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## A Study of Acidogenesis by the Microflora of Dental Plaque in Orthodontic Patients

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A STUDY OF ACIDOGENESIS BY THE MICROFLORA  
OF DENTAL PLAQUE IN ORTHODONTIC PATIENTS

by

John R. Riggs, D.D.S.

A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of  
the Requirements for the Degree of  
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1969

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## CHAPTER I

## INTRODUCTION

In the constant research against its number one nemesis, dental science recognizes the important and integral relationship of oral acidity and low pH to dental caries. The production of acid by bacteria depends, to a considerable extent, on the amount of fermentable carbohydrate and its persistence in the oral cavity. When carbohydrate solutions are present in the mouth, there is a rapid production of acids by the bacteria which results in the increase in hydrogen ion concentration in the bacterial plaque and saliva. (13,14)

It is conceivable that if an acidic pH is maintained for a period of time or reoccurs at frequent intervals in the region of the dental plaque because of the action of acidogenic bacteria upon the fermentable carbohydrates, the bacterial populations and the saliva could be altered, resulting in an increase in acidophilic and aciduric organisms and an increased metabolic activity leading to environmental conditions and metabolic capacity and ability more suited to the initiation and/or perpetuation of the carious lesion. (25)

The purpose of this thesis is to study and investigate the acidogenesis of the dental plaque in orthodontic patients to determine whether or not the flora of dental plaque has changed to a more aci-

dogenic flora after the insertion of orthodontic appliances. The amount of acid produced by the bacteria of the flora of the plaque before and after the insertion of the orthodontic appliances will be recorded, compared and evaluated.

## REVIEW OF THE LITERATURE

A. Bacteria-Acidogenesis-pH.

Acid production in the oral cavity and its relation to dental caries was first discussed in 1830 when Koecker <sup>(1)</sup> combined the inflammation theory and chemical theory of tooth decay and proposed the dissolution of the dentin by acids. In 1835, Robertson <sup>(2)</sup> suggested that acids might be formed by chemical decomposition. Pasteur's discovery of lactic acid production by bacteria, themselves, planted the seed for research on bacterial acidogenesis causing dental caries; and, in 1881, Milles and Underwood <sup>(3)</sup> stated clearly the hypothesis that the decalcification of tooth substance was due to acids secreted by the bacteria of the teeth growing on the carbohydrate residues of the mouth. In 1884, Miller <sup>(4)</sup> stated: "The first stage of dental caries consists in a decalcification of the tissues of the teeth by acids, which are for the greater part generated in the mouth by fermentation." Goadby <sup>(5)</sup> in 1903 reported two groups of oral flora: the fermenters and the liquefiers, according to whether they attacked the mineral structure with their acid or broke down the organic matter; he was probably the first to isolate from decayed teeth one of the organisms recognized today as playing a predominant role in the decalcification of enamel. In 1905, Miller <sup>(6)</sup> stated that "the present knowledge left no doubt that the oral mucous can become decidedly acidic and, if this occurs in the mouth, a definite caries



producing factor can be suspected." In 1915 Kligler (7) believed the streptococci of the oral flora, during dental decay, became an acid producing rod and he isolated Lactobacillus acidophilus which was capable of producing and withstanding large quantities of acid. In 1922, Mc Intosh and Lazarus-Barlow (8) produced an artificial carious lesion and reported that an acid must be stronger than pH 4 to decalcify tooth structure. They cultured bacteria from decayed areas with glucose broth and obtained pH values of 3.5 and 2.75. In 1922 Rodriguez (9) also produced artificial caries and obtained sugar broths of acidities from pH 3.9 to 2.9 within five days. In 1932 Enright, Friesell, and Trescher (10) reported the following conclusions: (1) Organic acids produced by fermentation of carbohydrates decalcify all human enamel in vitro; (2) The rate of decalcification varies; (3) A general oral acidic condition may predispose to caries; (4) Mucin plaques present a favorable environment for acids to accumulate and decalcify; (5) Weak alkaline salivary salts are not sufficient to control the acids present; (6) Soluble oral carbohydrates diffuse through plaque, and under favorable conditions, are changed to acids; (7) Plaques are practically insoluble in all solutions that can be tolerated orally. In 1936, Jay et al (11) testing the effect of increase carbohydrate intake in children showed a striking increase in the Lactobacillus acidophilus content of the saliva and this increase was followed by active caries in 44% of the cases. In 1937, Anderson

and Rettger<sup>(12)</sup> stated that a high hydrogen ion concentration may never be reached on the tooth surface, and, therefore, a large number of organisms producing small amounts of acid may be of as much importance as the highly acidogenic types; they found that oral streptococci in vitro were as effective as lactobacilli in decalcification of enamel, and yeast and leptothrix forms were observed in large enough numbers to make them highly suspected factors in caries production. In 1938, Miller and Kuntz<sup>(13)</sup> demonstrated the presence of lactic acid in carious tooth substance obtained from enamel lesions. Stephen<sup>(14)</sup>, also in 1938, reported plaque hydrogen ion concentration as low as pH 4.6 and stated that enamel solubility studies have indicated that enamel will actively dissolve at this pH under oral conditions. In 1940, Bradel and Blayney<sup>(15)</sup> submitted the following results after testing for bacterial involvements: of 3,920 cultures, 77.6% were positive for lactobacilli; in 18% of the patients, lactobacilli were present in less than 50% of the samples; in caries-free patients, of 333 specimens, 25.2% were positive for lactobacilli and these counts were low. In 1940 and 1941, Stephen<sup>(16)</sup>, Fosdick, Campaigne, and Francher<sup>(17)</sup> found that under the right conditions, acid is formed on localized surfaces of the teeth in sufficient potential to decalcify tooth structure, and the amount and rapidity of acid formation is predicated on the kind of fermentable carbohydrate in the mouth. In 1942, Becks<sup>(18)</sup> reported that by decreasing excessive amounts of refined sugars, the

Lactobacillus count is decreased and the carious process becomes arrested. In 1943, Dietz<sup>(19)</sup> reported that the carious lesion was, at least in part, a product of the acidogenic action of the bacteria as evidenced by the facts that only the enamel immediately beneath the plaque is affected, the zooglea of experimental plaque is far more adherent in saliva held within the lower pH ranges, the incipient lesion is more caries-like than later when the acid attack on the surface is more diffuse, and, other than lactobacilli, numerous streptococci were consistently found peripherally in enamel smears. In 1951, Bibby, Goldberg and Chen<sup>(20)</sup> reported that an individual's potential to produce acid orally will be exaggerated or reduced by the acid producing capacity of his saliva. In 1954, Krasse<sup>(21)</sup> reported a correlation between caries activity and the number of lactobacilli in some but not in others; a probable correlation between caries activity and the number of Candida; a definite correlation was seen between caries activity and the number of streptococci per mg. of plaque material; no relationship was found between dental caries and Streptococcus salivarius, however. In 1956, Crowley<sup>(22)</sup> et al showed that in in vitro studies with lactobacilli, streptococci, staphylococci, and yeast within 24 hours all the strains studied produced sufficient acid from glucose to decalcify teeth. In 1959, Stanmeyer and Adams<sup>(23)</sup> reported a positive correlation between bacterial action as measured by the pH of oral

plaque and tooth surface temperature: the lower the temperature, the less activity present. In 1960, Leach<sup>(24)</sup> stated that the reaction of acid on the tooth substance must be a result of the relative fraction of the tooth available to the acid at any stage of the process, and the relative diffusion rates of the acids and soluble ions of the minerals; there is a direct relationship between the drop in pH and the amount of mineral dissolved using a given buffer at a fixed initial pH; however, with the same pH but different molar concentrations, the amount of dissolution is not the same. In 1960, Andlow<sup>(25)</sup> stated that there is no direct correlation between the terminal pH, the amount of acid produced by the bacteria from foodstuffs in saliva, and the amount of enamel decalcification; he further suggested the possibility of different kinds of acids being produced by bacteria acting upon different foods because his results showed mixtures with similar terminal pH levels, but greatly varying titratable acidities, and vice versa. In 1960, Frostell<sup>(26)</sup> demonstrated that acid substances are continuously produced in vitro by suspensions of oral acidogenic organisms and dental plaque; this caused the suspensions to become more and more acidic when no additional substrate was added. Also, in 1960, Muhlemann<sup>(27)</sup> reported that in vitro attack of several acid buffers on intact enamel in replicae and in ground sections that surface etching and white spot formation were comparable to

the amount of phosphorus released during enamel demineralization. In 1961, Mac Gregor<sup>(28)</sup> stated that a relatively high percentage of acid was present in the early enamel carious lesion and suggested that the low pH in the enamel acted as an irritant to elicit the bacteria to produce toxins to alter the dentin. In 1961, Mörch<sup>(29)</sup> reported that his in situ experiments showed that the saliva has an important influence on the pH on the tooth surfaces and only rarely did the pH value on tooth surfaces go below 5.0, but relatively small amounts of carbohydrates were sufficient for a considerable drop in pH on tooth surfaces. In 1962, Shaw<sup>(30)</sup> reiterated that a source of readily fermentable carbohydrates are required in the oral cavity as a source of energy for various organisms which produce acids and are the causative factor for the carious lesion. In 1963, Buonocore and Sperber<sup>(31)</sup> reported that different patterns of enamel demineralization were produced, depending on the type of acid involved; agitation of the acid solutions caused severe and rapid cavitation; a "white-spot" lesion on the surface only seemed to result from a slow rate demineralization as with "inhibiting" acids. In 1964, Gibbons<sup>(32)</sup> stated that the majority of micro-organisms in the saliva are derived from the tongue and not from the dental plaque; however, large numbers of cariogenic organisms do exist in the plaque and some of the characteristics of these organisms appear to be the ability to form acid from carbohydrates and to form intracellular

polysaccharides. In 1965, Rosen and Weisenstein<sup>(33)</sup> stated that the pH of dental plaque in the caries-susceptible individuals generally was from .1 to .2 unit less than in the caries-free individuals. In 1965, Sims<sup>(34)</sup> reported that with a fermenting glucose solution, the rate of acid production on the tooth surface in vitro depended on the inherent ability of an organism to produce acid and on the concentration of bacterial cells covering the surface--each organism has a certain optimum density of bacterial cells on a surface which gives the maximum rate of acid production. In 1965, Magnusson<sup>(35)</sup> demonstrated the cariogenicity of certain liquid medicines with a low pH and showed that a 70% sucrose solution decalcified enamel in five cases. In 1965, Schole and Federick<sup>(36)</sup> placed tooth material into solutions of citric and asparic acids and because of the release of hydrogen ions from the hydroxyapatite complex, they proposed that this exchange in the mouth initiates the caries, and, then the subsequent lower pH perpetuates it. In 1965, Kuskova and Morozova<sup>(37)</sup> found a direct relationship between caries incidence, lactobacilli titer, and hyaluronidase activity in children from 7 to 18 years of age. In 1965 Bramstedt<sup>(38)</sup> stated that only low molecular sugars are cariogenic and this is caused by their breakdown into organic acids by microorganisms, especially streptococci, in the plaque, and in times of substrate deficiency, the streptococcus polysaccharides are broken down into acids. Fosdick

and Hutchinson<sup>(39)</sup> in 1965, stated that the living tooth is a semi-permeable membrane and does react with acid to tend to destroy itself; the carious lesion is a special case in the behavior of the membrane. Jenkins<sup>(40)</sup> again reiterated in 1965, that present evidence tends to consider acid, rather than chelators, as most instrumental in caries enamel decalcification, and Yardeni<sup>(41)</sup> in the same year, stated that acid action as a triggering mechanism applies to early caries, whereas chelation is suspected in progressing caries. In 1966, Frank and Brendel<sup>(42)</sup> using an electron microscope, observed that the bacteria first deposited on the plaque are coccus-like, rod shaped, and some rare filamentous forms. Arora<sup>(43)</sup> in 1966, stated that damaging acids result from the action of microbes on sugars and starch adjacent to tooth structure in protected areas and the rate of acid formation varies. In 1967, Vercellino and Chantel<sup>(44)</sup> tested caries affected and non-affected children from parafin stimulated saliva and found no correlation between caries activity and salivary pH. In 1967, Sharpenak<sup>(45)</sup> stated that proteolysis initiated dental caries and demineralization occurred at a more advanced stage. Newbrun<sup>(46)</sup> in 1967, stated that extracellular polysaccharides function both as a reservoir of fermentable carbohydrates for continuous acid production and as a structural component of the dental plaque "gluing" the bacteria to the teeth. In 1967, Steinle<sup>(47)</sup> et al used agar plating with lactobacillus corresponding with clinical lesions in 82% of the instances and suggested a

direct relationship between the presence of lactobacilli and the active carious lesion. In his studies of cariogenesis, Wannermacher<sup>(48)</sup> in 1967, found that the caries is a product of the interaction of the physiologic activity of the enamel surface membrane and the saliva and immediate environment adjacent to this surface; in caries a certain acid concentration must be present, especially in the form of a plaque, to disfavor remineralization and enhance the breakdown of the tooth material. In 1968, Rosen, Lenny, and O'Malley<sup>(49)</sup> did research with gnotobiotic rats and hypothesized that since dental caries developed only in the sulcal regions and plaque did not accumulate, it is likely that, in these experiments, caries was due to the production of acid.

#### B. Carbohydrates.

Carbohydrates, in general, and specific sugars have long been subjects of research concerning the integral role they play in the caries process. In 1947, Stephen and Hemmens<sup>(50)</sup> reported that when a high concentration of glucose was available, acid production was somewhat more rapid and lasted longer. In 1950, Shaw et al<sup>(51)</sup> revealed that with tube feeding, the absence of food in the mouth completely prevented tooth decay. Volker<sup>(52)</sup> in 1955, showed that glucose, sucrose, and fructose were each readily susceptible to the action of those enzymes in the saliva associated with acid production and acid production resulted within five minutes after the mixture of caries-active



patient's saliva with glucose and sucrose. In 1955, Williams<sup>(53)</sup> stated that the kinds of carbohydrates, their concentration, physical state, and periodic ingestion can affect the oral flora as evidenced with Lactobacilli studies. In 1957, Shaw<sup>(54)</sup> showed compelling evidence on experimental animals that carbohydrates, in general, and the monosaccharides and disaccharides, in particular, are responsible for the initiation and progression of carious lesions; when sucrose was consumed directly into the stomach and the rest of the diet consumed normally via the mouth, carious lesions were not initiated. In 1962, Rapp<sup>(55)</sup> stated that the fermentation process seems to be indispensable to the caries process by increasing the osmotic pressure gradients and drawing more nutrients from the inner tooth for the microbes. Pigman, Brosher, and Koulourides<sup>(56)</sup> reported, in 1962, that in studies using different sugars, oral plaque bacteria, and artificial conditions for eight hours to soften enamel surfaces, D-glucose and sucrose produced very similar rates of softening; slower rates were seen with lactose and galactose; the pH reading before and after decreased from 6.4-6.7 to 5.0-6.0, and an increase in titratable acidity was also noted. In 1965, Krasse<sup>(57)</sup> reported that when glucose was the main carbohydrate in the diet, no increase in streptococci or caries activity resulted, whereas, with sucrose, there was a noticeable increase in caries-inducing strepto-

cocci and a high degree of caries activity. In 1967, Hartles<sup>(58)</sup> stated that sucrose is indicated as the most potent cariogenic food stuff because of its ease of fermentation to acid and its ability to act as a substrate for levan and dextran formation within the dental plaque; and, in the same year, Newbrun<sup>(59)</sup> supported this by reporting that certain microbial strains can synthesize extracellular levans and dextrans from sucrose and the elimination of specific carbohydrates, especially sucrose, can prevent caries. Sharpenak<sup>(60)</sup> in 1967, also stated that nutrition is the most important factor in the origin of caries and the elimination of sucrose from the diet reduces, but does not completely prevent caries. In 1968, Rosen, Lenny, and O'Malley<sup>(61)</sup> reported causing caries in 13 of 16 Sprague-Dawley rats, 3 of 8 caries-resistant rats, and 1 of 2 caries-susceptible rats given a high sucrose (12000) diet. Winter<sup>(62)</sup> in 1968, reported the following conclusions concerning sucrose and cariogenicity: (1) in population groups prevalence of dental caries is more associated with sucrose levels of consumption; (2) in animal experimentation, sucrose is more cariogenic than other carbohydrates; (3) animal and human dietary sucrose results in more extensive plaque formation; (4) cariogenic streptococci produce more extracellular polysaccharides than non-cariogenic organisms when sucrose is the substrate; (5) a relationship has been established in young children between sucrose consumption, prevalence of dental caries, and the ability of oral microbial flora to produce intercellular polysaccharide material.

### C. The Effect of Orthodontics on the Oral Environment

The effect of orthodontic appliances in the oral cavity in relation to dental caries was studied by Noyes,<sup>(63)</sup> and in 1937, he reported that the preponderance of evidence suggested that the process of etching beneath an orthodontic band is similar, if not identical, to the process of dental caries and these appliances afford the acidogenic organisms an additional opportunity for growth. In 1941, Burrill<sup>(64)</sup> showed from chemical and bacteriologic testing of orthodontic patients that caries-susceptible patients tended to get less susceptible, while low susceptibility patients became more susceptible; it was suggested that increased supervision or oral hygiene aided the former, whereas an increase in food traps and stagnation from the appliances harmed the latter. In 1949, Owen<sup>(65)</sup> reported that: (1) the presence of orthodontic appliances does increase the Lactobacillus count; (2) the degree of increase is somewhat dependent upon the number of bands; and (3) there is a correlation between the total band months and the number of lactobacilli per cc. Dolce,<sup>(66)</sup> in 1950, stated that the orthodontic appliances can serve as a causative agent for dental caries in the sense that they influence the caries incidence by increasing a caries susceptible environment. In 1954, Bach<sup>(67)</sup> reported from clinical observation that after an average of 3 1/2 years of orthodontic treatment, patients consuming excessive carbohydrates showed a 1.3 per cent increase in decalcified areas per patient over the patients with a minimum

carbohydrate intake. In 1956, Quinn<sup>(68)</sup> related that, after banding a group of patients with proximal lesions on the teeth and comparing the progress with similar lesions on an unbanded control group, the caries may progress beneath the bands but the rate of progress is less rapid than in unbanded teeth. In 1962, Dikeman<sup>(69)</sup> reported: (1) orthodontic appliances tend to increase both Lactobacillus and Staphylococcus counts, but does not appreciably change yeast or Streptococcus counts; (2) a definite positive relationship exists between DMF teeth and Lactobacillus counts. In 1964, Bloem and Brown<sup>(70)</sup> measured seven types of anaerobic and aerobic microorganisms and reported that all categories showed a numerical increase after the placement of orthodontic appliances, especially Lactobacilli, which increased by 3,500 per cent, and stated that the greatest number of orthodontic bands and auxiliaries manifested the greatest quantitative increase in microbial populations studied. In 1965, Gzorgy<sup>(71)</sup> stated that not only the fixed type of orthodontic appliance, but also the removable appliances can be instrumental in causing caries if it is worn conscientiously, with a short or long time lapse according to the individual. Hureau,<sup>(72)</sup> in 1966, reported findings from research with 500 orthodontic patients and stated that when bands were not fitted correctly and the cement seal was broken, caries began within two weeks; the caries showed: Staphylococcus 5 per cent, Streptococcus 60 per cent, Spirillum 5 per cent, fusiforms 10 per cent, and bacilli 20 per cent; the lesions of the orthodontic

patients were similar in appearance and development to the regular carious lesion. In 1967, Sakamaki<sup>(73)</sup> reported from a study of the localization of oral lactobacilli in orthodontic patients that there was a considerable rise in Lactobacillus after orthodontic banding and, once the bands were removed, the count returned to the pretreatment level. In 1967, Adams<sup>(74)</sup> reported from his studies that fixed orthodontic appliances sufficiently altered the oral environment to significantly affect disk-type, bell-type, and total counts of both initial treatment and final treatment groups, at both the 1 per cent and 5 per cent levels of confidence; he suggested the changes in the bell-type lactobacilli concentrations might give a more accurate index of caries activity during orthodontic treatment and showed an increased tendency for caries activity upon fixed orthodontic appliance insertion and a decreased tendency upon appliance removal. In 1968, Balenseifen<sup>(75)</sup> demonstrated that after the placement of orthodontic bands the pH of the dental plaque became more acidic, the carbohydrate content increased 40 per cent, and the microbial population, viz. Lactobacillus, Streptococcus mitis and salivarius, increased significantly per mg. of plaque.

#### D. Titratable Acidity.

The titratable acidity of a culture can be measured by titration of a known volume of the media with sodium hydroxide to the predetermined end point as shown by a standardized glass electrode or by the

color of a suitable indicator. The titratable acidity is of importance, along with the final pH, in the comparison of high-acid-producing organisms. (76)

In 1955, Volker (77) utilized the method of titratable acidity as reported in his article. In 1960, Frostell (78) described his method of determining the total acid-production of a suspension of microorganisms by titration with an alkali or acid; the amount of acid or base required per unit time to keep the pH constant is a measure of the activity. Andlow, (79) in 1960, and Pignen, Brosher, and Koulourides, (80) in 1962, also reported the use of titratable acidity to determine experimental results, and, thus, attest to and verify its use as a valuable adjunct in research.

Research through the years has proceeded to not only discover bacterial acidogenesis in relation to dental caries and the role the bacteria play in relation to the carbohydrate residue left in the oral cavity, but also has proceeded to delineate specific strains of bacteria relative to acid production and enamel breakdown, and to demonstrate the potential of specific sugars to be used by the oral flora in altering the pH and hydrogen ion concentration. The presence of orthodontic appliances in the mouth has been shown to establish a favorable oral environment for an increase in bacterial populations, a more acidic saliva, and a lower oral pH on and around the dental units and in the dental plaque itself. The present investigation

related in this thesis, attempts to demonstrate that the insertion and presence of fixed orthodontic appliances changes the oral environment and enables the oral flora to become more acidic as evidenced by an increase in the amount of acid produced by the flora relative to the bacterial population before and after the orthodontic appliances are inserted.

## CHAPTER III

## METHODS AND MATERIALS

A. Subject Selection.

Ten patients who were to be treated orthodontically were randomly selected from the orthodontic clinic before any bands or appliances were placed in the mouth. These patients were to be treated with complete orthodontic banding and a light wire technique. The selection was made with complete disregard to age, sex and type of malocclusion. A plaque sample was collected from each patient before any bands or appliances were inserted, and then another plaque sample was obtained one month after the bands and appliances had been in place.

B. Plaque Collection.

Each patient, after not having brushed his teeth or rinsed his mouth for a minimum of three hours, had plaque scaled from around the dental units. The plaque was collected with a gracy curette from around the gingival sulcus and the interproximal areas of maxillary and mandibular anterior and posterior teeth. Lilly No. 5 gelatin capsules were preweighed to a ten-thousandth of a gram, and the collected plaque was immediately placed into the capsule.

C. Plaque Preparation.

The capsules and the plaque samples they contained were then immediately weighed again to determine the amount of plaque collected.



Each capsule was placed into a five milliliter Viritis\* microhomogenizer cup and one half a milliliter of sterile water per one milligram of plaque was added to the cup. After allowing the capsule to soak for approximately ten minutes, it was homogenized for from three to five minutes until which time no plaque particles could be distinguished in the liquid.

#### D. Cultivation of Plaque Microflora.

A cysteine trypticase sugar broth was used as the culturing media. The sugars chosen were glucose, lactose, and sucrose; and the media were prepared as follows:

Cysteine hydrochloride	0.5 gm/liter
The sugar	10.0 gm/liter
Trypticase	20.0 gm/liter
Sodium chloride	5.0 gm/liter
Sodium sulfide	0.5 gm/liter
Phenol red	.3 ml/liter

Cysteine was used for the preparation of the broth because the cystine was not soluble when the ingredients were mixed at room temperature. The cysteine converted to cystine when the broth was autoclaved, before the sugar was added and the pH adjusted.

The three sugar broths, the glucose cysteine trypticase broth, the lactose cysteine trypticase broth and the sucrose cysteine trypticase broth, were adjusted using either hydrochloric acid or sodium hydroxide to a pH of 7.03 at 25° Centigrade and filter sterilized with a Millipore† filter of .45 micron pore size. Each of the above

\*Virtis Research Equipment, Gardiner, New York  
 †Millipore Filter Corp., Bedford, Mass.

three broths was divided into 100 ml. sterile portions. One one-hundred milliliter portion of each type of sugar broth was used for each patient. The glucose, lactose, and sucrose cysteine broths were then each inoculated with a 0.1 ml. of the homogenized plaque sample. The inoculated broths were incubated for twenty-four hours at thirty-seven degrees Centigrade.

#### E. Determining Bacterial Population.

The bacterial populations of the sugar broths were determined by means of serial dilution. A 0.1 ml. sample was taken from each broth and was diluted with 0.9 ml. of sterile water. This serial dilution procedure was continued to a  $10^{-10}$  dilution for each of the three broths.

A double plating was done and BBL\* Trypticase Soy Agar was used as the media. When the agar solidified, the petri dishes were inverted and incubated for seventy-two hours at 37° Centigrade. After incubation, all the colonies on the dishes were counted and the numbers were recorded. The number of bacteria per ml. of 24 hour culture was calculated.

#### F. Determining Bacterial Acid Production.

After twenty-four hours of incubation, the 100 mls. of culture were used to determine the amount of titratable acidity produced by the bacteria of the plaque sample from each patient. The volume of each portion was recorded. A blank or uninoculated control portion

\*Baltimore Biological Laboratories, Cockeysville, Maryland

was run for each of the Cysteine Trypticase sugar broths as a check to see that the sterility and the pH were maintained after the incubation period. This also verified the validity of the supposition that any and all alterations in the sugar broths were due entirely to the bacterial inoculant. The pH of the control was used as a reference to which the broth sample could be neutralized back to during the titratable acidity procedure.

After the entire volume of the broth portion had been recorded, the broth was put into a beaker. Using a Coleman Metrian IV pH meter,\* the terminal pH produced after twenty-four hours of incubation was obtained and recorded. The titration technique used to determine the acidity of the broth was the same as that defined and described in the Manual of Microbiological Methods.<sup>(76)</sup> The neutralization was done with 1.0N sodium hydroxide solution.

A five milliliter microburette was filled with the 1.0N sodium hydroxide and placed over the beaker of sugar broth. The electrode ends of the pH meter were in the broth and the meter was left on while, simultaneously, the sodium hydroxide was dropped into the broth and the beaker was gently swirled to evenly stir and distribute the sodium hydroxide into the broth. When the reference pH of the control broth was reached, no more sodium hydroxide was added into the beaker. The amount of sodium hydroxide needed to neutralize the broth to the

\*Coleman Instruments, Maywood, Illinois

reference pH was then obtained and recorded to one one-hundredth of a milliliter.

#### G. Analysis.

The first group of data was collected and recorded before the placement of any bands or appliances; the second group after the bands and appliances had been in the patients' mouths for one month. Each group of data consisted basically of two parts. One part was to determine the number of bacteria present in the broth cultures, and this was accomplished using the serial dilution and agar plating procedures. The other part was to determine the acidogenic ability of this number of bacteria in the broth sample using different sugars or carbohydrates as substrates. This latter part was accomplished using the glucose, lactose, and sucrose broths as culture media and the titratable acidity technique.

The two groups of data collected from the ten subjects before and after banding were then correlated. The amount of acid produced in each of the three different sugar broths by the bacteria of the plaque sample before and after banding was contrasted according to the amount of base required to neutralize the acidic broths back to a neutral reference pH. The data was integrated to determine the amount of 1N.NaOH required to neutralize the acid produced by  $10^{11}$  bacteria in 100 ml. of the specific sugar broth.

The statistical analysis of the data was done by using the "Related or Paired Samples" technique. The ratio of the average difference to

the standard error of the average difference is  $t = \frac{\bar{d}}{SE_{\bar{d}}}$ . This ratio is distributed in the t-curve with (N-1) degrees of freedom. The t values were computed and checked to determine whether or not a statistically significant change had occurred in the oral acidogenesis of the bacteria before and after the insertion of orthodontic appliances.

## CHAPTER IV

## RESULTS

The data for all ten subjects was collected before and after banding, and after this data was prepared, calculated, and computed, the results were available.

A. Titratable Acidity.

The data using the glucose media appears in Table I. The mean difference was  $-.01154$ , the standard deviation was  $.01194$ , and the t-value was  $-.96634$ . The latter figure gives a probability of  $>.40$ , and suggests no significant change was present before and after banding.

The data using lactose broth as the media appears in Table II. The mean difference was  $.00027$ , the standard deviation was  $.00036$ , and the t-value was  $.74503$  and gave a probability of  $>.20$ , which suggested no significant change before and after banding.

The data using the sucrose broth as the media appears in Table III. The mean difference was  $.00010$  and the standard deviation was  $.00034$ . The t-value of  $.30665$  showed a probability of  $>.30$ , and suggested no significant change was present before and after banding.

The results of this investigation have shown that there is no alteration in the acidogenesis of the bacteria of a sample of dental plaque taken before the insertion of orthodontic appliances and then after the placement of the appliances and cultured in glucose, lactose and sucrose sugar broths.

B. Terminal pH.

The terminal pH values produced by the bacteria cultured in each of the three sugar broths was recorded before and after orthodontic band insertion. Table IV lists the recorded pH values for observation. This data was not subjected to statistical computation and no attempt was made to determine any statistically significant alteration in pH in the different sugar broths before and after banding. Visual observation indicates little difference in terminal pH among the different cultures.

TABLE I

TITRATABLE ACIDITY PRODUCED BY BACTERIA FROM PLAQUE  
OF ORTHODONTIC PATIENTS CULTIVATED IN A GLUCOSE MEDIUM

<u>Patient No.</u>	<u>Ml. of 1 N. NaOH per 10<sup>11</sup> Bacteria</u>		<u>Difference</u>
	<u>Before Banding</u>	<u>After Banding</u>	
1	.0107	.0115	.0008
2	.0073	.0097	.0025
3	.0173	.0161	-.0012
4	.0047	.0047	.0000
5	.0061	.0064	.0003
6	.0128	.0113	-.0015
7	.0105	.0109	.0004
8	.1348	.0159	-.1189
9	.0182	.0191	.0009
10	.0113	.0127	.0014
Mean	.02336	.01182	-.01154
Standard Deviation	.01246	.00140	.01194

t-value ..... -.96634

P ..... >.40



TABLE II

TITRATABLE ACIDITY PRODUCED BY BACTERIA FROM PLAQUE  
OF ORTHODONTIC PATIENTS CULTIVATED IN A LACTOSE MEDIUM

<u>Patient No.</u>	<u>ML. of 1 N. NaOH per 10<sup>11</sup> Bacteria</u>		<u>Difference</u>
	<u>Before Banding</u>	<u>After Banding</u>	
1	.0121	.0125	.0004
2	.0109	.0136	.0026
3	.0150	.0146	-.0004
4	.0036	.0037	.0001
5	.0061	.0064	.0003
6	.0169	.0150	-.0019
7	.0085	.0091	.0006
8	.0117	.0116	-.0000
9	.0176	.0186	.0010
10	.0058	.0059	.0001
Mean	.01083	.01110	.00027
Standard Deviation	.00152	.00149	.00036
t-value .....	.74503		
P .....	>.20		

TABLE III

TITRATABLE ACIDITY PRODUCED BY BACTERIA FROM PLAQUE  
OF ORTHODONTIC PATIENTS CULTIVATED IN A SUCROSE MEDIUM

<u>Patient No.</u>	<u>ML. of 1 N. NaOH per 10<sup>11</sup> Bacteria</u>		<u>Difference</u>
	<u>Before Banding</u>	<u>After Banding</u>	
1	.0103	.0108	.0006
2	.0123	.0129	.0006
3	.0102	.0116	.0014
4	.0035	.0039	.0004
5	.0048	.0051	.0003
6	.0118	.0121	.0004
7	.0099	.0072	-.0027
8	.0114	.0112	-.0002
9	.0143	.0146	.0003
10	.0038	.0039	.0000
Mean	.00923	.00933	.00010
Standard Deviation	.00121	.00126	.00034
t-value .....	.30665		
P .....	.30		

TABLE IV

TERMINAL pH PRODUCED BY BACTERIA FROM PLAQUE  
OF ORTHODONTIC PATIENTS IN  
CYSTIENE TRYPTICASE SUGAR BROTHS

<u>Patient No.</u>	<u>Glucose</u>		<u>Lactose</u>		<u>Sucrose</u>	
	<u>Before Banding</u>	<u>After Banding</u>	<u>B.B.</u>	<u>A.B.</u>	<u>B.B.</u>	<u>A.B.</u>
1	4.91	4.87	5.00	4.91	5.00	4.93
2	5.08	5.10	5.19	5.21	5.10	5.08
3	5.08	4.99	5.20	5.01	5.10	4.90
4	4.99	4.98	5.11	5.02	5.00	4.95
5	5.15	5.10	5.28	5.18	5.20	5.08
6	5.00	4.98	5.10	5.00	5.60	5.52
7	5.11	5.05	5.13	5.10	5.10	4.99
8	4.85	4.80	5.05	4.99	5.10	5.02
9	4.99	4.91	5.00	4.98	4.90	4.80
10	5.30	5.22	5.23	5.22	5.30	5.24

## CHAPTER V

## DISCUSSION

From the very inception of investigations into the etiology of dental caries, acid formation and involvement has been a prime suspect. Koecker,<sup>(1)</sup> Robertson,<sup>(2)</sup> Miller,<sup>(4)</sup> Milles and Underwood<sup>(3)</sup> all spoke of acidic decalcification of the tooth material and its contributory effect upon the carious lesion. In the decades which followed, men like Mc Intosh, Lazarus-Barlow,<sup>(8)</sup> Rodriquez,<sup>(9)</sup> Enright, Friesell, Trescher,<sup>(10)</sup> and Stephen<sup>(14)</sup> produced artificial caries, found that acids are produced introrally from carbohydrate fermentation by bacteria, and reported that acids produced must attain a certain pH to decalcify tooth enamel. Concurrent with the research concerning acid production was the research involving the bacteria themselves, and more specifically, the acidogenic and aciduric strains. Bradel and Blayney<sup>(15)</sup> reported a high correlation between the presence of Lactobacillus and dental caries. Following this investigation, Dietz,<sup>(19)</sup> Krasse,<sup>(21)</sup> and Crowley<sup>(22)</sup> et al substantiated the findings concerning lactobacilli, and further showed a definite correlation between dental caries activity and streptococcal strains; it was also reported that lactobacilli, streptococci, staphylococci, and yeast all produced sufficient acid within 24 hours to decalcify teeth in vitro. Men like Bibby<sup>(20)</sup> and Andlow<sup>(25)</sup> reported production of different kinds of acids from foodstuffs with

varying acid production potentials. Research along the above mentioned lines continued and became more sophisticated and more specific. Buonocore and Sperber<sup>(31)</sup> stated that different patterns of enamel demineralization were produced depending on the type of acid involved. Sims<sup>(34)</sup> reported that the acid production on the tooth surface in vitro depended on the inherent ability of an organism to produce acid and on the concentration of the bacteria covering the surface because each organism has an optimum density of bacterial cells which gives the maximum rate of acid production.

Through the entire gamut of research, glucose and sucrose have remained in the top position on the list of contributory carbohydrates in dental caries. Stephen, Hemmens,<sup>(50)</sup> Volker,<sup>(52)</sup> Pigman, Brosher, and Koulourides<sup>(56)</sup> all attest to the use of these two sugars by the oral microorganisms during acidogenesis. Very recently, men like Krasse,<sup>(57)</sup> Sharpenak,<sup>(60)</sup> Newbrun<sup>(59)</sup> and Winter<sup>(62)</sup> have reported evidence to show that sucrose is the most potent cariogenic foodstuff because of its ease of fermentation to acid and its ability to act as a substrate for the extracellular production of dextran and levan within the dental plaque.

When the orthodontic profession became involved, Noyes<sup>(63)</sup> and Burrill<sup>(64)</sup> reported that the existence of appliances in the mouth created a susceptible environment because of an increase in food traps and areas of stagnation. Owen<sup>(65)</sup> reported an increase in the

Lactobacillus count after the insertion of bands and a correlation between the number of bands and the amount of time they are in the mouth. Dikeman<sup>(69)</sup> reported an increase in Lactobacillus and Staphylococcus counts after banding; Bloom and Brown<sup>(70)</sup> reported a statistically significant increase in the Lactobacillus after banding. Sakamaki<sup>(73)</sup> went one step further and reported a rise in oral lactobacilli in localized areas in orthodontic patients after banding. Balenseifen<sup>(75)</sup> reported that after the insertion of orthodontic appliances, the dental plaque became more acidic, the carbohydrate content increased 40 per cent, and there was a statistically significant increase in the Lactobacillus, Streptococcus mitis, and Streptococcus salivarius counts.

The increase of microorganisms in plaque accompanied by a drop in pH is the result of an environmental change produced in dental plaque as a result of orthodontic banding, and this new environment is obviously more cariogenic. This investigation concerns itself with the changes in bacterial flora. If the microflora of plaque shifted to a flora capable of producing greater quantities of acid, not only increases of bacteria but also increased acidogenesis by the bacteria would favor a cariogenic potential.

This investigation has shown that the overall ability of the bacteria in dental plaque to produce acid is unchanged, therefore, suggesting the lower pH observed in the plaque of orthodontic patients is the result of increased numbers of bacteria and increases in fermentable carbohydrate.

The acidogenesis produced by the bacteria in plaque is the effect of many different types of bacteria in a broth. An increase of one acidogenic group of bacteria may be overcome by a decrease of another group of bacteria. A pure culture study of the organisms in plaque is an involved procedure but may indicate the increase of specific acidogenic groups of bacteria. It should also be kept in mind that the ecological relationship of the bacteria to each other is different in the culture medium than in dental plaque. The synergistic effect between two bacteria which is necessary for fermentation may not exist in the artificial culture media.

When an artificial environment is used to collect data, the results obtained must be interpreted in light of the conditions of the experiment. The fermentation process is effected by the available substrate in the culture medium and the environmental conditions such as oxygen and carbon dioxide tension, pH, and incubation temperature. The only bacteria that grew in the culture are those which are favored by these conditions. Cysteine trypticase broth is a rich medium and it has been shown that even the most fastidious microorganisms will grow on it. It is impossible to be sure, however, that all the bacteria in the plaque will grow on it.

All cultivation in this investigation was performed under aerobic conditions. This means the anaerobic bacteria were not a part of this investigation since they will not grow under the conditions provided in this project. Since anaerobic bacteria make up a good portion of the plaque flora, this might make a good investigation in the future.

Facultative anaerobic bacteria will grow under aerobic conditions, and this means they are a part of the organisms cultivated in the cultures of this investigation. In plaque there is a more anaerobic environment than in the cultures of this investigation, and it is possible that greater acidogenesis could be present under the anaerobic conditions of plaque.

When titratable acidity is studied, the buffer capacity of the medium must be taken into consideration. In this investigation the same medium was used throughout, therefore, equalizing the effect of the medium's buffer capacity on both groups. The acidogenesis of the microflora might have been more apparent if a different medium were used. A medium with a small amount of buffering might have shown a distinguishable difference.

The amount of titratable acidity was reported in mls. of NaOH per  $10^{11}$  bacteria. This was necessary to take into account the population differences in the broth cultures. Even though a standard inoculum was used, the plaque sample may have varied in terms of the number of cultivatable bacteria present. Depending on the generation time of the individual bacteria species, the number of bacteria in the culture after 24 hours incubation will effect the amount of acid produced. It was for this reason the titratable acidity was recorded with respect to the number of bacteria in the culture medium.



Three sugars were used in this investigation. Bacterial fermentation varies from species to species on different sugars. It was necessary to include these sugars in order to increase the opportunity of observing a significant difference. Besides the fact that there were no differences between the before and after groups, there was also very little difference in the amount of acid produced on each of the three substrates.

The importance of this investigation lies in the fact that it has been demonstrated that the drop in pH seen in the plaque or orthodontic patients is not the result of a change in the bacterial flora to a more acidogenic type of bacteria but, most likely, to an increase in the number of bacteria and the amount of substrate for fermentation. This reemphasizes the importance of good oral hygiene in the control of dental caries in the orthodontic patient.

## CHAPTER VI

## SUMMARY

An investigation of the acidogenesis of the oral flora of dental plaque before and after the insertion of orthodontic appliances was made. The microbial population of the plaque sample was cultivated in glucose, lactose, and sucrose sugar broths and the amount of acid produced by these bacteria was determined by titratable acidity. By means of agar plating, the number of bacteria was determined, and this number was correlated with the amount of acid produced to determine the individual bacterial acidogenesis. This procedure was carried out on ten patients before and after the placement of bands and the findings were compared. The results of this investigation demonstrated no statistically significant alteration in the acidogenesis of the bacteria of the dental plaque before and after the insertion of orthodontic appliances.

## CHAPTER VII

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## CHAPTER VIII

## APPENDIX

TABLE V

TITRATABLE ACIDITY PRODUCED BY BACTERIA FROM THE  
PLAQUE OF ORTHODONTIC PATIENTS  
BEFORE BANDING

Patient No.	Total Aerobic Bacterial Count x 10 <sup>11</sup>			Titratable Acidity of 100 ml. Culture in ml. of 1N. NaOH		
	Glucose*	Lactose	Sucrose	Glucose	Lactose	Sucrose
1	281.6	244.1	291.1	3.01	2.95	2.99
2	321.5	196.3	178.1	2.33	2.15	2.20
3	127.1	125.3	198.3	2.20	1.88	2.02
4	510.7	578.7	639.7	2.39	2.09	2.21
5	332.1	311.9	423.9	2.01	1.90	2.02
6	230.3	160.9	171.4	2.95	2.73	2.02
7	208.4	247.1	323.3	2.19	2.09	2.21
8	25.3	258.9	262.5	3.41	3.03	3.00
9	137.9	125.5	207.3	2.51	2.21	2.96
10	161.7	327.8	484.3	1.83	1.91	1.86

\* The amount of acid indicated by the titratable acidity using sodium hydroxide does not necessarily indicate the amount of acid required to change the pH from 7 to the terminal pH, due to the action of media buffers, the heterogeneous nature of the acids produced and their individual pKa, and the inherent experimental error.

TABLE VI

TITRATABLE ACIDITY PRODUCED BY BACTERIA FROM THE  
PLAQUE OF ORTHODONTIC PATIENTS  
AFTER BANDING

<u>Patient No.</u>	<u>Total Aerobic Bacterial Count x 10<sup>11</sup></u>			<u>Titratable Acidity of 100 ml. Culture in ml. of 1N. NaOH</u>		
	<u>Glucose</u>	<u>Lactose</u>	<u>Sucrose</u>	<u>Glucose</u>	<u>Lactose</u>	<u>Sucrose</u>
1	290.5	256.5	296.1	3.33	3.20	3.21
2	238.6	154.9	184.7	2.32	2.10	2.39
3	148.6	136.3	190.5	2.39	1.99	2.21
4	521.1	580.9	660.8	2.44	2.16	2.55
5	328.0	314.2	430.6	2.09	2.00	2.19
6	280.3	200.5	232.1	3.16	3.01	2.82
7	200.6	241.7	310.7	2.19	2.20	2.23
8	220.1	276.3	291.5	3.49	3.21	3.27
9	130.6	119.8	201.8	2.50	2.23	2.94
10	140.7	320.1	465.5	1.79	1.90	1.80

## APPROVAL SHEET

This thesis submitted by Dr. John R. Riggs has been read and approved by the members of his thesis committee.

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

This thesis is, therefore, accepted in partial fulfillment of the requirements for the Degree of Master of Science in Oral Biology.

May 23, 1969

Date

John V. Madonia D.D.S., Ph.D.

Signature of Advisor