of mouthrinses was defined by the Procter and Gamble Co., Cincinnati, Ohio. Each patient was assigned into one of the two groups based upon gender.

Two randomization tables of random numbers were used, one for the male population and one for the female population, so as to assign the subjects into one of the two treatment groups. This random assignment gave each patient a unique identification number which was retained throughout the study. These measures ensured that neither the investigators nor the patients knew if they were in the experimental group or the placebo group, but there were equal numbers of subjects in each of the two groups at the commencement of this study.

4. Drug Administration

Both the 0.12% chlorhexidine gluconate and the placebo mouthrinses were bottled by The Procter and Gamble Company, Cincinnati, Ohio. The mouthrinses were dispensed at Case Western Reserve University School of Dentistry to the patients of the Graduate Orthodontic Department involved in this study. Each subject received six 16 ounce bottles, enough mouthrinse for the duration of this 12 week study.

5. Dosage Regimen

Each patient in this study was provided with three toothbrushes, three tubes of Crest^{R} toothpaste with fluoride, a thirty second egg timer, and six 16 ounce bottles of either 0.12% chlorhexidine gluconate mouthrinse or a placebo mouthrinse. These quantities were determined to be sufficient for a three month investigation.

The patients were all instructed to use one toothbrush every month. They were only to brush with Crest^{R} toothpaste with fluoride once in the morning after breakfast and once in the evening before

bedtime. They were all told that they had to brush a minimum of three minutes to ensure thorough brushing.

The mouthrinses were then to be used after toothbrushing according to the following regimen:

Number of Subjects	Dose 1	Dose 2
18 Placebo Subjects	½ oz. of placebo for 30 seconds after breakfast	½ oz of placebo for 30 seconds before bedtime
18 Experimental Subjects	½ oz. of 0.12% Chlorhexidine Gluconate for 30 seconds after breakfast	½ oz of 0.12% Chlorhexidine Gluconate for 30 seconds before bedtime

The subjects were then instructed not to take any liquid or food into their mouth for at least thirty minutes after using the prescribed mouthrinse. The reason for this was twofold. First, a bitter taste could have occurred in the mouth immediately after rinsing with chlorhexidine. Second, the substantivity of the 0.12% chlorhexidine gluconate could be decreased, thereby, diminishing the effect of the drug.

6. Reports and Records

Gingival inflammation, plaque accumulation, bleeding tendency, and the intensity and the area of stain were recorded at baseline, six weeks and finally at the end of the study which was at 3 months. Intraoral photographs were taken of all of the subjects at all three time periods to serve as photographic evidence.

The following four indices were utilized:

A. GINGIVAL INDEX: Used to determine the health and degree of inflammation of the marginal gingiva.

The mesiofacial, facial, distofacial and lingual surfaces of six teeth, #3, #9, #13, #19, #25, and either #28 or #29 depending upon which bicuspid was extracted for orthodontic purposes (Ramfjord, 1967) were scored as indicted by Loe (1967).

0 = Normal gingivae

1 = Mild inflammation - slight change in color, slight edema. No bleeding on probing.

2 = Moderate inflammation - redness, edema and glazing. Bleeding on probing.

3 = Severe inflammation - marked redness and edema. Ulceration. Tendency to spontaneous bleeding.

The results will be recorded as GI occurrence (the proportion of diseased sites, i.e., grades, 1, 2, or 3, with reductions representing an absence of gingivitis), and GI severity (the average extent of the disease, with reductions representing a lessening of the disease (Grossman et.al., 1986).

B. PLAQUE INDEX: Used to determine the amount of plaque on the tooth surface. The mesiofacial, facial, distofacial and lingual surfaces of the same teeth used in the gingival index were scored as described by Loe (1967). The following criteria were used:

0 = No plaque in the gingival area.

1 = A film of plaque adhering to the free gingival margin and adjacent area of the tooth recognized by running

a probe across the tooth.

2 = Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin or adjacent tooth surface.

3 = Abundance of soft matter within the gingival pocket and on the gingival margin and adjacent tooth surface.

C. BLEEDING INDEX: Used to determine either the presence or the absence of bleeding as described by Polson and Caton (1986) as The Eastman Interproximal Bleeding Index. The same six teeth that were assessed in the gingival and plaque indices were assessed in this index. Only the mesial surfaces of these teeth were tested by using a wooden interdental cleaner (Stim-U-Dent, Johnson and Johnson) between the teeth from the facial aspect in such a way as to depress the gingival papilla 1 to 2 mm. The path of insertion was horizontal, with care being taken not to direct the point in an apical direction. The interdental cleaner was inserted and removed 4 times. The scores were determined as follows:

NO = The absence of bleeding within 15 seconds.

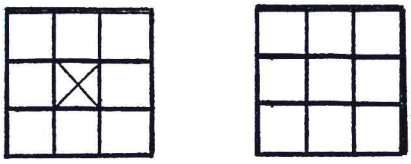
YES = The presence of bleeding within 15 seconds.

This was recorded as a percentage.

D. STAIN INDEX: Created at Case Western Reserve University School of Dentistry; Department of Periodontics. The tooth is divided into 18 sections:

three buccal sections, three lingual sections, and twelve interproximal sections. Because an orthodontic bracket was bonded to the middle buccal section, this section had to be eliminated. Both the intensity and the area of stain was recorded as follows:

Buccal Lingual



0 = no stain

1 = light stain

2 = moderate stain

3 = severe stain

Recordings:

1) Total Tooth Surfaces (TTS) = $\frac{\text{stain recording}}{17}$

2) Interproximal Tooth Surfaces (ITS) = $\frac{\text{stain recording}}{12}$

3) Buccal-Lingual Surfaces (BLS) = $\frac{\text{stain recording}}{5}$

E. Cooperation: was measured by the following:

$$\frac{\text{number of times patients used the mouthrinse}}{\text{number of days patients was in the study x2}} \quad x100$$

F. Tannic Acid: was measured as a percentage of the number of

subjects who drank coffee, tea, port wine or smoked as:

$$\frac{\text{number of tannic acid users}}{\text{total number of subjects}} \times 100$$ for each group.

G. Actinomyces and Streptococcus mutans levels. The indices for these two groups are expressed as colony forming units per total plaque specimen (CFU). The calculation for the organism count/plaque specimen is as follows:

Number of organisms/plaque specimen (tot.10 ml. volume)=
Number of organisms on original plate x 100
Number of organisms on dilution #n x 10^{n+2}

An example of this would be:
60 CFU on dilution #3 = $60x10^5$ or $6x10^6$ /plaque specimen.

The gingival index and the plaque index were scored by the primary investigator, and the stain index and bleeding index were scored by a second investigator, who was a senior dental student. This was done to minimize bias, so that, the investigator who was scoring the GI and PI was not influenced by the degree of extrinsic stain that is a common side effect of chlorhexidine.

7. Study Design

After each subject received an identification number, a baseline sample of plaque was taken from two different teeth. The right mandibular central incisor and the right mandibular first molar were chosen to show plaque accumulation representing all areas

were chosen to show plaque accumulation representing all areas of the mouth (Briner et.al., 1986). After the baseline plaque sample was taken, all of the subjects were given a thorough prophylaxis and instructed in oral hygiene procedures. All subjects were instructed to use the Bass technique of toothbrushing, and to brush with the Crest^{R} toothpaste that was provided. They were then instructed on the usage of mouthrinse, and they were all given a cooperation table on which to mark only the times that they completed the mouthrinsing. They were told not to mark the table if they forgot to use the mouthrinse.

The right mandibular central incisor and first molar were isolated with cotton rolls, and the supragingival plaque was removed from these teeth as completely as possible with sterile scalers. Each plaque sample was placed into 10 milliliters of cold, sterile reduced transport fluid (RTF), and it was kept refrigerated for up to 24 hours before processing (Briner et.al., 1986a; van Palenstein-Helderman and Winkler, 1985) (Appendix I).

The specimens were then processed as follows:

1. 10 ml. of RTF specimen was sonicated on a Cole Parmer Ultrasonic Homogenizer (Model 4710). The probe was placed 2/3 of the way down the test tube, and the test tube was kept on ice to make sure that it stayed cold. The output control was set to 50 and sonicated for two minutes.
2. Five tubes containing 0.9 ml. cold RTF were prepared, and 0.1 ml. of sonicate was added to the first tube and vortexed to mix. Serial dilutions were made by adding 0.1 ml. to the next tube.

3. 0.1 ml. from each tube were plated out onto:

   a. Streptococcus mutans selective medium (modified HLR with 20% sucrose, bacitracin and polymycin) (Appendix II) - incubated at 35° anaerobically for 48 hours, followed by aerobically for 24-48 hours (Ritz 1967).

   b. Actinomyces selective medium (containing CdSO4, NaF, neutral acriflavin, tellulrite, and basic fuschin) (Appendix III) - incubated in CO_2 incubator at 35° C for four days (10% CO_2) (Zylber and Jordan, 1980).

The specimens were then read as follows:

1. Streptococcus mutans - medium was selective for Streptococcus mutans, allowing little else to grow. It was subcultured and confirmed with viridans strep identification from two colonies on the plate counted (Setterstrom et.al., 1979).

2. Actinomyces - medium inhibited other organisms, but it was not entirely selective for Actinomyces, only. Actinomyces colonies were bright white and were the whitest colonies which grew on the plates. Three colonial varients are described as:

   a. Smooth round.
   b. Irregular round margin, "hilly" surface.
   c. Irregular shaped, irregular margin, "hilly" surface - smaller than other two types.

   Colonies were usually 1 mm in diameter. Two colonies were Gram stained from the plate that was counted to confirm the morphology of Actinomyces. The calculation for the organism count/plaque specimen were then made as outlined in the reports and records section.

8. Statistical Analysis

This study was designed to provide a minimal power of 87% for detecting a clinically important difference to be statistically significant at the 0.05 probability level.

The mean and standard deviations were determined for the plaque, gingival, and stain indices. Mean differences were calculated and recorded as a percentage difference for all of the groups. The bleeding index was expressed as a percentage of the number of sites that tested positive for bleeding.

Statistical significance was determined using independent groups and paired t-tests. A pooled variance estimate was used for all of the baseline comparisons between the chlorhexidine group and the placebo group. A separate variance estimate was used for all six week and twelve week comparisons between the chlorhexidine group and the placebo group. Significant change over time, was assessed as the difference from baseline to six weeks and to twelve weeks.

Initial descriptive data revealed that the microbiologic data was not normally distributed, which would disallow parametric testing. The Kolmogorov - Smirnov 2-Sample test, a non-parametric test, was used to compare the distribution of Streptococcus mutans and Actinomyces to something expected over the normal distribution.

Arithmetic transformations to the log Base 10 were performed on all of the microbiologic data to assume a normal distribution. Independent t-tests were then used to compare mean values of the chlorhexidine and placebo groups. Non-parametric tests (Wilcoxon

Rank Sum Test) were performed to the above equivalent t-tests, and the results proved to be comparable in both situations, so the parametric test findings were reported.

IV. RESULTS

Thirty-six subjects participated in this clinical trial at the onset of this experiment. Double blinded, randomization procedures produced two evenly divided groups with eighteen individuals in each group. Two patients, one male and one female, were dismissed from this study because of their inability to cooperate. Both of these patients were rinsing with the chlorhexidine mouthrinse, but they only participated in this study for under one week. Therefore, it was decided by the primary investigator that these patients should not be considered as subjects in this clinical trial. As a result, sixteen subjects remained in the chlorhexidine group, and eighteen subjects were in the control group. The mean age of the chlorhexidine group was 14.88 ± 1.78, and the mean age of the placebo group was 14.78 ± 1.52 at the beginning of the experimental period. The mean percent cooperation for the chlorhexidine group was 94.73 ± 4.6 and 93.89 ± 6.1 for the placebo group. The cooperation percentage was determined from the daily logs each subject was keeping during the experimental period.

Baseline data for all of the indices used in this study shows that the two groups were comparable at the onset of the experimental period (Table I).

Comparison of the six week data between the chlorhexidine group and the placebo group showed statistically significant differences in the plaque index, all of the Streptococcus mutans and Actinomyces levels (Table II), and all of the stain recordings, except for the mandibular bucco-lingual stain (Table IV). The gingivitis index did not show a significant difference at this test period,

although, clinically, the mean of the chlorhexidine group was lower than the mean of the placebo group by 11.0%. Table III shows the gingivitis severity and occurrence. Although the severity was reduced by only 11%, the occurrence of normal gingiva in the chlorhexidine group was twice that of the placebo group.

Tables VII and VIII demonstrate the baseline to six week data of the chlorhexidine group and the placebo group (intra-group differences). Within the chlorhexidine group, all of the results were statistically significant at $p < .001$, except for the bleeding index which was statistically significant at $p < .01$. The baseline to six week data for the placebo group also showed statistically significant differences, but only for the plaque index, the gingival index, and the maxillary total stain index. Although these placebo indices demonstrated statistical significance, a much greater statistical significance and clinical difference was demonstrated in the chlorhexidine group.

Staining of the teeth from baseline to six weeks was twice as severe on the mandibular teeth than on the maxillary teeth for the chlorhexidine group.

The microbiological data (Table VIII) for the chlorhexidine group showed significant reductions, in the 99% range, for the baseline to six week results. The placebo group, for this same time period, showed a 16.7% to a 114% difference in these levels, none of which were statistically significant.

Table IX demonstrates a comparison between the chlorhexidine group and the placebo group after twelve weeks of treatment. This table shows that all of these intergroup comparisons were statistically

significant except for the Streptococcus mutans at the incisor site. There are many possible reasons as to why this site did not show a statistically significant difference, such as, the inherent error with the sampling and culturing techniques, and the decreased number of retention sites the incisor possesses compared to the molar.

As seen in Table III, the gingivitis severity became statistically significant at twelve weeks at the $p < .001$ significance level. The percent difference between the six week (11%) data and the twelve week data (56.1%) was approximately 45%. The gingival occurrence at the twelve week interval was 54.2% for the chlorhexidine group, and only 11.1% for the placebo group. There was also a concomitant decrease in gingival severity. Only 5% of the sites in the chlorhexidine group had a G.I.= 2, whereas, 22% of the placebo group had this score on the gingival occurrence index.

Table IV demonstrates the inter-group differences at baseline, six weeks, and at twelve weeks. Plaque severity decreased and became statistically more significant at twelve weeks when compared to baseline or six week data. Plaque occurrence for the number of sites with no detectable plaque (P.I. =0) at twelve weeks was 60.7% for the chlorhexidine group and 13.0% for the placebo group. This represented a 47.7% difference between these two groups.

Tables V and VI show significantly more stain at twelve weeks ($p < .001$) for all of the sites measured. The difference in the percentage of sites with a stain index of 2 (moderate stain), at twelve weeks, between the chlorhexidine group and the placebo group was 10.5%. More severe stain was observed lingually in the maxillary

arch and interproximally in the mandibular arch.

Tables VII and VIII also demonstrate the baseline to twelve week intra-group differences for the chlorhexidine group and the placebo group. There was a statistically significant difference at $p < .001$, for all of the indices measured in the chlorhexidine group. The percent difference from the amount of plaque present was 64.9% for this time period. This means that the severity of plaque decreased significantly from baseline to twelve weeks (Figure I). The gingivitis severity has a similar improvement (Figure II). The mean improvements for the PI and GI were 0.40 and 0.50, respectively. These clinical improvements may be seen in Figures III and IV. Gingival bleeding also decreased significantly from baseline to twelve weeks (Figure V).

Total stain increased more in the mandibular arch than in the maxillary arch (Figure VI). The baseline measurements for both the maxillary and mandibular arches was 0.51 and 0.56, respectively. The maxillary twelve week level increased to 1.41 (152%) which was a mild to moderate stain occurring predominantly on the lingual surfaces, and the mandibular arch increased to 1.86 (265%) which was more of a moderate stain. Figures VII through XVI show the clinical results of the chlorhexidine group.

All of the microbiological data for the baseline to twelve week interval for the chlorhexidine group proved to be significant at $p < .001$ (Table VIII). In fact, the data from this time period did not differ significantly from the baseline to six week data. Both of the twelve week incisor sites slightly increased in the number of bacteria present, and the molar site slightly decreased

in the number of Streptococcus mutans present at twelve weeks, when compared to the baseline to six week results (Figure XVIII). The molar site for Actinomyces remained constant when comparing these time periods (Figure XVII).

The baseline to twelve week results for the placebo group showed statistically significant results at p .05, for both the plaque and gingival indices (Figures I and II). Although these results showed statistical significance, clinically, no true difference existed (Figure XIX-XXII).

Stain also increased for this time period in the placebo group, but the increase shown is minor. Both the total maxillary and total mandibular stain only increased 0.10, and this is only slightly numerically different from the baseline to six week results. The significance both statistically and clinically for the chlorhexidine group, far outweighs that of the placebo group.

None of the microbiological data for the placebo group showed any significance for the baseline to twelve week data, as expected. These results remained quite constant over time (Table VIII).

TABLE I

Comparison of Baseline Data

	Chlorhexidine (n=16) $\bar{X}$ (S.D.)	Placebo (n=18) $\bar{X}$ (S.D.)
Plaque Index	1.14(0.26)	1.03(0.19)
Gingival Index	1.25(0.22)	1.23(0.17)
Bleeding Index % of 96 sites	52.1%	50.9%
Maxillary Total Stain	0.56(0.32)	0.51(0.25)
Mandibular Total Stain	0.51(0.31)	0.54(0.32)
S. mutans (Log/tooth)		
incisor	6.51(2.21)	5.66(2.32)
molar	8.00(1.13)	7.06(2.03)
Actinomyces (Log/tooth)		
incisor	9.01(0.91)	9.03(0.58)
molar	8.65(0.88)	8.11(0.73)

+ no comparisons significant at $p < .05$

(Means were calculated from sites/test period)

TABLE II

Comparison of Six Week Data

	Chlorhexidine (n=16) $\bar{X}$ (S.D.)	Placebo (n=18) $\bar{X}$ (S.D.)	%-Difference
Plaque Index	0.75(.24)	0.95(.17)**	20.5%
Gingival Index	1.03(.24)	1.15(.21)	11.0%
Bleeding Index (% of 96 sites)	36.5%	49%	35.8%
Maxillary Total Stain	0.95(.61)	0.56(.28)*	-71.2%
Mandibular Total Stain	1.25(.68)	0.63(.39)**	-98.4%
S. Mutans (Log/tooth)			
incisor	3.41(1.21)	5.51(2.34)**	99.2%
molar	4.69(2.44)	7.39(2.59)**	99.8%
Actinomyces (Log/tooth)			
incisor	5.38(2.80)	8.95(.60)***	99.9%
molar	5.87(2.67)	8.27(.66)**	99.6%

* $p < .05$

** $p < .01$

*** $p < .001$

$$\% \text{ Difference} = \frac{(\text{Placebo-Chlorhexidine})}{\text{Placebo}} \times 100$$

(Means were calculated from sites/test period)

(Antilogs were used to calculate the percent differences for the microbiological data)

TABLE III

Gingivitis Severity and Occurrence

		$\bar{X}$(S.D.)	%-Difference	Gingival Index Score*			
				0	1	2	3
Baseline	CHX	1.25(.22)		1.6%	71.6%	26.8%	0%
	PL	1.23(.17)		1.6%	74.1%	24.3%	0%
6 weeks	CHX	1.03(.24)	11.0%	14.3%	69.8%	15,9%	0%
	PL	1.15(.21)		7.4%	71.1%	21.5%	0%
12 weeks	CHX	0.50(.27)	56.1%***	54.2%	40.6%	5.2%	0%
	PL	1.14(.28)		11.1%	66.9%	22.0%	0%

CHX= Chlorhexidine

PL= Placebo

* $p < .05$ significant by T-test comparison of means

** $p < .01$ significant by T-test comparison of means

*** $p < .001$ significant by T-test comparison of means

N= 384 gingival sites/test period for CHX group

N= 432 gingival sites/test period for PL group

TABLE IV

Plaque Severity and Occurrence

		$\bar{X}$(S.D)	% Difference	Plaque Index Score*			
Baseline	CHX	1.14(.26)		8.9%	68.2%	22.9%	0%
	PL	1.03(.19)		14.8%	70.4%	14.8%	0%
6 weeks	CHX	0.75(.24)	20.5%*	28.1%	68.5%	3.4%	0%
	PL	0.95(.17)		14.4%	76.8%	8.8%	0%
12 weeks	CHX	0.40(.24)	57.4%***	60.7%	38.8%	0.5%	0%
	PL	0.94(.18)		13.0%	79.6%	7.4%	0%

CHX= Chlorhexidine

PL= Placebo

* $p < .05$ Significant by T-test comparison of means

** $p < .01$ Significant by T-test comparison of means

*** $p < .001$ Significant by T-test comparison of means

N= 384 plaque sites/test period for CHX

N= 432 plaque sites/test period for PL

TABLE V

Stain Severity and Occurrence

	Maxillary Total Stain $\bar{X}$ (SD)	Mandibular Total Stain $\bar{X}$ (SD)	Stain Index Score* 0	1	2	3
CHX	0.51(.32)	0.51(.31)	82.4%	17.3%	0.3%	0%
Baseline						
PL	0.51(.25)	0.54(.32)	82.8%	16.9%	0.3%	0%
CHX	0.95(.61)	1.25(.68)	69.9%	23.8%	6.3%	0%
6 weeks	*	**				
PL	0.56(.28)	0.63(.39)	81.3%	17.8%	1.0%	0%
CHX	1.41(.69)	1.86(.92)	58.0%	30.5%	11.1%	0%
12 weeks	***	***				
PL	0.61(.32)	0.64(.34)	80.2%	19.2%	0.6%	0%

CHX = chlorhexidine

PL = Placebo

* $p < .05$

** $p < .01$

*** $p < .001$

N= 816 stain sites/test period for CHX

N= 918 stain sites/test period for PL

TABLE VI

Comparison of Changes of Interproximal and

Bucco-lingual Stain Severity and Occurrence

		Maxillary		Mandibular	
		ITS	BLS	ITS	BLS
		$\bar{X}$(S.D)	$\bar{X}$(S.D.)	$\bar{X}$(S.D.)	$\bar{X}$(3.0)
Baseline	CHX	0.41(.31)	0.94(.49)	0.43(.31)	0.74(.44)
	PL	0.35(.28)	0.86(.35)	0.38(.32)	0.90(.48)
6 weeks	CHX	0.79(.62) *	1.34(.78) **	1.18(.75) ***	1.35(.75)
	PL	0.40(.33)	0.90(.36)	0.43(.35)	1.07(.63)
12 weeks	CHX	1.26(.74) ***	1.78(.92) **	1.91(.98) ***	1.88(1.23)
	PL	0.44(.34)	0.39(.09)	0.43(.30)	1.17(.55)

CHX= Chlorhexidine

PL = Placebo

ITS= Interproximal Stain N=576 stain sites/test period for CHX;
N=648 stain sites/test period for PL

BLS= Bucco-lingual stain N=240 stain sites/test period for CHX;
N=270 stain sites/test period for PL

* $p < .05$

** $p < .01$

***$p < .001$

TABLE VII COMPARISON OF BASELINE DATA TO SIX WEEK DATA AND BASELINE DATA TO TWELVE WEEK DATA

		Chlorhexidine (N=16)				Placebo (N=18)			
		X (S.D.)		% -Difference		X (S.D.)		%-Difference	
Plaque Index	Baseline	1.14	(.26)	***	***	1.03	(.19)	*	*
	6 weeks	0.75	(.24)	34.2%		0.95	(.17)	7.8%	8.7%
	12 weeks	0.40	(.24)		64.9%	0.94	(.18)		
Gingival Index	Baseline	1.25	(.22)	***	***	1.23	(.17)	*	*
	6 weeks	1.03	(.24)	17.6%		1.15	(.21)	6.5%	7.3%
	12 weeks	0.50	(.27)		60%	1.14	(.28)		
Bleeding Index	Baseline		52.1%	**	***	50.9%		3.6%	
	6 weeks		36.5%	40.3%	77.2%	49.0%			9.1%
	12 weeks		11.5%			47.2%			
Maxillary Total Stain	Baseline	0.56	(.32)	***	***	0.51	(.25)	*	**
	6 weeks	0.95	(.61)	-69.6%	-152%	0.56	(.28)	-9.8%	-19.6%
	12 weeks	1.41	(.69)			0.61	(.32)		
Mandibular Total Stain	Baseline	0.51	(.31)	***	***	0.54	(.32)		**
	6 weeks	1.25	(.68)	-145.1%	-264.7%	0.63	(.38)	-16.7	
	12 weeks	1.86	(.92)			0.64	(.34)		-18.5%

* $p < .05$

** $p < .01$

*** $p < .001$

$$\%\text{-Difference} = \frac{\text{Baseline-Time}}{\text{Baseline}} \times 100$$

TABLE VIII MICROBIOLOGICAL COMPARISON OF BASELINE DATA TO SIX WEEK DATA AND BASELINE DATA TO TWELVE WEEK DATA

		Chlorhexidine (n=16) $\bar{X}$ (S.D.)		%-Difference		Placebo (n=18) $\bar{X}$ (S.D.)		%-Difference	
S. mutans (Log/tooth)									
incisor	Baseline	6.51	(2.21)	*** 99.9%	*** 99.7%	5.66	(2.32)	29.2%	59.3%
	6 weeks	3.41	(1.21)			5.51	(1.21)		
	12 weeks	3.98	(2.21)			5.27	(2.26)		
molar	Baseline	8.00	(1.13)	*** 99.9%	*** 99.9%	7.06	(2.03)	-114%	60.2%
	6 weeks	4.69	(2.44)			7.39	(2.44)		
	12 weeks	4.29	(2.13)			6.66	(2.58)		
Actinomyces (Log/tooth)									
incisor	Baseline	9.01	(0.91)	*** 99.9%	*** 99.9%	9.03	(.58)	16.7%	-35.5%
	6 weeks	5.38	(2.80)			8.95	(.60)		
	12 weeks	5.80	(2.65)			9.16	(.61)		
molar	Baseline	8.65	(0.88)	*** 99.9%	*** 99.9%	8.11	(.73)	-44.2%	-134%
	6 weeks	5.87	(2.67)			8.27	(.66)		
	12 weeks	5.82	(2.54)			8.48	(.67)		

* $p < .05$
** $p < .01$
*** $p < .001$

Antilogs were used to determine %-Difference

$$\%\text{-Difference} = \frac{\text{Baseline-Time}}{\text{Baseline}} \times 100$$

TABLE IX

Comparison of Twelve Week Data

	Chlorhexidine (n=16) $\bar{X}$ (S.D.)	Placebo (n=18) $\bar{X}$ (S.D.)	% Difference
Plaque Index	0.40(.24)	0.94(.18)***	57.5%
Gingival Index	0.50(.27)	1.14(.28)***	56.1%
Bleeding Index % of 96 sites	11.5%	47.2%***	74.2%
Maxillary Total Stain	1.41(.69)	0.61(.32)***	-131.1%
Mandibular total Stain	1.86(.92)	0.64(.34)***	-190.6%
S. mutans (Log/tooth)			
incisor	3.98(2.21)	5.27(2.26)	94.9%
molar	4.29(2.13)	6.66(2.58)*	99.6%
Actinomyes (Log/tooth)			
incisor	5.80(2.65)	9.16(.61)**	99.9%
molar	5.82(2.54)	8.48(.67)***	99.8%

* $p < .05$

* $p < .01$

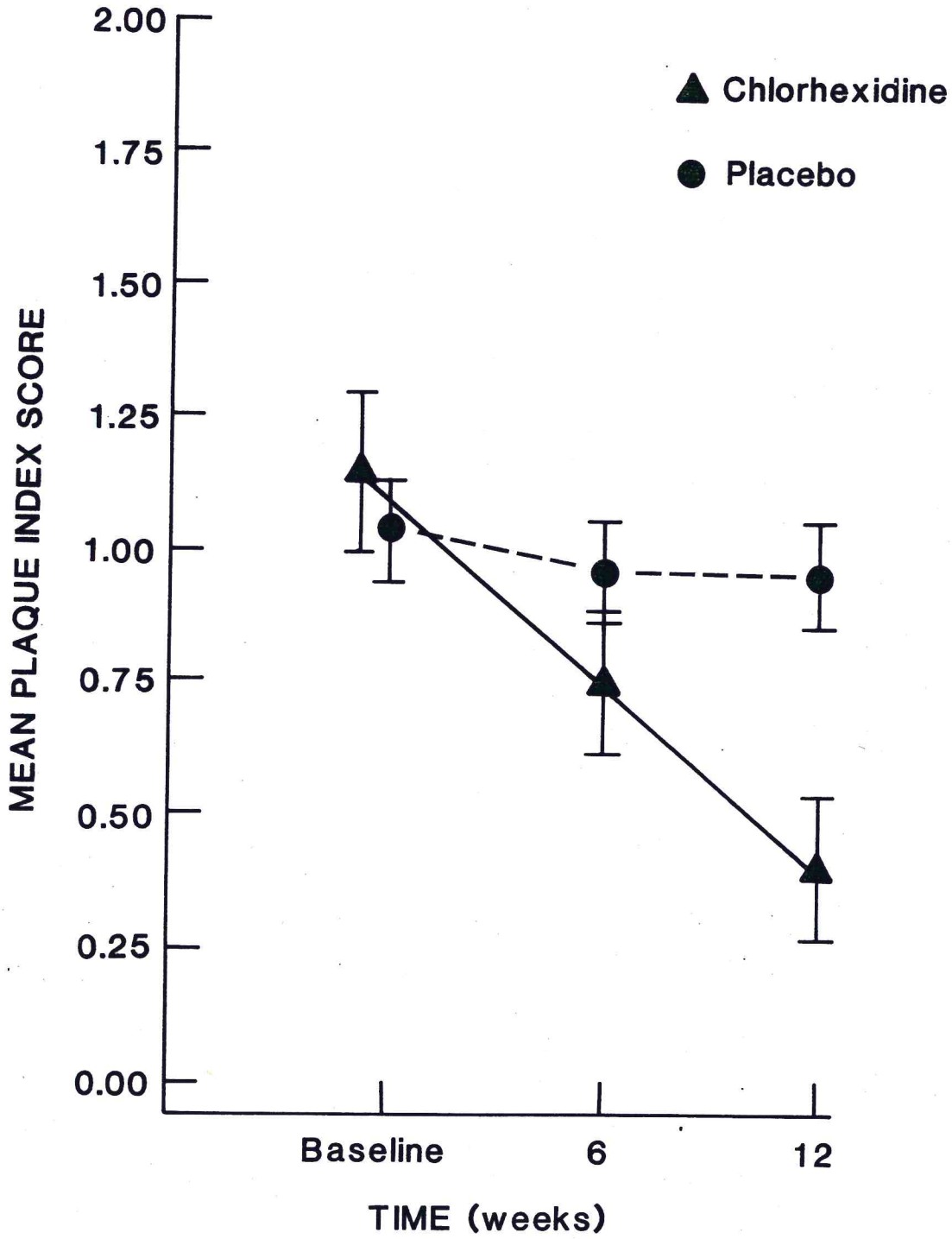
* $p < .001$

$$\%\text{-Difference} = \frac{\text{Placebo-Chlorhexidine}}{\text{Placebo}} \times 100$$

(Mean were calculated from sites/test period)

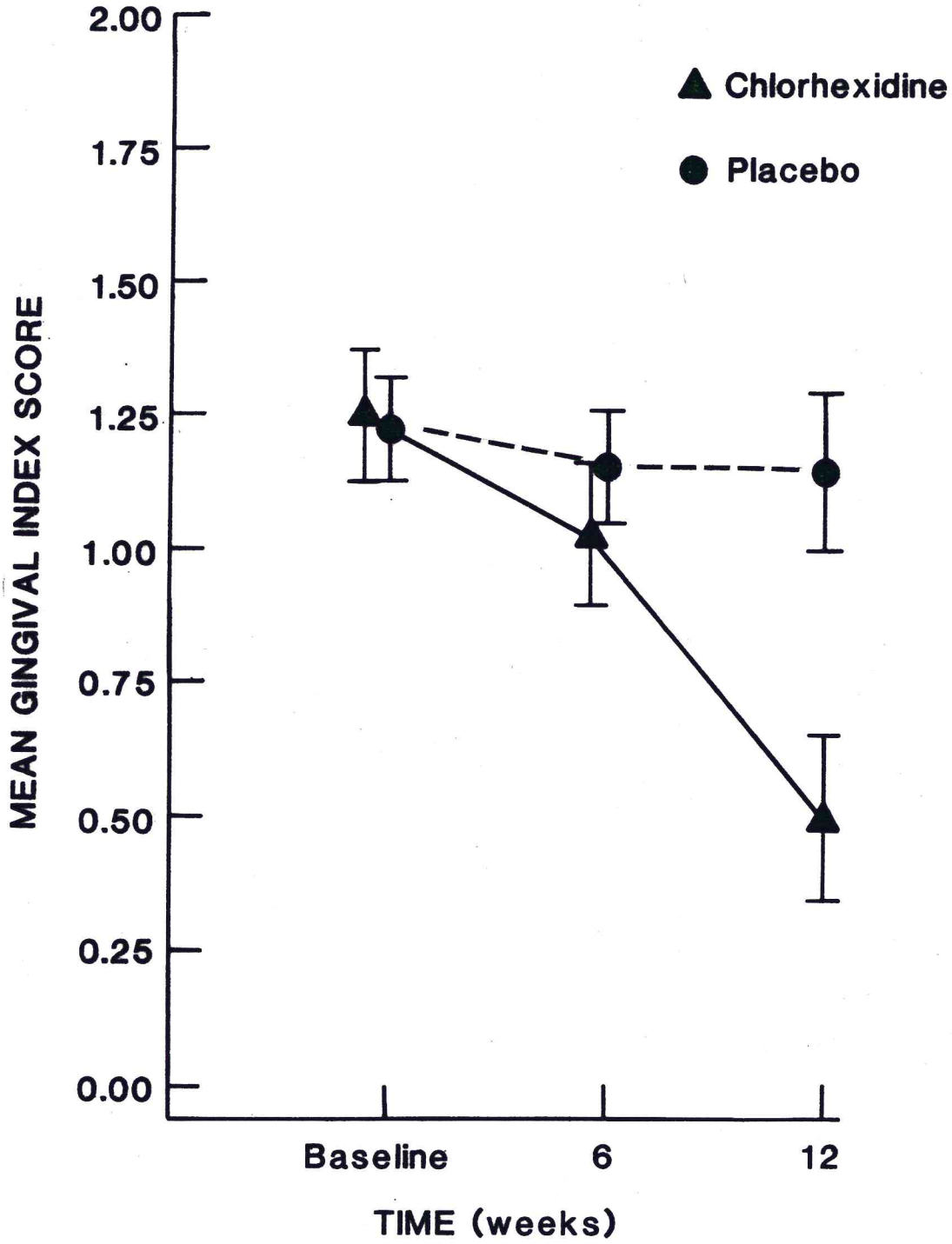
(Antilogs were used to calculate the percent differences for the microbiological data)

FIGURE I



*95% Confidence Interval Around Mean

FIGURE II



*95% Confidence Interval Around Mean

FIGURE III

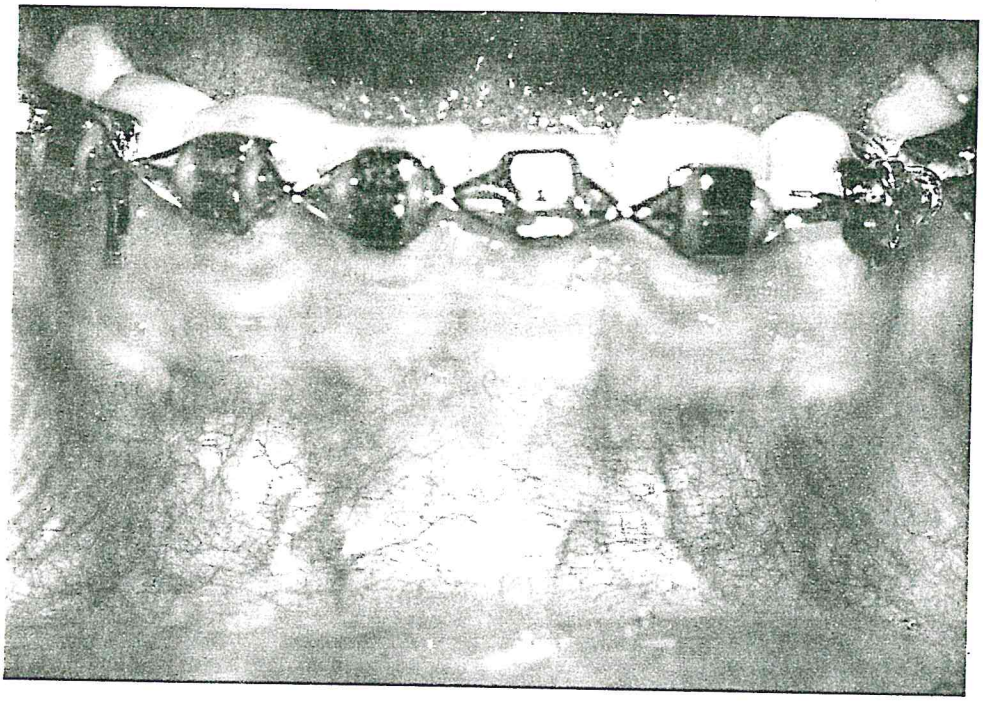
Baseline of patient using chlorhexidine mouthrinse
Note: Presence of gingival inflammation

Patient # 1

(Tannic acid user)

FIGURE IV

3 months of using chlorhexidine mouthrinse
Note: Absence of gingival inflammation and slight increase of yellowish-brown stain



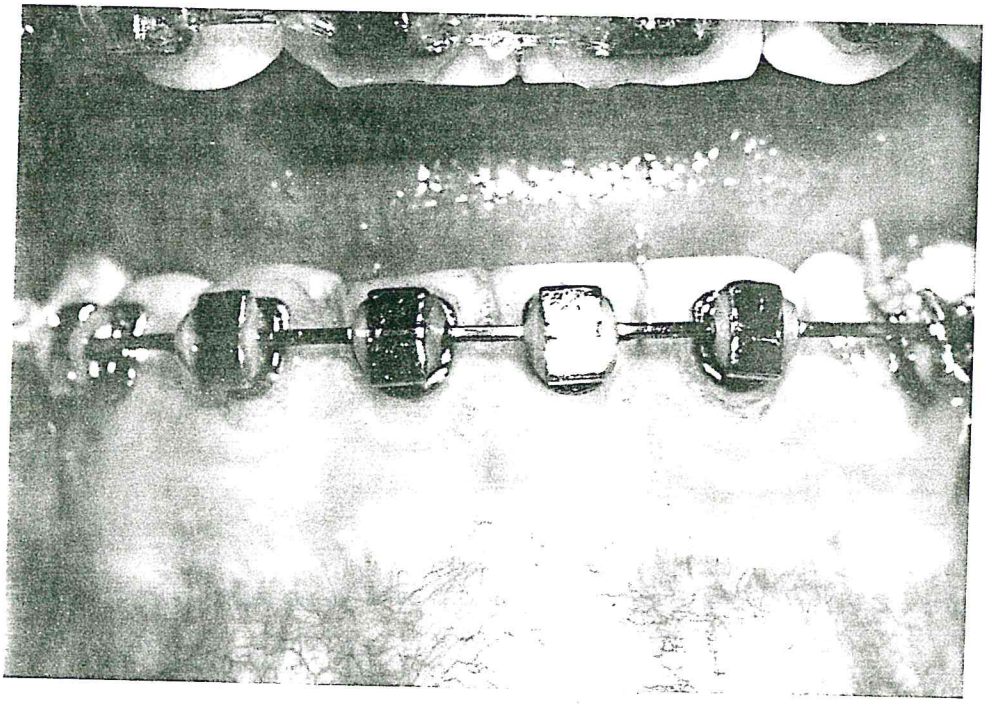
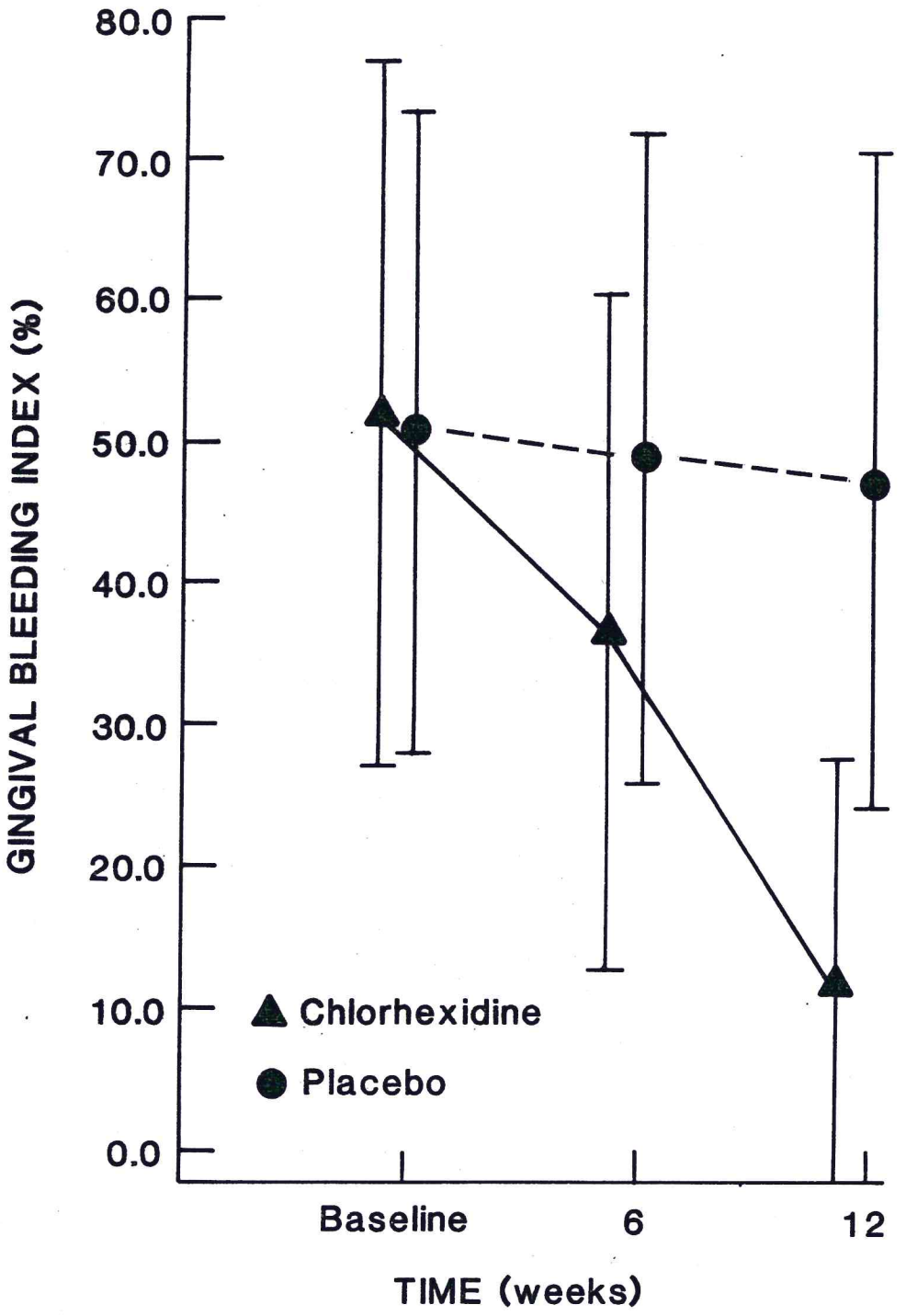
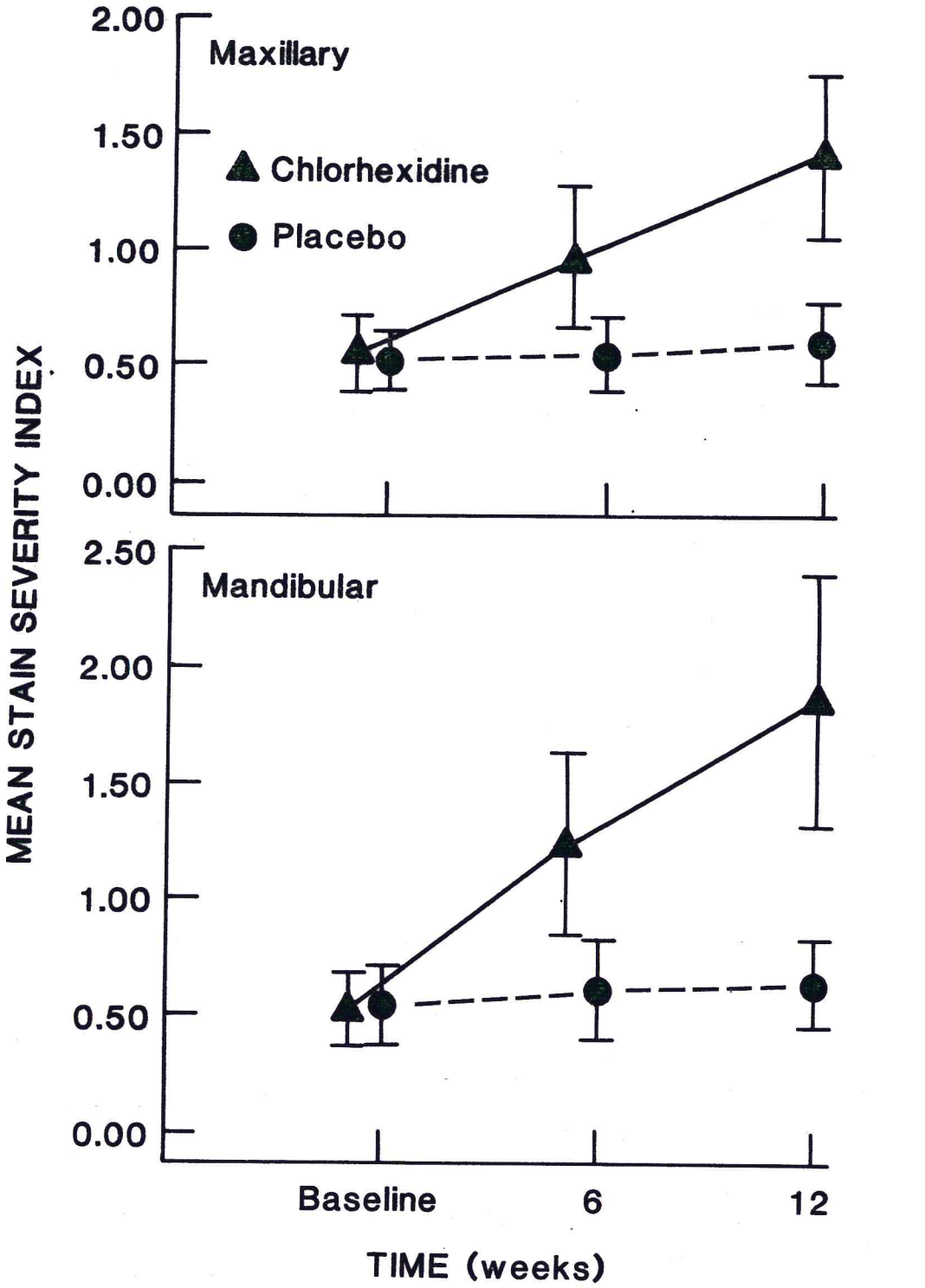


FIGURE V



*95% Confidence Interval Around Mean

FIGURE VI



*95% Confidence Interval Around Mean

FIGURE VII

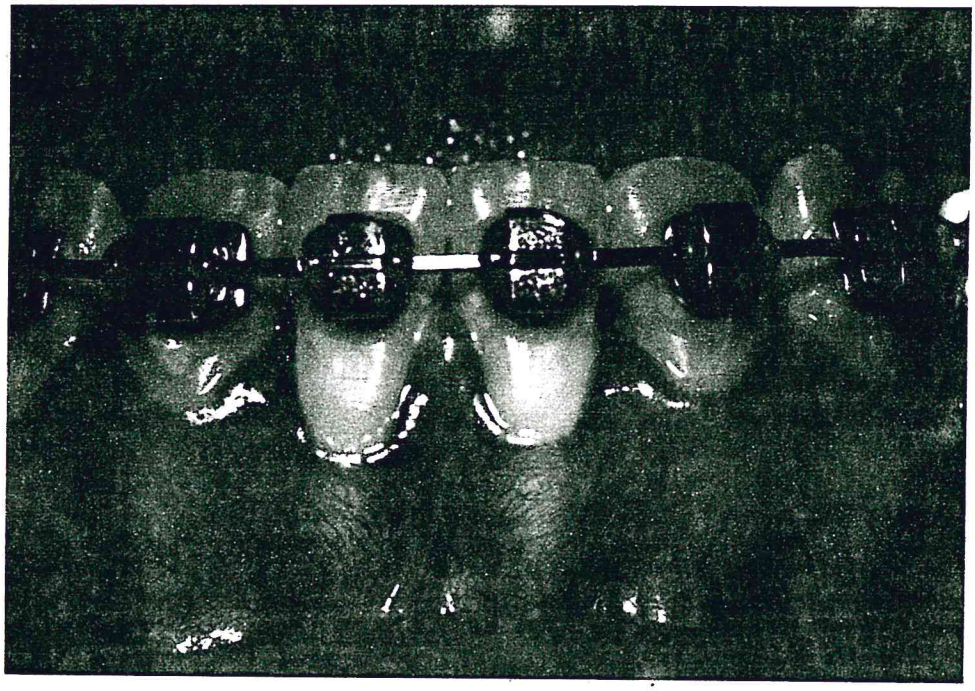
Baseline of patient using chlorhexidine mouthrinse
Note: Gingival inflammation

Patient #35

(non-tannic acid user)

FIGURE VIII

3 months of using chlorhexidine mouthrinse
Note: Reduction of gingival inflammation, very slight increase of stain, and calculus formation



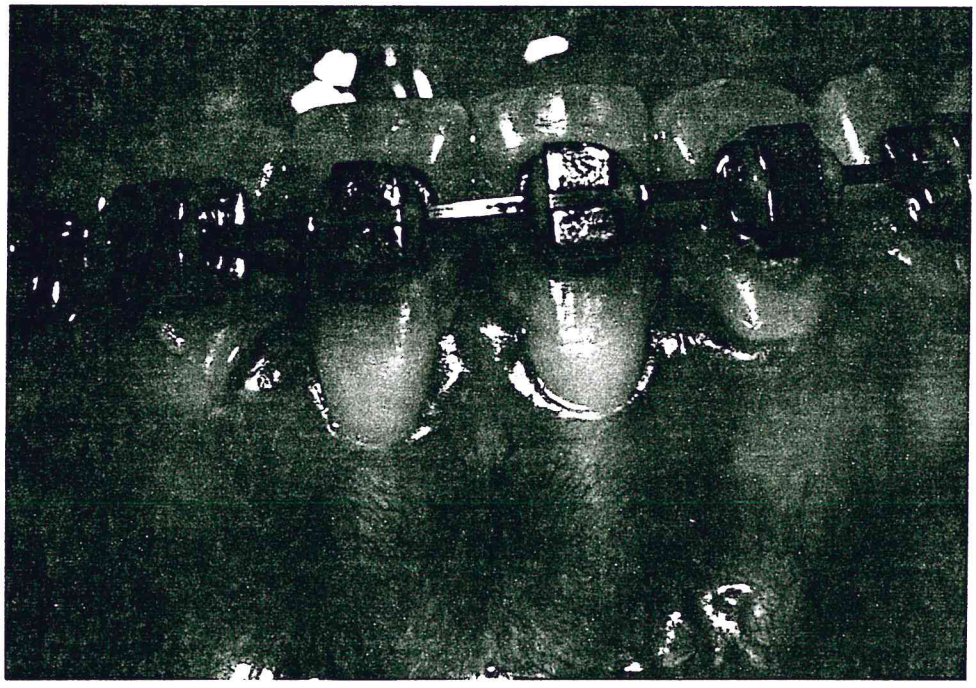


FIGURE IX

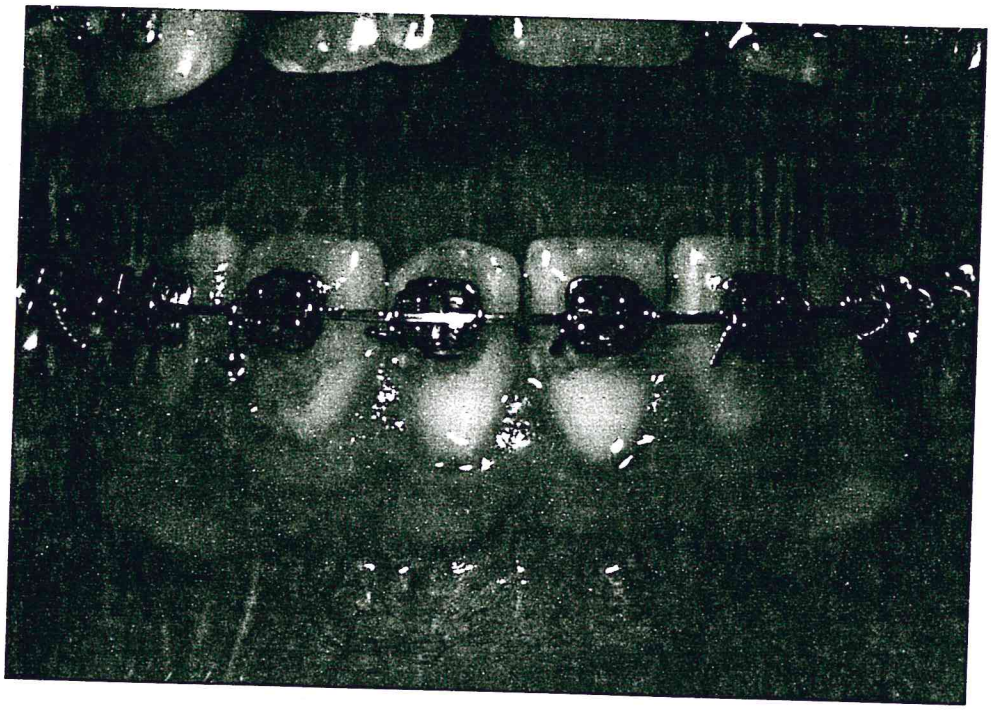
Baseline of patient on chlorhexidine mouthrinse
Note: Presence of gingival inflammation and slight extrinsic staining

Patient #26

(Tannic acid user)

FIGURE X

3 months of using chlorhexidine mouthrinse
Note: Reduction of gingival inflammation and brownish stain



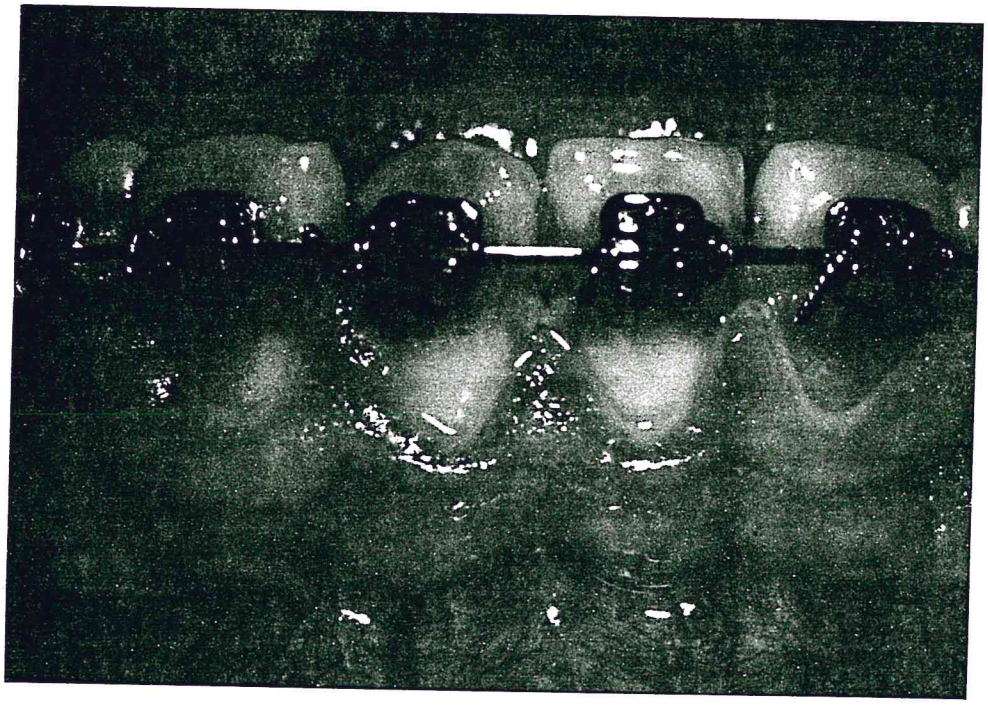


FIGURE XI

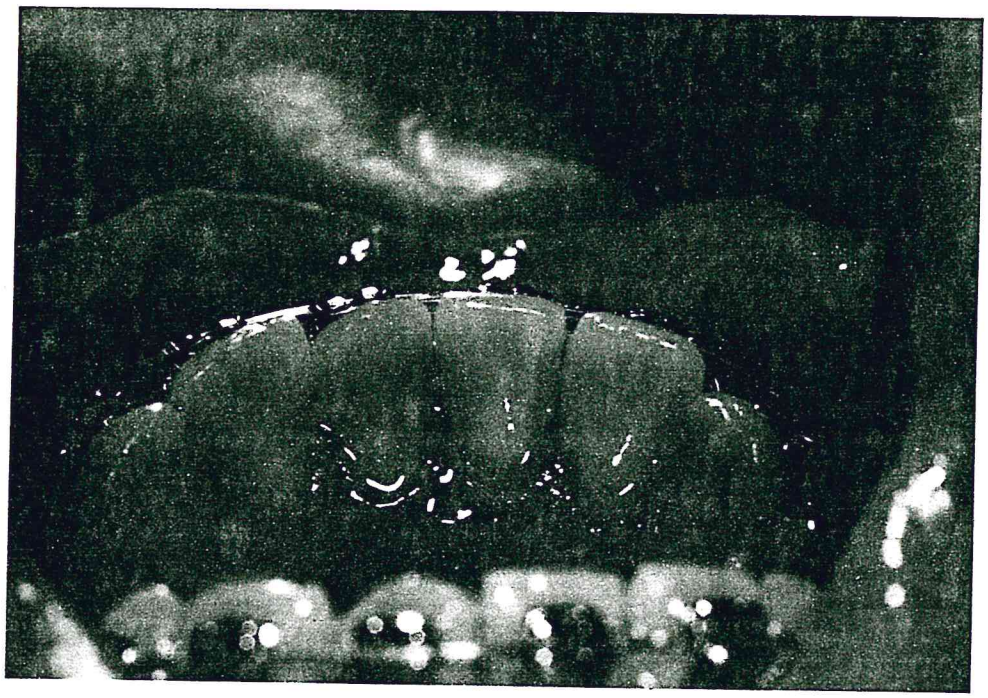
Baseline of patient on chlorhexidine mouthrinse
Note: Gingival inflammation, slight plaque accumulation, and slight staining

Patient #26

(Tannic acid user)

FIGURE XII

3 months of using chlorhexidine mouthrinse
Note: Absence of gingival inflammation, presence of yellowish-brown stain, and calculus formation



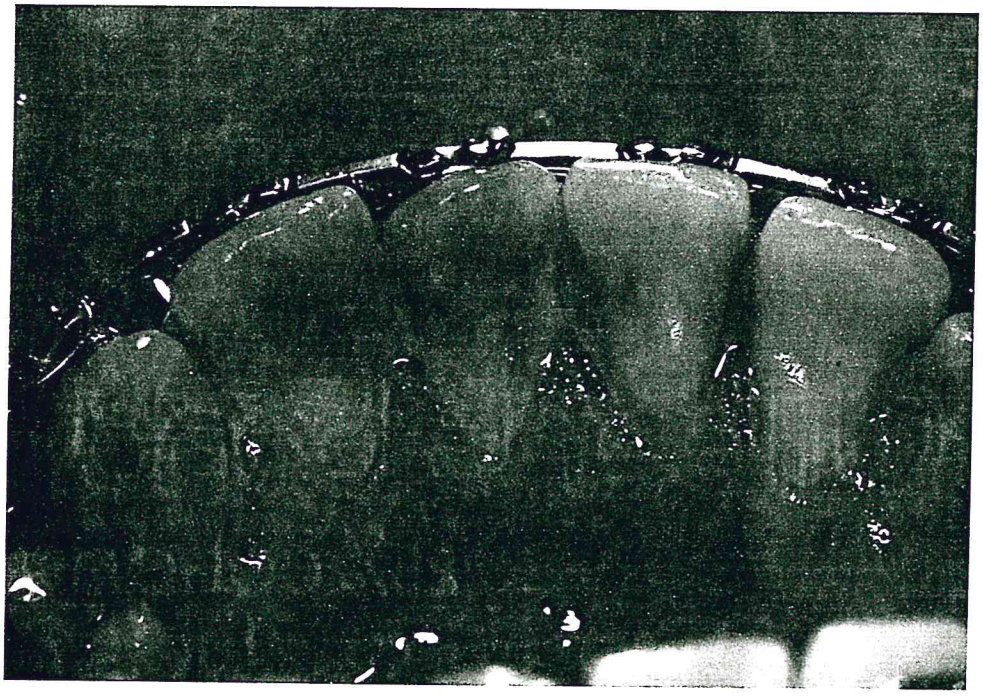


FIGURE XIII

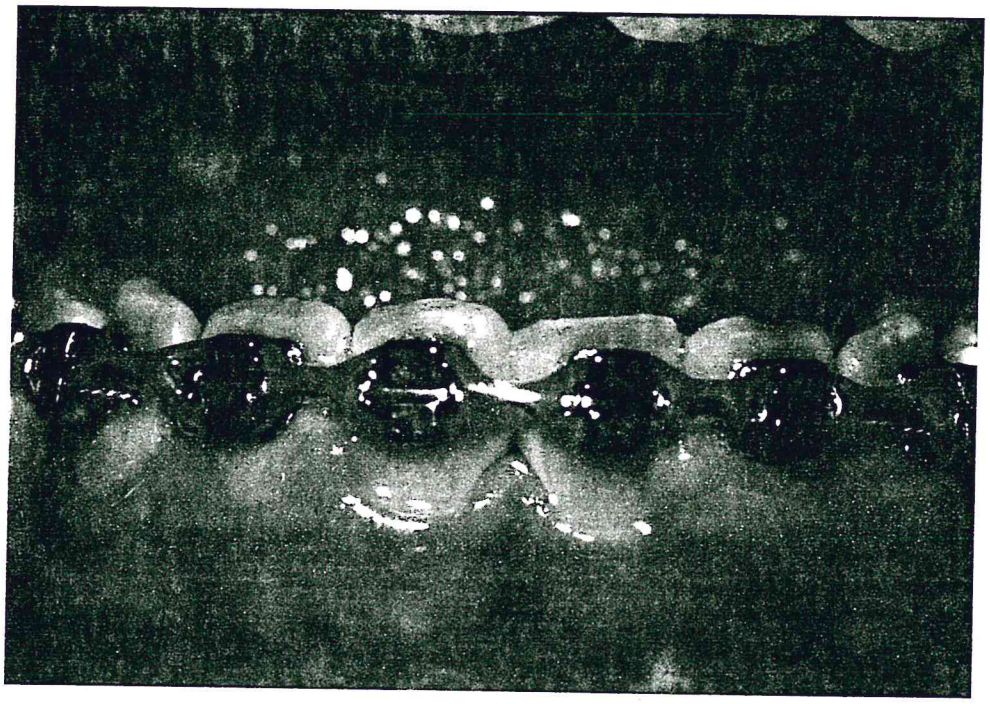
Baseline of patient using chlorhexidine mouthrinse
Note: Presence of gingival inflammation

Patient #19

(Tannic acid user)

FIGURE XIV

3 months of using chlorhexidine mouthrinse
Note: Reduction of gingival inflammation
and yellowish-brown stain



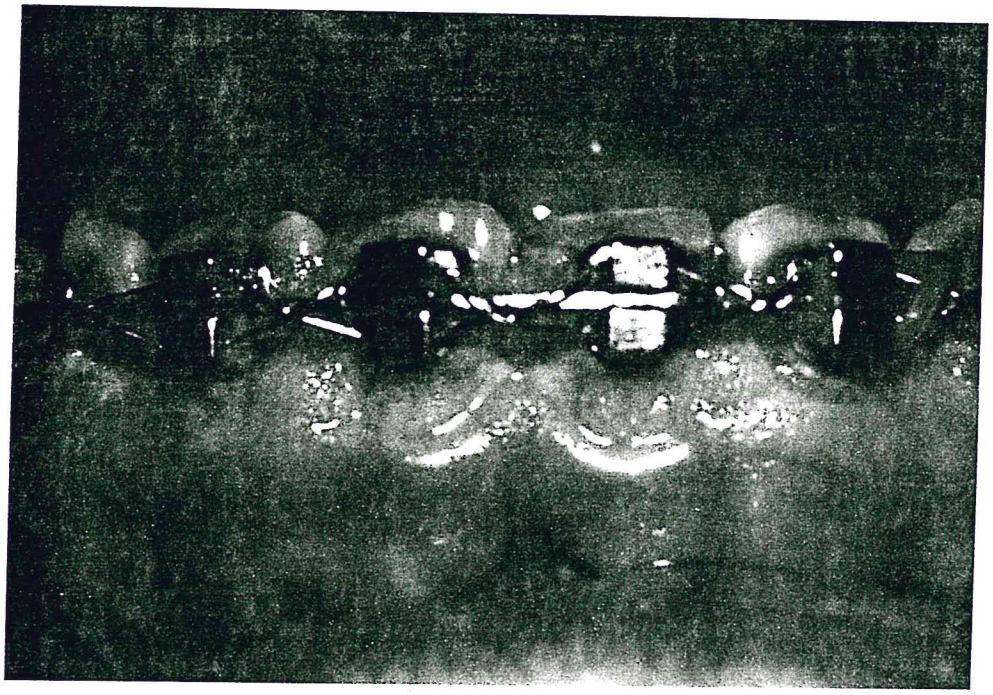


FIGURE XV

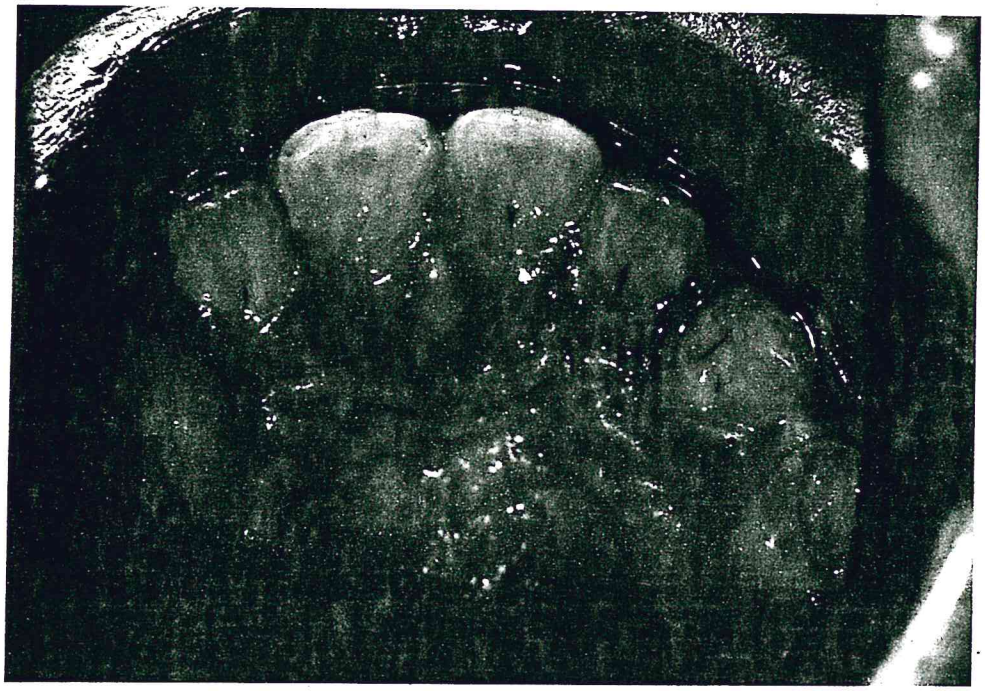
Baseline of patient using chlorhexidine mouthrinse
Note: Presence of some gingival inflammation and some slight stain

Patient #10

(Tannic acid user)

FIGURE XVI

3 months of using chlorhexidine mouthrinse
Note: Reduction of gingival inflammation and increased staining of the lingual and interproximal surfaces



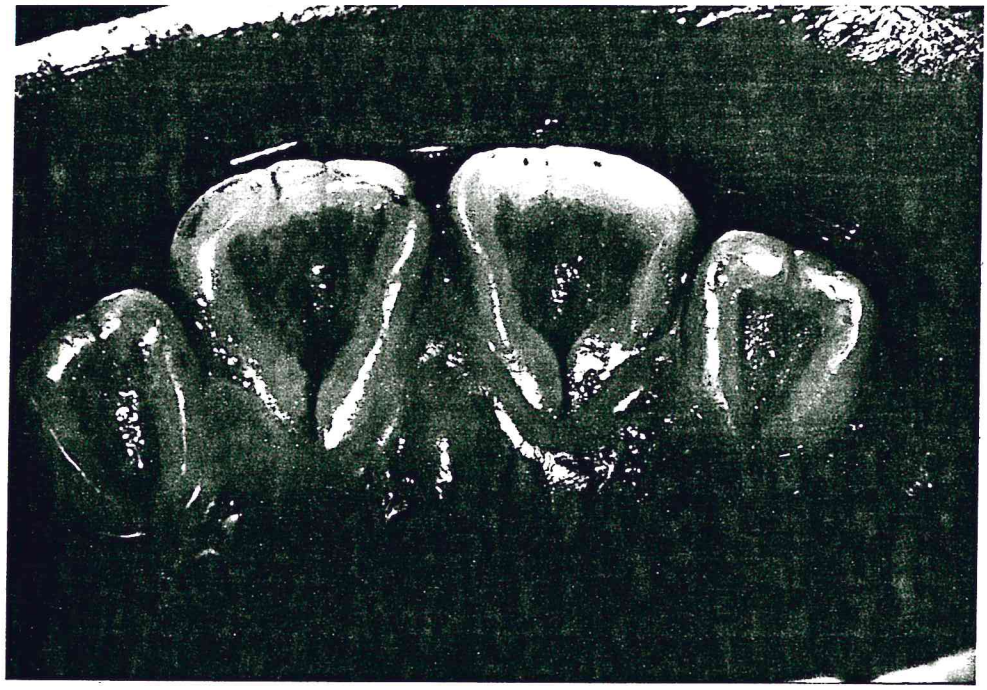
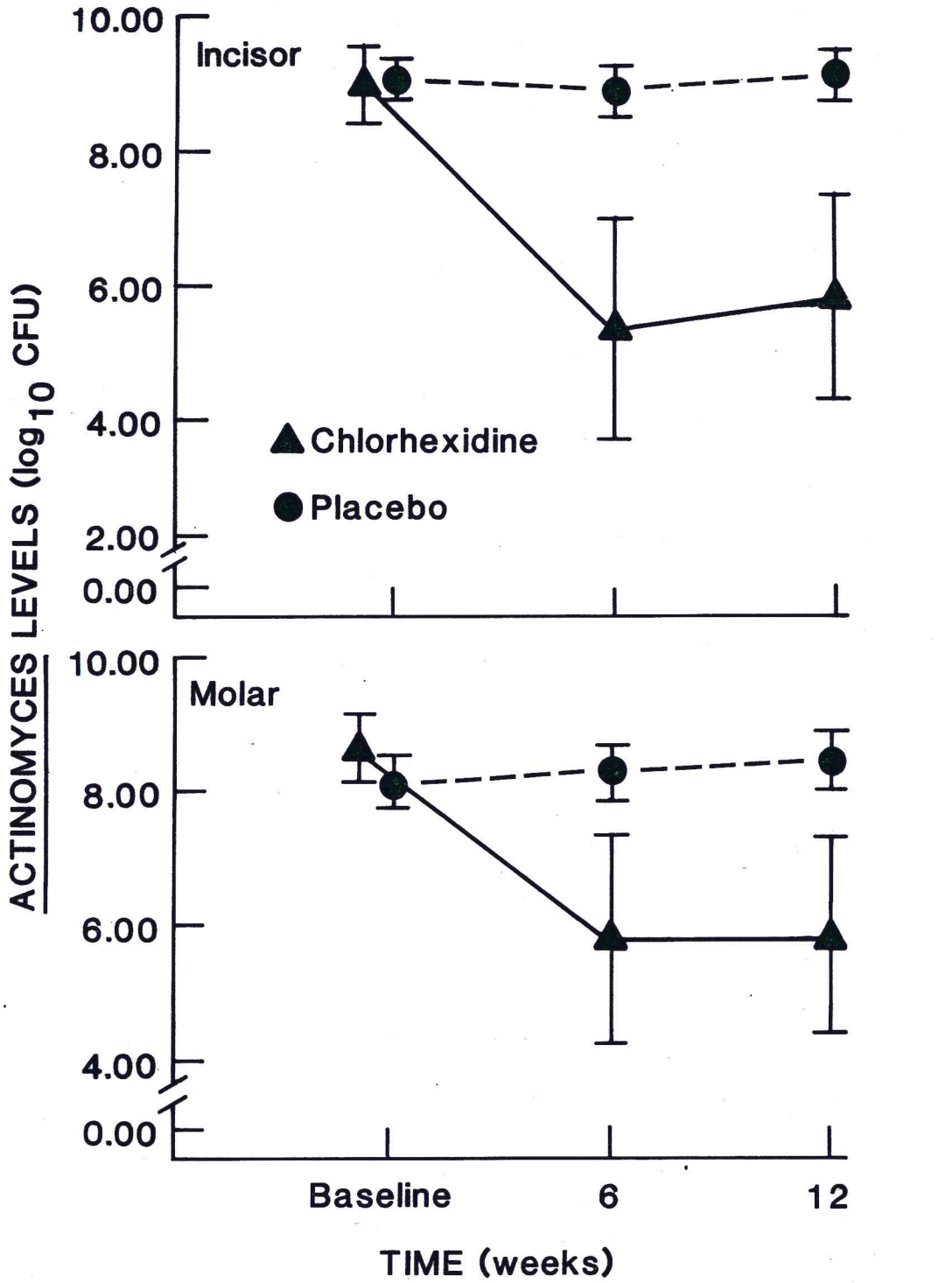
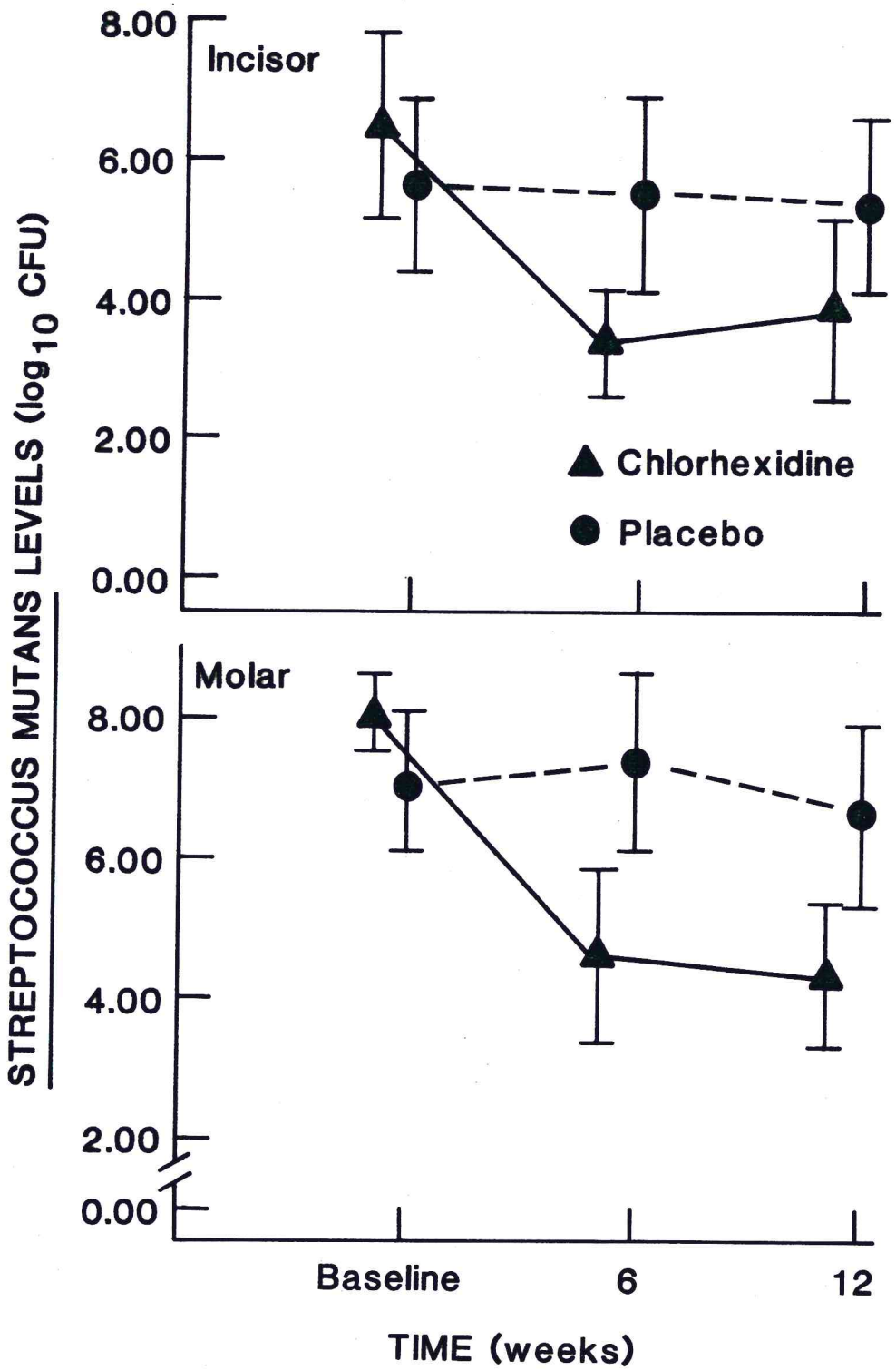


FIGURE XVII



*95% Confidence Interval Around Mean

FIGURE XVIII



*95% Confidence Interval Around Mean

FIGURE XIX

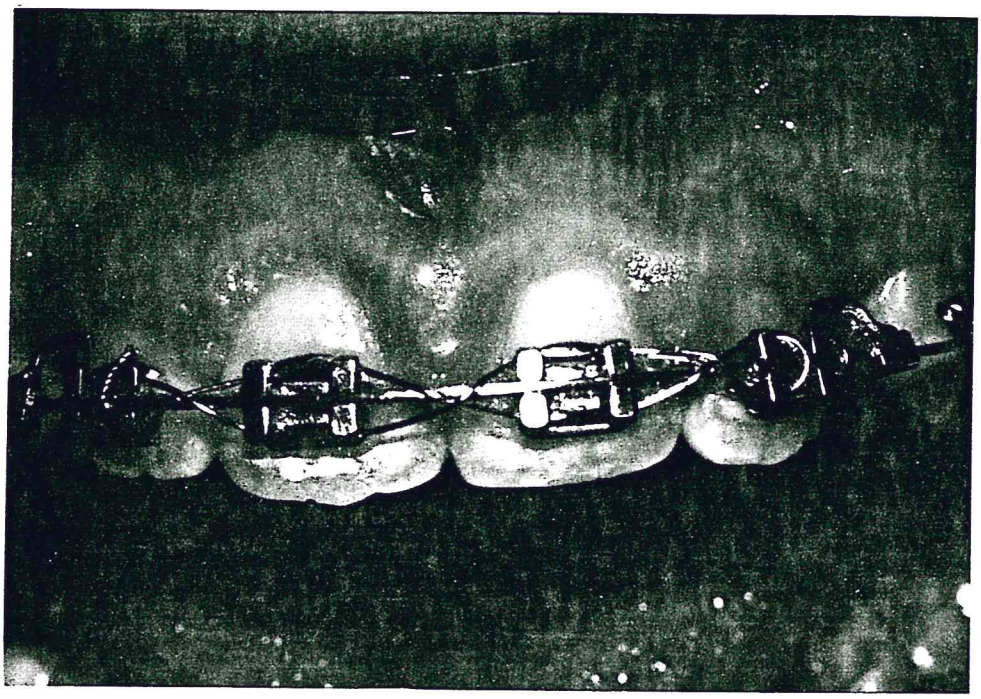
Baseline of patient using placebo mouthrinse
Note: Gingival inflammation, especially interproximally

Patient #18

(non-tannic acid user)

FIGURE XX

3 months of using placebo mouthrinse
Note: Continued presence of gingival inflammation, as seen above



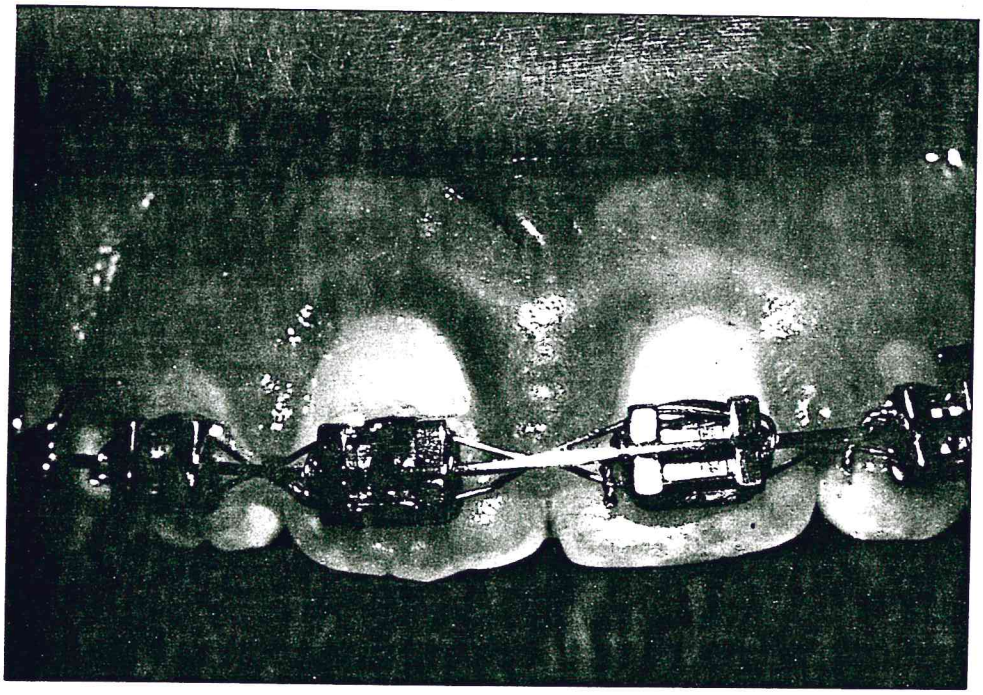


FIGURE XXI

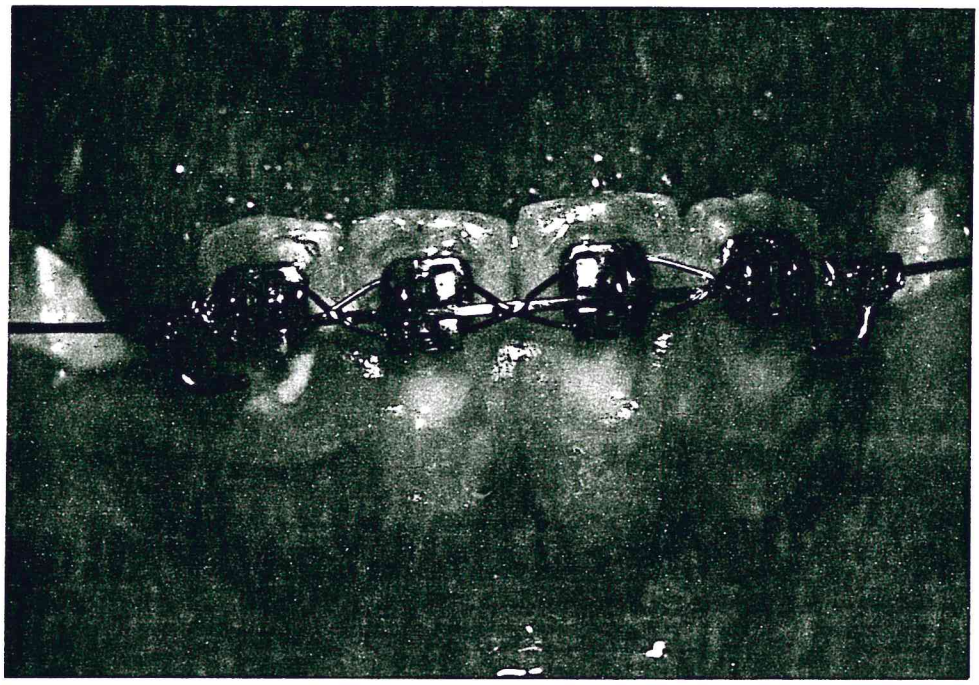
Baseline of patient using placebo mouthrinse
Note: Presence of gingival inflammation and plaque accumulation

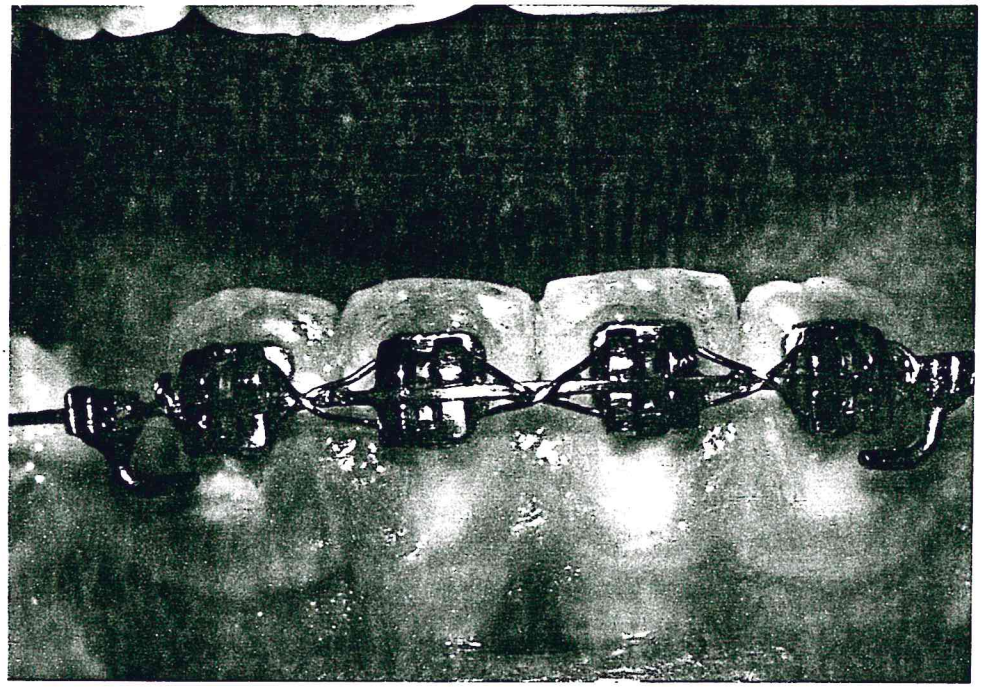
Patient #18

(non-tannic acid user)

FIGURE XXII

3 months of using placebo mouthrinse
Note: Continued presence of gingival inflammation and plaque accumulation





DISCUSSION

For the past two decades, there have been many clinical and animal studies that have documented the beneficial effects of chlorhexidine in the treatment of plaque related diseases (Loe and Schiott, 1970; Flortra et.al., 1972; Loe et.al., 1976; Briner et.al., 1980; Lang et.al., 1982; Briner et.al.; 1986 a, b; Grossman et.al., 1986). The use of chlorhexidine in orthodontic patients, however, has not been widely documented (Lundstrom and Krausse, 1987).

This study was conducted to evaluate the clinical and microbiological efficacy of a 0.12% chlorhexidine gluconate mouthrinse in an orthodontic population, aged eleven through seventeen, during three months of twice daily use. The major side effect of chlorhexidine, which is tooth stain, was also monitored.

The difference in the gingival index, plaque index, bleeding index and the Streptococcus mutans and Actinomyces levels among individuals using experimental and placebo mouthrinses, indicate that a 0.12% chlorhexidine gluconate mouthrinse, used twice daily, effectively reduces plaque accumulation, gingival inflammation and gingival bleeding. Significant reductions in Streptococcus mutans and Actinomyces levels were also observed.

Subjects participating in this study presented with gingivitis and plaque ranging from the absence of gingivitis and plaque to moderate gingivitis and plaque. There was never any severe gingivitis (G.I.=3) or plaque (P.I.=3) present at any time during this study. The gingival and plaque indices were initially greater than those seen in a non-banded population because of the presence of orthodontic appliances. These increased index values correspond with values

from other studies involving fully banded orthodontic patients (Zachrisson and Zachrisson, 1972).

The reduction in the occurrence of gingivitis was much greater from baseline to three months in the chlorhexidine group than in the placebo group. This indicated that the chlorhexidine subjects had a significantly greater proportion of healthy gingival sites. These results correspond to similar studies that have recently been conducted. Segreto et.al. (1986) found an average of 28% less gingival occurrence in a three month study. Grossman et.al. (1986), during a six month experimental period, found an average of a 29% decrease in gingival occurrence after three months, and an average of a 37% decrease at the conclusion of this six month study.

Both the plaque and gingival indices had comparable reductions in severity over the three month experimental period. Comparisons of the six week data showed that there was a more immediate, significant decrease in the amount of plaque present, and this decrease was even more profound at twelve weeks. The gingivitis severity took more time to show a significant reduction, but at twelve weeks, when there was a significant reduction, it paralleled the reduction in the plaque index. A possible explanation for this result is that it takes longer for chlorhexidine to show a tissue response of the gingiva, than it takes to show a clinical reduction in the amount of plaque present. This result also corresponds with the significant reductions in the microbiological counts as early as six weeks.

Although both the chlorhexidine group and the placebo group

showed statistically significant reductions in the plaque and gingival indices, the placebo group's results were not nearly as profound as the chlorhexidine group's results. The reductions found in the chlorhexidine group were clinically significant and the reductions found in the placebo group were not of clinical significance.

The reduction of bleeding sites was greater at the twelve week period than at the six week period. This corresponds to the greater reduction in the gingival index at twelve weeks than at six weeks of time. This index proved to be quite significant showing a 77% reduction in the total number of bleeding sites in the chlorhexidine group. This result correlated directly with similar studies by Segreto et.al (1986) and Grossman et.al. (1986), where they found an average of 53% reduction and a 44% reduction in the number of bleeding sites, respectively.

The reductions in the plaque, gingival, and bleeding indices all corresponded with the concomitant reductions in both Actinomyces and Streptococcus mutans levels found at both the baseline to six week and the baseline to twelve week time periods in the chlorhexidine group. By the end of this study, there were no statistically significant differences between the incisor site and the molar site, in the chlorhexidine group. Significant differences remained throughout the treatment period in the placebo group. The molar site had consistently more Streptococcus mutans at all time periods than did the incisor site in the placebo group. However, the incisor site had slightly higher levels of Actinomyces than the molar site. This result could explain the increased caries rate commonly seen in the molar site over the incisor site, and the fact that the

molar has a greater number of retention sites than the incisor.

The reductions in both the levels of Actinomyces and Streptococcus mutans in the chlorhexidine group were similar, in this study, to the results of Briner et.al. (1986 a,b). The reductions were both very significant at $p < .001$ when comparing the baseline to the twelve week values. A study on the levels of Streptococcus mutans in orthodontic patients (Lundstrom and Krasse, 1987), found that the number of Streptococcus mutans could be significantly suppressed through the use of chlorhexidine, and therefore, decrease the caries risk in this population of patients.

Syed and Loeshe (1978) stated that either the length of exposure of the gingival tissues to the plaque accumulation or the development of more virulent bacteria in the flora, not the plaque mass, per se, is responsible for the development of gingivitis. In this study, a decrease in the plaque biomass, most likely from use of the 0.12% chlorhexidine gluconate mouthrinse, paralled the clinical observations of a decrease in the plaque, gingival and bleeding indices.

Stain was evaluated in this study because it is the primary side effect of chlorhexidine, and it can be a major esthetic concern to the patients using this drug. In this study, both the chlorhexidine group and the placebo group showed significant increases in the degree of stain, but the chlorhexidine group had a much more significant amount of stain. Siegrist et.al. (1986) also found staining in both the chlorhexidine and placebo groups. Dietary factors are a probable cause that staining is observed in the placebo group. Tannic acid, and in particular tea, has been found to be a causitive

agent in producing increased degrees of stain in chlorhexidine users. (Prayitno et.al., 1979a; Addy et.al., 1979; Prayitno et.al., 1979b). Although this study involved a teenage population, the percent of iced tea drinkers approximated 50% in both the groups in this study.

The stain in the chlorhexidine group was significantly greater in the mandibular arch than in the maxillary arch. This was probably due to gravity. The stain was concentrated in the bucco-lingual areas in the maxillary arch, and in the mandibular arch, the stain was evenly divided between the bucco-lingual areas and the interproximal areas. Clinically, the amount of stain present was mild to moderate (Stain Index = 1 to 2). The placebo group showed slight stain in the less than mild range, and the severity of change only increased .10 during the entire experimental period. This was hardly a clinically significant observation.

A 0.12% chlorhexidine gluconate mouthrinse is recommended as a plaque reducing agent. Its use in full-banded/bonded orthodontic patients to reduce plaque accumulation, gingivitis, gingival bleeding, Actinomyces and Streptococcus mutans levels has been demonstrated in this study over a six to twelve week period.

SUMMARY AND CONCLUSIONS

The concept of a chemical agent to enhance oral health has long been considered, and the importance for such an agent is even greater with orthodontic patients with established gingivitis. It was the purpose of this study to determine the efficacy of a 0.12% chlorhexidine gluconate mouthrinse as an antiplaque and anti-gingivitis agent, providing substantial clinical reductions in gingivitis occurrence, severity, and bleeding, in a teenage orthodontic population.

Thirty-four patients, sixteen experimental and eighteen control, used a chlorhexidine or a placebo rinse, respectively, for a three month period. The results showed a significant reduction of plaque accumulation, gingival inflammation, gingival bleeding, and Actinomyces and Streptococcus mutans levels could be attained while using the chlorhexidine gluconate mouthrinse. Although optimal levels of gingival health were not obtained in all subjects while using chlorhexidine gluconate, the reduction of plaque was associated with a reduction of gingival inflammation.

Based on these observations, chlorhexidine gluconate can be considered a safe, effective, and acceptable agent to reduce plaque formation. Recommendations for the use of chlorhexidine gluconate are suggested to decrease plaque accumulations and improve the health of the gingival tissue in patients undergoing active orthodontic treatment. The staining seen with the use of chlorhexidine gluconate is significant to a mild to moderate degree, and it can be removed with a dental prophylaxis. Although, the taste may be bitter to some, the advantages of this drug far outway the disadvantages,

and make it an acceptable chemical agent to reduce plaque and gingivitis.

Therefore, it can be concluded that 0.12% chlorhexidine gluconate, proved to be an important therapeutic agent in controlling gingival inflammation, gingival bleeding, plaque accumulation, and in reducing selective bacterial levels in orthodontic patients. Its effect on these clinical and microbiological levels, in this study, was found to be best when used for the full three months of therapy.

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VIII. APPENDIX

APPENDIX 1

RTF with Asolectin

	Lot #	Wt/L
Ingredients		
Yeast Extract		5.00 gm
Asolectin (See Premix below)		0.30 gm
Bacto-peptone		1.00 gm
NaCl		8.50 gm
L(+) Cysteine HCl monohydrate		0.50 gm
Na_2HPO_4(Anhydrous)		0.868 gm
KH_2PO_4(Anhydrous)		0.528 gm
Tween 80		1.00 gm
Glycerine		150.0 ml
+DRO Water q.s. (or good quality Distilled H_2O)		1000.0 ml
pH Adjustment IN NaOH to pH 6.9-7.1		

Record pH_____

*Asolectin - 95% purified soy phosphetides distributed by:
Associated Concentrates
32-34 61st Street
Woodside, L.I., New York 11377

Directions: Medium is heated to near boiling while N is bubbled through the medium. The hot medium is dispensed into screw cap vials while bubbling N_2into the vials. Caps are replaced tightly. The vials are autoclaved for 15 minutes at 121°C and 15 p.s.i. Pressure to sterilize.

Pre-Mix: Dissolve Tween 80 in 10% of DRO H_2O, then add asolectin with stirring and heating until a cloudy solution results. (Heat to approximate 80 C). Then the rest of the ingredients should be added slowly, with stirring.

APPENDIX II

S. mutans HLR - MODIFIED

Ingredient	Wt./L
Trypticase Soy Agar	40.0 g
Sucrose	200.0 g
Autoclave Crystal Violet[1]	1.0 ml
Distilled Water	1000.0 ml
Add aseptically at 50°C	
Polymixin B, Sulfate[2]	1.0 ml
Bacitracin[3] 200 units/	0.4 ml
Autoclave Pressure _____	
Temp _____	
Time _____	
Temp at aseptic addition _____	
Delivery setting _____	
Yield _____	

1) Crystal Violet Solution (500 ppm) add 0.05 gm crystal violet per 100 ml distilled water. Filter sterilize and refrigerate.

2) Polymixin B. Sulfate Solution (Aerosporin product of Pfizer Inc.), add 0.0875 gm of Aerosporin per 50 ml distilled water, filter sterilize.

APPENDIX III

Actinomycetes Media (laked blood)

Ingredient (Lot #)	Wt/L
Trypoticase Soy Broth	30.0
Glucose (Dextrose)	5.0
Agar (granulated)	15.0
$CdSO_4$	0.013
Autoclave Naf	0.080
Neutral acriflavin[1]	1.0ml
K-tellurite[2]	1.0ml
Basic Fuchsin [3]	0.125 ml
Distilled Water	850.0ml
Add aseptically 50°C	
Lake Sheep Blood[4]	30.0ml
Sheep Serum	40.0ml
Autoclave Pressure_____	
Temp _____	
Time _____	
Temp. at aseptic addition _____	
Delivery setting_____	
Yield_____	

[1]Neutral acriflavin solution 0.113 gm Neutral acriflavin per 100 ml H_2O.

[2]K-tellurite solution 0.25gm of K-tellurite per 100ml H_2O.

[3]Basic fuchsin solution 0.2% basic fuchsin. 0.2 gm of basic fuchsin/100ml H_2O.

[4]Add 20.0ml of sterile distilled water to 10.0ml difibrinated Sheep Blood. Distribute to sterile centrifuge tubes and centrifuge 15 minutes @ 7 on Backman Centrifuge. Record _____rpm.

DISCUSSION

For the past two decades, there have been many clinical and animal studies that have documented the beneficial effects of chlorhexidine in the treatment of plaque related diseases (Loe and Schiott, 1970; Flortra et.al., 1972; Loe et.al., 1976; Briner et.al., 1980; Lang et.al., 1982; Briner et.al.; 1986 a, b; Grossman et.al., 1986). The use of chlorhexidine in orthodontic patients, however, has not been widely documented (Lundstrom and Krausse, 1987).

This study was conducted to evaluate the clinical and microbiological efficacy of a 0.12% chlorhexidine gluconate mouthrinse in an orthodontic population, aged eleven through seventeen, during three months of twice daily use. The major side effect of chlorhexidine, which is tooth stain, was also monitored.

The difference in the gingival index, plaque index, bleeding index and the Streptococcus mutans and Actinomyces levels among individuals using experimental and placebo mouthrinses, indicate that a 0.12% chlorhexidine gluconate mouthrinse, used twice daily, effectively reduces plaque accumulation, gingival inflammation and gingival bleeding. Significant reductions in Streptococcus mutans and Actinomyces levels were also observed.

Subjects participating in this study presented with gingivitis and plaque ranging from the absence of gingivitis and plaque to moderate gingivitis and plaque. There was never any severe gingivitis (G.I.=3) or plaque (P.I.=3) present at any time during this study. The gingival and plaque indices were initially greater than those seen in a non-banded population because of the presence of orthodontic appliances. These increased index values correspond with values

from other studies involving fully banded orthodontic patients (Zachrisson and Zachrisson, 1972).

The reduction in the occurrence of gingivitis was much greater from baseline to three months in the chlorhexidine group than in the placebo group. This indicated that the chlorhexidine subjects had a significantly greater proportion of healthy gingival sites. These results correspond to similar studies that have recently been conducted. Segreto et.al. (1986) found an average of 28% less gingival occurrence in a three month study. Grossman et.al. (1986), during a six month experimental period, found an average of a 29% decrease in gingival occurrence after three months, and an average of a 37% decrease at the conclusion of this six month study.

Both the plaque and gingival indices had comparable reductions in severity over the three month experimental period. Comparisons of the six week data showed that there was a more immediate, significant decrease in the amount of plaque present, and this decrease was even more profound at twelve weeks. The gingivitis severity took more time to show a significant reduction, but at twelve weeks, when there was a significant reduction, it paralleled the reduction in the plaque index. A possible explanation for this result is that it takes longer for chlorhexidine to show a tissue response of the gingiva, than it takes to show a clinical reduction in the amount of plaque present. This result also corresponds with the significant reductions in the microbiological counts as early as six weeks.

Although both the chlorhexidine group and the placebo group

showed statistically significant reductions in the plaque and gingival indices, the placebo group's results were not nearly as profound as the chlorhexidine group's results. The reductions found in the chlorhexidine group were clinically significant and the reductions found in the placebo group were not of clinical significance.

The reduction of bleeding sites was greater at the twelve week period than at the six week period. This corresponds to the greater reduction in the gingival index at twelve weeks than at six weeks of time. This index proved to be quite significant showing a 77% reduction in the total number of bleeding sites in the chlorhexidine group. This result correlated directly with similar studies by Segreto et.al (1986) and Grossman et.al. (1986), where they found an average of 53% reduction and a 44% reduction in the number of bleeding sites, respectively.

The reductions in the plaque, gingival, and bleeding indices all corresponded with the concomitant reductions in both Actinomyces and Streptococcus mutans levels found at both the baseline to six week and the baseline to twelve week time periods in the chlorhexidine group. By the end of this study, there were no statistically significant differences between the incisor site and the molar site, in the chlorhexidine group. Significant differences remained throughout the treatment period in the placebo group. The molar site had consistently more Streptococcus mutans at all time periods than did the incisor site in the placebo group. However, the incisor site had slightly higher levels of Actinomyces than the molar site. This result could explain the increased caries rate commonly seen in the molar site over the incisor site, and the fact that the

molar has a greater number of retention sites than the incisor.

The reductions in both the levels of Actinomyces and Streptococcus mutans in the chlorhexidine group were similar, in this study, to the results of Briner et.al. (1986 a,b). The reductions were both very significant at $p < .001$ when comparing the baseline to the twelve week values. A study on the levels of Streptococcus mutans in orthodontic patients (Lundstrom and Krasse, 1987), found that the number of Streptococcus mutans could be significantly suppressed through the use of chlorhexidine, and therefore, decrease the caries risk in this population of patients.

Syed and Loeshe (1978) stated that either the length of exposure of the gingival tissues to the plaque accumulation or the development of more virulent bacteria in the flora, not the plaque mass, per se, is responsible for the development of gingivitis. In this study, a decrease in the plaque biomass, most likely from use of the 0.12% chlorhexidine gluconate mouthrinse, paralled the clinical observations of a decrease in the plaque, gingival and bleeding indices.

Stain was evaluated in this study because it is the primary side effect of chlorhexidine, and it can be a major esthetic concern to the patients using this drug. In this study, both the chlorhexidine group and the placebo group showed significant increases in the degree of stain, but the chlorhexidine group had a much more significant amount of stain. Siegrist et.al. (1986) also found staining in both the chlorhexidine and placebo groups. Dietary factors are a probable cause that staining is observed in the placebo group. Tannic acid, and in particular tea, has been found to be a causitive

agent in producing increased degrees of stain in chlorhexidine users. (Prayitno et.al., 1979a; Addy et.al., 1979; Prayitno et.al., 1979b). Although this study involved a teenage population, the percent of iced tea drinkers approximated 50% in both the groups in this study.

The stain in the chlorhexidine group was significantly greater in the mandibular arch than in the maxillary arch. This was probably due to gravity. The stain was concentrated in the bucco-lingual areas in the maxillary arch, and in the mandibular arch, the stain was evenly divided between the bucco-lingual areas and the interproximal areas. Clinically, the amount of stain present was mild to moderate (Stain Index = 1 to 2). The placebo group showed slight stain in the less than mild range, and the severity of change only increased .10 during the entire experimental period. This was hardly a clinically significant observation.

A 0.12% chlorhexidine gluconate mouthrinse is recommended as a plaque reducing agent. Its use in full-banded/bonded orthodontic patients to reduce plaque accumulation, gingivitis, gingival bleeding, Actinomyces and Streptococcus mutans levels has been demonstrated in this study over a six to twelve week period.

SUMMARY AND CONCLUSIONS

The concept of a chemical agent to enhance oral health has long been considered, and the importance for such an agent is even greater with orthodontic patients with established gingivitis. It was the purpose of this study to determine the efficacy of a 0.12% chlorhexidine gluconate mouthrinse as an antiplaque and anti-gingivitis agent, providing substantial clinical reductions in gingivitis occurrence, severity, and bleeding, in a teenage orthodontic population.

Thirty-four patients, sixteen experimental and eighteen control, used a chlorhexidine or a placebo rinse, respectively, for a three month period. The results showed a significant reduction of plaque accumulation, gingival inflammation, gingival bleeding, and Actinomyces and Streptococcus mutans levels could be attained while using the chlorhexidine gluconate mouthrinse. Although optimal levels of gingival health were not obtained in all subjects while using chlorhexidine gluconate, the reduction of plaque was associated with a reduction of gingival inflammation.

Based on these observations, chlorhexidine gluconate can be considered a safe, effective, and acceptable agent to reduce plaque formation. Recommendations for the use of chlorhexidine gluconate are suggested to decrease plaque accumulations and improve the health of the gingival tissue in patients undergoing active orthodontic treatment. The staining seen with the use of chlorhexidine gluconate is significant to a mild to moderate degree, and it can be removed with a dental prophylaxis. Although, the taste may be bitter to some, the advantages of this drug far outway the disadvantages,

and make it an acceptable chemical agent to reduce plaque and gingivitis.

Therefore, it can be concluded that 0.12% chlorhexidine gluconate, proved to be an important therapeutic agent in controlling gingival inflammation, gingival bleeding, plaque accumulation, and in reducing selective bacterial levels in orthodontic patients. Its effect on these clinical and microbiological levels, in this study, was found to be best when used for the full three months of therapy.

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VIII. APPENDIX

APPENDIX 1

RTF with Asolectin

	Lot #	Wt/L
Ingredients		
Yeast Extract		5.00 gm
Asolectin (See Premix below)		0.30 gm
Bacto-peptone		1.00 gm
NaCl		8.50 gm
L(+) Cysteine HCl monohydrate		0.50 gm
Na_2HPO_4(Anhydrous)		0.868 gm
KH_2PO_4(Anhydrous)		0.528 gm
Tween 80		1.00 gm
Glycerine		150.0 ml
+DRO Water q.s. (or good quality Distilled H_2O)		1000.0 ml
pH Adjustment 1N NaOH to pH 6.9-7.1		

Record pH_____

*Asolectin - 95% purified soy phosphetides distributed by:
Associated Concentrates
32-34 61st Street
Woodside, L.I., New York 11377

Directions: Medium is heated to near boiling while N is bubbled through the medium. The hot medium is dispensed into screw cap vials while bubbling N_2into the vials. Caps are replaced tightly. The vials are autoclaved for 15 minutes at 121°C and 15 p.s.i. Pressure to sterilize.

Pre-Mix: Dissolve Tween 80 in 10% of DRO H_2O, then add asolectin with stirring and heating until a cloudy solution results. (Heat to approximate 80 C). Then the rest of the ingredients should be added slowly, with stirring.

APPENDIX II

S. mutans HLR - MODIFIED

Ingredient	Wt./L
Trypticase Soy Agar	40.0 g
Sucrose	200.0 g
Autoclave Crystal Violet[1]	1.0 ml
Distilled Water	1000.0 ml
Add aseptically at 50°C	
Polymixin B, Sulfate[2]	1.0 ml
Bacitracin[3] 200 units/	0.4 ml
Autoclave Pressure _____	
Temp _____	
Time _____	
Temp at aseptic addition _____	
Delivery setting _____	
Yield _____	

1) Crystal Violet Solution (500 ppm) add 0.05 gm crystal violet per 100 ml distilled water. Filter sterilize and refrigerate.

2) Polymixin B. Sulfate Solution (Aerosporin product of Pfizer Inc.), add 0.0875 gm of Aerosporin per 50 ml distilled water, filter sterilize.

APPENDIX III

Actinomycetes Media (laked blood)

Ingredient (Lot #)	Wt/L
Trypoticase Soy Broth	30.0
Glucose (Dextrose)	5.0
Agar (granulated)	15.0
$CdSO_4$	0.013
Autoclave Naf	0.080
Neutral acriflavin[1]	1.0ml
K-tellurite[2]	1.0ml
Basic Fuchsin [3]	0.125 ml
Distilled Water	850.0ml
Add aseptically 50°C	
Lake Sheep Blood[4]	30.0ml
Sheep Serum	40.0ml
Autoclave Pressure_____	
Temp _____	
Time _____	
Temp. at aseptic addition _____	
Delivery setting_____	
Yield_____	

[1]Neutral acriflavin solution 0.113 gm Neutral acriflavin per 100 ml H_2O.

[2]K-tellurite solution 0.25gm of K-tellurite per 100ml H_2O.

[3]Basic fuchsin solution 0.2% basic fuchsin. 0.2 gm of basic fuchsin/100ml H_2O.

[4]Add 20.0ml of sterile distilled water to 10.0ml difibrinated Sheep Blood. Distribute to sterile centrifuge tubes and centrifuge 15 minutes @ 7 on Backman Centrifuge. Record _____rpm.