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## A Study of Dental Plaque in Orthodontic Patients

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# A STUDY OF DENTAL PLAQUE IN

## ORTHODONTIC PATIENTS

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following:

To John Vincent Madent, D.D.S., Ph.D., my thesis advisor, for his guidance in preparing this thesis.

To Donald C. Hilgers, A.S., M.D.S., M.S., Chairman of the Department of Orthodontics, who gave me the opportunity to do this work.

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Finally, I want to emphasize the important role of my mother, Pearl I. Balenseifen and my father, the late Walter M. Balenseifen, for their love, encouragement and help in writing this thesis.

**A Thesis Submitted to the Faculty of the Graduate School**

**of Loyola University in Partial Fulfillment of**

**the Requirements for the Degree of**

**Master of Science**

**June**

**1968**

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## ACKNOWLEDGEMENTS

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To my wife, Winnie, for her love, devotion, encouragement, and secretarial assistance in the preparation of this thesis.

Finally, I want to emphasize the important role of my mother, Pearl B. Balenseifen and my father, the late Homer M. Balenseifen, for their love, encouragement and help in making this all possible.

## LIFE

Jack W. Balenseifen was born in Watonga, Oklahoma on October 20, 1939. He graduated from Watonga High School in June, 1958 and entered Southwestern State College in June of the same year. He graduated from Southwestern State College in August, 1961 with the degree of Bachelor of Science.

He entered the University of Missouri at Kansas City, School of Dentistry in 1961 and graduated in June 1965 with the degree of Doctor of Dental Surgery.

He served as a staff dentist with the Oklahoma Public Health Department from 1965 to 1966. He has been enrolled in the Department of Oral Biology at Loyola University working toward a degree of Master of Science since June, 1966.



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CHAPTER I

ACTIVITY OF THE ORAL FLORA

INTRODUCTION

MICROORGANISMS IN THE ORAL CAVITY

In the past seventy years, dental scientists have done an enormous amount of research on dental caries. The Scientists (5,6,30) have found several types of bacteria that are directly related to dental caries. (29,30,31) Most dental scientists believe that the incipient dental cavity is covered by dental plaque in the beginning of the carious process. The researchers (24,30) have studied dental plaque from a bacteriolo-

gical point many times, but some research has been done on the composition of the dental plaque in carious and non-carious teeth. The pH (17) of dental plaque in carious teeth has been found to be more acidic than the pH of non-carious teeth. The pH (19) of dental plaque becomes more acidic when the patient eats carbohydrates.

The purpose of this thesis is to study changes in the characteristics of the dental plaque in orthodontic patients. The quantity of lactobacilli and streptococci in the plaque, the pH of the plaque, and the total carbohydrate content of the plaque will be recorded before and after the patients teeth are banded. The data will be evaluated for correlations between the content of lactobacilli and streptococci with the pH and carbohydrate content of the plaque.

The discovery of microorganisms in the oral cavity led to the association of microorganisms with dental caries. In 1822 Cutler (3) described the microorganism found in carious dentin of the tooth. Cutler described the microorganism as globular or slipper shaped, measuring 1/50,000 to 1/10,000 of inch in diameter. In 1876 Pierce (4) morphologically identified rods, spheres, and other forms of bacteria taken from carious dentin.

## CHAPTER II

## REVIEW OF LITERATURE

MICROORGANISMS

The first notable observations on bacteria were recorded in 1675 by Anton Van Leeuwenhoek<sup>(1)</sup> 1632 - 1723. With an optical instrument made of a biconvex lens, Leeuwenhoek discovered bacteria in various body fluids, in water, in pepper infusion, in yeast, in beer, and in plaque. The discovery of the microscope opened a new field for study of the causation of disease.

Robert Koch<sup>(2)</sup> 1843 - 1910 is conceded to be the "Father of Bacteriological Technic," for he discovered and developed the use of solid culture media, and isolate mixed cultures of microorganisms. He set forth criteria for establishing the etiology of disease. These are known as Koch's postulates and state: (1) A specific organism must always be associated with a disease. (2) It must be isolated in pure culture. (3) When inoculated into a healthy susceptible animal it must always produce the disease. (4) It should be obtained again in pure culture.

MICROORGANISMS IN THE ORAL CAVITY

The discovery of microorganism in the oral cavity led to the association of microorganisms with dental caries. In 1872 Cutler<sup>(3)</sup> described the microorganism found in carious dentin of the teeth. Cutler described the microorganism as globular or slipper shaped, measuring 1/60,000 to 1/10,000 of inch in diameter. In 1876 Pierce<sup>(4)</sup> morphologically identified rods, vibrios, and other forms of bacteria taken from carious dentin.

W. D. Miller<sup>(5)</sup> 1890 compared "life conditions" of bacteria in vitro with the conditions prevailing in the human mouth and he stated "the oral cavity must be an excellent breeding place for these organisms."

In 1903 Goadby<sup>(6)</sup> classified oral microorganism as liquefiers and acid formers, and he named Streptococcus as one of the acid forming organism.

Kligler<sup>(7)</sup> in 1915 classified oral microorganisms into three divisions (A) protozoa, (B) spirochetes, and (C) bacteria. He divided the bacteria of the mouth into anaerobic and aerobic groups.

### MICROORGANISMS AND DENTAL CARIES

In 1884 Underwood<sup>(8)</sup> observed histologically that microorganisms are always present in carious lesions and are not present in normal intact tooth substance.

W. D. Miller<sup>(5)</sup> 1853 - 1907 is conceded to be the father of oral microbiology. As the formulator of the chemico-parasitic theory of dental caries he was the first to apply a basic science to the solution of dental disease. He described the chemico-parasitic process as two stages: (A) decalcification and (B) dissolution of the softened residue. He believed that certain microorganisms found in the human mouth possessed the power to produce acid in the presence of food. He never named any one bacteria as the cause of dental decay. In 1897 Williams<sup>(9)</sup> showed that oral acid forming bacteria are the etiological factor in cause of dental decay.

Kligler<sup>(10)</sup> in 1915 described oral microorganism in material taken from three places: (A) between the teeth, (B) on tooth surfaces, and (C)

valuable material from the dental plaque. They found bacteria in the

from carious dentin. He believed that in dental decay the characteristics of the oral flora changed from that of Streptococcus to an acid producing rod. Kligler isolated from the carious material a non-spore forming bacillus, which he called Bacillus acidophilus, now called Lactobacillus acidophilus. He showed that the bacillus was capable of producing and with standing a greater amount of acid than that produced by any of his other isolates. In 1922 McIntosh, Janes, and Lazarus-Barlow<sup>(11)</sup> noted the constant presence of Bacillus acidophilus odontolyticus in carious material. Bunting and Parmerley<sup>(12)</sup> in 1925 isolated Lactobacillus acidophilus in every initial carious lesion, and they found the organism to be highly pleomorphic. In 1928 Bunting, Nickerson, Hard, and Crowley<sup>(13)</sup> ran a survey on 1,335 children to find out if there was a correlation between Bacillus acidophilus and dental decay. The group found the correlation to be very high. In 1933 Enright, Frisell, and Trescher<sup>(14)</sup> determined that the active agent of carious lesions must be able to withstand a acidity of pH 5.0. They found Lactobacillus acidophilus to be the only microorganism in direct contact with carious plaque and exhibit both aciduric and acidogenic characteristics at pH 5.0.

In 1941 Fosdick and Starke<sup>(15)</sup> found that Lactobacillus acidophilus was able to catalyze the various steps in carbohydrate degradation. Fosdick and Starke notice that lactobacilli accomplished the reduction of pyruvic acid to lactic acid very quickly.

Hemmens, Blayney, Eradel, and Harrison<sup>(16)</sup> in 1946 studied the cultivable microbial flora of the dental plaque. They found Lactobacillus to

be the only genus of bacteria for which there was definite evidence of increased incidence with the association of a developing carious lesion.

(17)  
In 1940 Stephan studied the changes in hydrogen-ion concentration on tooth and in carious lesions. Stephan stated "The pH of plaque and open cavities may be lowered by ingestion of certain carbohydrates. These pH changes can be demonstrated directly in the mouth with the antimony electrode."

(18)  
In 1948 Stralfors used an antimony electrode with an electric pH meter, to measure the changes in pH of the dental plaque and at the same time he made Lactobacillus counts of the saliva. He showed a significant relation to acidic pH and high Lactobacillus counts. The dental plaque at normal pH had fewer lactobacilli.

(19)  
D. E. Jager and Ockerse 1965 studied the pH of saliva and dental plaque of tooth surfaces before and after glucose rises by school children in high and low caries incidence area. They found the pH of the saliva to be more acidic in the children with a high incidence of caries, but the pH of the plaque was not significantly different in the two groups.

(20)  
Harrison in 1944 studied the plaque of six hundred patients before caries appeared and continued the study on the plaque of three hundred patients which had become carious. He found acid producing and acid tolerating streptococci and lactobacilli both to be closely related to dental decay.

(21)  
Besser in 1950 studied plaque material, and he found that lactobacilli were always present in plaque of carious teeth. He also noted

other forms of bacteria in the plaque.

Hemmens, Blayney, Bradel, and Harrison<sup>(22)</sup> in 1946 found Lactobacillus was the only bacterium to increase in the plaque with the developing of carious lesion.

Krasse<sup>(23)</sup> in 1954 found that the lactobacilli must be correlated with carbohydrate consumption and caries activity at the same time.

In 1954 Orland<sup>(24)</sup> demonstrated that germ free rats do not develop dental caries even when maintained on diets which are cariogenic for their conventional counterpart.

In 1955 Orland<sup>(25)</sup> and his co-workers demonstrated that caries can be induced in germ free rats infected with combinations of enterococcus and either a proteolytic aerobic rod or an anaerobic pleomorphic rod. Keyes<sup>(26)</sup> 1960, Fitzgerald<sup>(27)</sup> and Keyes, 1961, and Gibbons<sup>(28)</sup> 1966 showed that animals harbouring cariogenic bacteria develop bacterial plaques whereas little or none accumulate in animals harbouring non-cariogenic bacteria.

Dawes and Jenkins<sup>(29)</sup> in 1962 found that the dental plaque had a high concentration of calcium and phosphorus in caries high animals.

Fitzgerald and Keyes<sup>(30)</sup> 1960 showed that dental caries was induced in a strain of albino "caries inactive" hamsters by oral inoculation of single or pooled cultures of streptococci isolated from carious lesions in a hamster. He also demonstrated that pooled cultures of six strains of diphtheroid organisms, or six strains of lactobacilli isolated from the

positive relationship exists between the plaque and lactobacillus counts.

oral cavity of "caries active" hamsters were without effect when similarly inoculated as were six strains of streptococci isolated from caries inactive hamsters.

Fitzgerald, Jordan, and Archard<sup>(31)</sup> 1966 were able to demonstrate that lactobacilli could produce caries in germ free rats when the rats were fed a diet of coarse particle with high sugar content.

Gibbon and Banghart<sup>(32)</sup> 1967 using a combination of serologic and isotope dilution technique estimated that dextran comprised almost 2% of the total dry weight of pooled plaque samples. He speculated that the plaque had a much higher percentage of dextran in the matrix of plaque.

#### ORTHODONTICS AND MICROORGANISMS

In 1948 Owen<sup>(33)</sup> studied the saliva of his orthodontic patients before and during orthodontic treatment. Owen stated the following: (1) "The presence of orthodontic appliances in the mouth does increase the Lactobacillus count. (2) The degree of increase is dependent some what upon the number of bands. (3) There is a correlation between the total band months and the number of lactobacilli per cc."

Dikeman<sup>(34)</sup> in 1962 studied the microorganisms of saliva in orthodontic and non-orthodontic patients. He reported the following: (1) "The presence of stainless steel orthodontic appliances tends to increase both Lactobacillus and Staphylococcus counts but does not appreciably change yeast or Streptococcus counts. (2) The greater the milk consumption, the lower the Lactobacillus and yeast counts. (3) A definite positive relationship exists between DMF teeth and Lactobacillus counts."



Bloom and Brown<sup>(35)</sup> 1964 studied the effect of orthodontic appliances on the oral microbial flora. They measured seven types of microorganisms, including anaerobic and aerobic types. They stated that "all categories showed a numerical increase after the placement of orthodontic appliances, only the lactobacilli increase by a statistically significant amount." The Lactobacillus increase by 3500% or an average of 90,000 Lactobacillus per milliliter of saliva. They also found "the greatest number of orthodontic bands and auxiliaries manifested the greatest quantitative increase in microbial population studied."

The preceding literature review proves that the orthodontic patient can be used for a study of the dental plaque. The patient can supply a measurable amount of dental plaque, so that bacteriological studies can be done.

The patients were required to brush their teeth for five minutes before the study. The patients were instructed to brush their teeth for five minutes using the modified brushing technique after eating. The patients were not allowed to eat anything during the hour following the brushing. The patients were instructed to brush their teeth for five minutes using the modified brushing technique after eating.

The patients' plaque samples and measurements were recorded one week before the study was started. The pH of the plaque was recorded in situ. The plaque was then removed for the lactobacilli and streptococci counting technique. The last part of the study was the quantitative determination of acidogenicity in the plaque by using the acidogenicity test.

### CHAPTER III

#### METHODS AND MATERIALS

##### (A) Selection of Subject

Twenty-one patients between ten and sixteen years of age were selected for this study from the Orthodontic Clinic at Loyola University School of Dentistry. The patients had Class I and Class II (Angle) malocclusion, and they are being treated with light, resilient wires and light forces. The patients were checked before the bands were placed, and rechecked one month after the bands and arches were inserted.

##### (B) Oral Hygiene

The patients were required to brush their teeth for five minutes using a Modified Stillman technique (occlusal-gingival cycle). The plaque samples and measurements were collected one hour after the supervised brushing. The patients were not allowed to eat anything during the hour following the brushing. The patients were instructed to brush their teeth for five minutes using the Modified Stillman technique after eating.

##### (C) Experimental Procedure

The patients' plaque samples and measurements were recorded one week before the teeth were banded. The pH of the plaque was recorded in situ. The plaque was then prepared for the Lactobacillus and Streptococcus counting technique. The last test of the plaque samples was the quantitative microdetermination of carbohydrate in the plaque by using the modified anthrone test.

The second group of plaque samples and measurements were recorded four to five weeks after the bands and arch wires were attached to the teeth. The pH of the plaque was recorded in situ. The plaque was then prepared for the Lactobacillus and Streptococcus counting technique. The last test of the plaque samples was the quantitative microdetermination of carbohydrate in the plaque by using the modified anthrone test.

The first group of data was recorded before the bands were placed. The second group of data was recorded after the bands and arch wires were placed. Comparisons were made between the first group of data and the second group of data. The measurements that were compared were: (1) pH, (2) Lactobacillus content, (3) Streptococcus mitis content, (4) Streptococcus salivarius content, (5) total Streptococcus content, and (6) Carbohydrate.

(D) pH Measurement

The pH meter was standardized for the two electrodes by using a buffer of known pH solution before each test. Comparative tests were made with the exploring (antimony - silver) electrode and the Coleman glass electrode in known pH solutions at 25° c. The maximum variation was less than 0.10 pH units in the range of 4.0 to 7.0 pH. This variation is comparable with that found by Parks and Beard. (36) Before the Antimony - Silver electrode was used in the mouth, it was standardized with a buffer of pH 6.0, so that reading could be made directly on the pH scale of the potentiometer - electrometer.

The pH readings were taken at various sites on the teeth. The plaque was usually found on the buccal of the maxillary molars, lingual of the maxillary lateral incisors, lingual of the mandibular molars, and the lingual of the mandibular incisors. A minimum of three readings were recorded for each patient at a session.

A Coleman Metrion IV pH meter was used to read the pH of the patients. The exploring electrode was specially built for this type of pH measurement, and was similar to the one used by Stephan<sup>(36)</sup> in 1940. It consisted of a glass tube,  $\frac{1}{4}$  " x 5 ", which was tapered on one end (Fig. 1). An Antimony - Silver tip was placed at the tapered end of the tube (Fig. 2). The Antimony - Silver tip was cemented with dental compound to the end of the glass tube. A bare copper wire was inserted into the glass tube and the glass tube was then filled with mercury, and the other end of the tube was sealed with dental compound. The exploring electrode was then attached to the pH meter. A standard glass calomel electrode was used for the reference electrode.

The reference electrode was inserted into a saturated solution of potassium chloride. The patient immersed his hand into the potassium chloride, and the investigator inserted the tip of the exploring electrode into the dental plaque. The pH reading was then recorded. The tip was the only part of the electrode placed in contact with the plaque.

#### (E) Collecting and Weighing the Plaque

The plaque was removed from the teeth with a number 6-7 gracy curette. The plaque is placed in a Lilly No. 5 gelatin capsules. The gelatin capsules weigh approximately sixteen milligrams after they were reduced in length. The capsules were cut to reduce the weight. The gelatin capsules were preweighted and placed into small vials which had the patients' name on them. The removal of the plaque from the teeth, and the collecting of the plaque in the gelatin capsule required approximately five minutes. The total weight of the filled capsule is then determined, and the actual weight of the plaque is calculated.

The plaque and gelatin capsule were inserted into a five milliliter Viritis microhomogenizer cup. A recorded quantity of water was added to the plaque and capsule. The plaque to water ratio was approximately three milligram of plaque to one milliliter of water. The total capsule was homogenatized in the water at 400 r.p.m. for five minutes.

#### (F) Serial Diluting and Plating

One-tenth of milliliter of the homogenized sample was diluted into a test tube with nine-tenth of a milliliter of sterile water. The serial dilution procedure was continued until the sample was diluted to  $10^{-15}$ .

The plating of the diluted sample was done on two different media. The selective media for streptococci was BBL\* Mitis-Salivarius agar. The selective media for lactobacilli was BBL Lactobacillus Selective Agar.\* One-tenth of a milliliter of the diluted sample from each of the fourteen tubes of water was placed on the selective streptococci agar. The sample

\*Baltimore Biological Laboratories, Baltimore, Maryland

was then evenly distributed on the surface of the agar by using a sterile right-angle glass rod. Lactobacillus selective media was heated at  $212^{\circ}$  F until it became molten. It was cooled to  $53^{\circ}$  C before using. One-tenth of a milliliter of the diluted sample was placed on the inner surface of a sterile petri dish, and the Lactobacillus selective agar was poured into the dish as the dish was gently rotated. The samples for the lactobacilli selective agar were taken from  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilutions. All of the petri dishes were inverted and incubated for forty-eight hours at  $37^{\circ}$  C. The total Streptococci, Streptococci mitis, Streptococcus salivarius, and Lactobacillus colonies were counted and recorded after the incubation (Fig. 3 and 4). The Streptococcus salivarius appear on mitis salivarius agar is large, clear, soft, mucoid colonies about two to three millimeters in diameter (Fig. 3). The Streptococcus mitis appeared as flat, non-elevated, pin pointed colonies (Fig. 3).

#### (G) Carbohydrate determination

One milliliter of homogenized sample was pipetted into a test tube containing five milliliters of anthrone sulfuric acid reagent. One milliliter of distilled water was pipetted into a second test tube containing five milliliters of anthrone-sulfuric acid reagent. One milliliter of a glucose-water solution was pipetted into a third test tube containing five milliliters of anthrone-sulfuric acid reagent. The concentration of the glucose-water solution was 0.1 milligram of dextrose per milliliter of water. The test tubes were gently swirled to mix the reagents with

the samples. The three test tubes were placed in boiling water for exactly five minutes. The tubes are removed from the boiling water and placed in an ice-water solution for one minute. The spectrophotometer is set at 620 mu. The third test tube was used to calibrate the spectrophotometer at zero optical density. The first test tube was read first, and the second test tube was recorded last on the spectrophotometer.

Determining of the carbohydrate content was done by a modification of the technique used by K. J. Weeks<sup>(37)</sup>.

The reagent used was a 0.2% solution of purified anthrone in 98% (v/v) sulphuric acid. The reagent solution had a three day shelf life.

Five milliliters of the reagent solution were pipetted into a 6 " X 1 " rimless test tube. Overlayed on the surface of the reagent was a one milliliter aliquot of the solution to be analysed. This was done carefully to prevent mixing of the layers. The tubes were then swirled gently to mix the solution and the reagent. The mixture was immediately placed in a boiling water bath for exactly five minutes, where upon it was removed and cooled rapidly to room temperature. The optical density of the solution was determined in a Coleman Model 6135 Junior II Spectrophotometer at 620 mu, where maximum absorption of the color complex occurs.

With each determination, or series of determinations, it was necessary to run a blank which contained one milliliter of water and a standard with one milliliter of a glucose solution containing 100 mg. of

glucose per ml. The amount of carbohydrate in the test solution in term of glucose was calculated as followed: Carbohydrate ( as glucose) per milliliter test solution=

$$\frac{\text{Absorbance of test solution} - \text{Absorbance of blank}}{\text{Absorbance of std. glucose soln.} - \text{Absorbance of blank}} \times \frac{100}{1}$$

The relation between color development in the reaction and glucose concentration was linear between 0 and 100 glucose per milliliter and it is not until a concentration of 125 was reached that the deviation from linearity is sufficient to produce an appreciable error.

This method gave results accurate to 0.5 microgram consistantly according to Weeks. (37) Because of the strong acid conditions used in the anthrone - carbohydrate reaction, all types of carbohydrate material can be determined directly and the result obtained will always be measure of the total carbohydrate present in terms of the standard sugar solution used. (38) When only one sugar is present then the result can be converted into terms of the sugar by either, applying a correction factor for the difference in color development of the standard sugar and the test sugar, or by using as a standard a solution of the sugar present in the test solution.

The reaction between carbohydrate anthrone under strongly acid conditions produces a blue-green which is specific for carbohydrates. (38) The only other compound which will yield a blue-green color is furfural, but in this case, the color soon becomes brown after a short heating period. (37)



There are some substances which will produce interfering effects in the determination of carbohydrate in biological material. Weeks<sup>(37)</sup> found protein and amino acids are the most usually encountered substances which will produce interference. However, interference due to protein does not make itself felt until the sugar content of the sample falls to about five per cent of the protein content. Below this level Weeks states that clarification procedures must be employed to remove the protein.

Before the investigator used the modified anthrone technique for determining the carbohydrate content of the plaque the investigator went through a series of trial tests with known values of glucose per milliliter of water. The test was run three separate times. The first test was done with samples containing 0.02, 0.04, 0.06, 0.08, and 0.10 milligram of glucose per milliliter of water (Fig. 5).

The second test was like the first test, except for one extra test sample. The extra test sample contained a solution of 0.058 milligram of protein per milliliter water with 0.058 milligram of glucose per milliliter water. This experiment showed that the protein at a 1 : 1 ratio with the carbohydrate had little effect on the accuracy of the test (Fig. 6).

The third test was like the first test, except for two extra test samples. One of the extra test sample was a solution containing 0.058 milligrams of gelatin per milliliter of water and 0.058 milligrams of glucose per milliliter of water. The other extra sample was a solution

containing 0.275 milligrams of gelatin per milliliter of water and 0.045 milligrams of dextrose per milliliter of water. The third experiment showed the protein could be five times as concentrated and not significantly alter the accuracy of the test (Fig. 7).

#### (H) Statistical Analysis

The data from the two groups of experiments were collected and prepared for statistical analysis. In the experiment the same group of patients is subjected to two different situations. The data was obtained as two series of measurements for the same group of individuals, so that the measurements in each series are not independent of, but, in fact, correlated with, each other.

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  $t = \frac{\bar{d}}{SE_d}$ . This ratio is distributed in the  $t$  - curve with  $(N - 1)$  degrees of freedom. The  $t$  value was computed and checked for a 1% and 5% level of significance.

The mean pH of the patients' plasma before loading the teeth was 7.38. The mean pH of the plasma for teeth after attaching bands and arch wires was 7.41. The difference in the mean pH was 0.03. The standard error of the plasma pH for individual patients was 0.1, more acidic than the mean pH of the plasma before banding.

The pH value was lowered in all of the blood specimens following the orthodontic intervention. This is shown graphically in Fig. 8.

## CHAPTER V

### RESULTS

The plaque samples and measurements were collected from twenty-one patients before banding. Nine of the original twenty-one patients were eliminated from the experiment due to orthodontic complications. The samples and measurements were collected from twelve patients after the orthodontic intervention.

The comparisons of the data were done on the final twelve patients with respect to their recorded measurements. The data appears in Table 1 and Table 2. A tabulation of the individual measurements for the six categories investigated is presented in Table 3. The pH measurement and the carbohydrate content of the plaque are shown graphically in Fig. 8 and Fig. 9. The logarithmic counterparts of each bacteria category are shown graphically in Fig. 10, 11, 12, and 13.

#### (A) pH comparisons

The mean pH of the patients' plaque before banding the teeth was 6.8. The mean pH of the plaque one month after attaching bands and arch wires was 6.4. The difference in the mean pH was 0.4. The median pH of the plaque after orthodontic intervention was 0.4 more acidic than the mean pH of the plaque before banding.

The pH value was lowered in each of the twelve patients following the orthodontic intervention. This is shown graphically in Fig. 8.

Patient number eight had the greatest pH difference. The difference was 0.80 pH units. Patient number one with a difference of 0.10 pH units had the smallest difference.

The before pH measurement for each patient was paired with the after pH measurement. The difference was calculated for all twelve patients. The ratio of the average difference to the standard error of the average difference is  $t = \frac{\bar{d}}{\frac{SE}{\sqrt{d}}}$ . The + value was 8.36 which is statistically significant at the 1% level.

The results of this investigation have established that there is a numerical decrease in the pH of the dental plaque following the placement of orthodontic appliances.

#### (B) Carbohydrate content comparison

The amount of total carbohydrate in the test solution was calculated in terms of glucose. The mean carbohydrate content of the patients' plaque before banding of the teeth was 0.0277 mg./mg. of plaque. The mean carbohydrate content of the plaque one month after attaching bands and arch wires was 0.377 mg./mg. of plaque. The mean carbohydrate content of the plaque was 0.0100 mg./mg. of plaque higher after attaching the bands and arch wires for one month.

The carbohydrate content of the dental plaque increased in nine of the twelve patients (Fig. 9). The highest increase was 0.0347 mg. of plaque. The highest decrease was 0.0127 mg. of carbohydrate per mg. of

plaque (Table 3).

The before carbohydrate content for each patient was paired with the after carbohydrate content. The difference was calculated for all twelve patients. The + value was 2.35 which was not statistically significant at the 1% level, but it was statistically significant at the 5% level. This investigation has shown that there is a statistically significant increase in the carbohydrate content of the dental plaque after insertion of orthodontic appliances.

(C) Lactobacillus content comparsion

The mean Lactobacillus content of the plaque in the before sample was  $4.35 \times 10^2$  lactobacilli/mg. plaque. The mean Lactobacillus count in the after sample was  $9.81 \times 10^4$  lactobacilli/mg. of plaque. The mean Lactobacillus count in the after sample was  $9.80 \times 10^4$  lactobacilli/mg. plaque higher than the before sample.

The Lactobacillus count of the plaque increased in eleven of the twelve patients after the placement of orthodontic appliances (Fig. 10). The highest increase in Lactobacillus count was in patient number ten. The increase was  $4.98 \times 10^4$ . Patient number two with an increase of  $9.0 \times 10^1$  had the smallest increase in Lactobacillus content (Table 3).

The before Lactobacillus count was statistically analysed with respect to the after Lactobacillus count. The + value was 6.33 which is statistically significant at the 1% level.

(D) Streptococcus content comparsion

Each of the streptococci categories studied manifested an increase in its population mean after the placement of orthodontic appliances (Table 1). The Streptococcus mitis showed the greatest increase of the streptococci. The mean Streptococcus mitis increase was  $1.50 \times 10^{14}$  cells/mg. of plaque.

The Streptococcus mitis content of the plaque increased in all twelve patients (Fig. 11). Patient number four had the largest increase in Streptococcus mitis. The increase was  $1.12 \times 10^{15}$ . Patient number two with an increase of  $1.13 \times 10^5$  had the smallest increase (Table 3). The Streptococcus mitis increase was statistically significant ( $P < 0.01$ ).

The Streptococcus salivarius content of the plaque increased in eleven of the twelve patients (Fig. 12). Patient number one with an increase of  $1.16 \times 10^{12}$  had the largest increase. Patient number eight with a decrease of  $5.8 \times 10^7$  was the only patient to have a decrease in Streptococcus salivarius content (Table 3). This increase in the Streptococcus salivarius was found to be statistically significant ( $P < 0.01$ ).

The total streptococci content of the dental plaque increased in all twelve patients (Fig. 13). The increases varied from  $1.16 \times 10^{15}$  to  $1.12 \times 10^{15}$  (Table 3). The increase in the total streptococci was found to be statistically significant ( $P < 0.01$ ). Although all bacterial categories manifested a rise in population concurrent with the placement of appliances, the total streptococci were found to increase by a far greater amount than any other category.

## CHAPTER VI

## DISCUSSION

In any attempt to understand the fundamental nature of dental caries, one is confronted by the question "What is the mechanism by which enamel and dentin are dissolved from teeth, under the conditions that exist in the mouth?"

The mass of evidence which led W. D. Miller<sup>(5)</sup> to formulate the "chemicoparasitic" theory of caries offers a logical starting point for seeking an answer to this question. We may summarize his findings and theory thus: Caries is a process of dissolution of the calcium salts from enamel and dentin by acids, formed in the mouth by fermentation of carbohydrates by bacteria lodged on tooth surfaces. This process is followed by disintegration of the organic matrix by proteolytic action of the same or other bacteria.

Miller's experiments did not simulate beginning caries of enamel. His experiments did not seem to explain why the acid produced on tooth surfaces was not neutralized by saliva. To fill this gap in the chemicoparasitic theory, others conceived of a "plaque" or "film" on the tooth surface which confined acid to the tooth surface and prevented neutralization by saliva.

Williams<sup>(39)</sup> demonstrated adherent bacterial plaques on surfaces of teeth, including some with beginning caries of the enamel. Black<sup>(40)</sup> presented evidence of "gelatinous plaques" produced by gelatinizing

bacteria on those surfaces of teeth which were not cleaned either by a brush or in mastication.

Kirk<sup>(41)</sup> presented evidence that mucin was precipitated from the saliva to form a "mucin plaque."

Regardless, however, of whether the nature of the caries producing "plaque" is bacterial, gelatinous, mucinous, composed of food particles or any combination of these, theoretically it is necessary for the plaque to develop a sufficient hydrogen-ion concentration to dissolve enamel, in order for the carious lesion to develop.

There have been many investigations of the factors governing the solubility of the enamel. McClelland,<sup>(42)</sup> Enright, Friesell, Fosdick and Starke,<sup>(15)</sup> Tresher,<sup>(43)</sup> Kanthak,<sup>(44)</sup> Chase,<sup>(45)</sup> and others have all indicated that the hydrogen-ion concentration is the chief factor in the solubility of enamel. The evidence indicates that, in a solution containing as much calcium phosphate as saliva, the enamel will not dissolve above a pH of about 5.0 to 5.5, but will dissolve below this pH. Since the pH of saliva is almost always from 6.0 to 7.8, it is obvious why the saliva does not dissolve enamel. Since enamel is dissolved in beginning caries, it would seem logical that something on the enamel surface attains a pH less than 5.0 - 5.5. The measurement of this pH is important in order to determine the immediate activity of the carious process.

There are several methods of measuring pH, none of which is without some disadvantages.



The microcolorimetric technic used by Stephan<sup>(17)</sup> in a reported study showed variations in pH of plaques from 4.6 to 7.0, and the carious debris from 4.2 to 7.0. This method required considerable time and patience, and therefore it was difficult to make many tests on material from tooth surfaces at one time. The material tested was removed from the mouth, and only one measurement was possible.

Electrometric methods have proved applicable for repeated direct measurements of pH in vivo. The glass electrode has the fewest errors. Microglass electrodes small enough to be applicable for pH readings on tooth surfaces have two serious limitations: 1. The electrodes are very fragile, and they break with slight pressure. 2. Their electrical resistance is so high that thorough electrostatic shielding is necessary. Thus far, these limitations have hindered their use directly on tooth surfaces. The antimony electrode has neither of these limitations.

Parks and Beard<sup>(36)</sup> have shown that a polished stick electrode, in the presence of air, and with a vacuum tube potentiometer-electrometer which would prevent polarization of the electrodes, followed the theoretical Nernst equation from pH 2-7 at 25° C., the greatest deviation being 0.2 pH unit.

T. R. Ball<sup>(46)</sup> showed that an antimony sulfide electrode functions between pH 1.8 pH 10, with results reproducible to 0.09 pH unit. Citrates rendered the electrode useless, but 10 per cent sugar, 2 per cent starch

and 0.5 molar sodium nitrate were without effect.

For many years acids produced by microorganisms have been considered a causative factor of dental caries; in fact even today this theory influences dental caries research to a very great extent. It seems reasonable to assume that, if it is correct, the acidity could be measured, the pH determined, and a fall in pH to the critical pH or beyond should be demonstrated in dental plaque or in carious cavities.

The caries research done on gnotobiotic rats by Orland<sup>(24)</sup> 1954 showed that germ free rats do not develop dental caries even when maintained on diets which are cariogenic for their conventional counterpart. Fitzgerald<sup>(27)</sup> 1960 demonstrated the etiologic role of streptococci in experimental caries in the hamster. Recently, Orland<sup>(47)</sup> 1964 reported that oral lactobacilli of human origin became cariogenic for rats after being maintained in three successive generation of gnotobiotic rats. The active role of lactobacilli and streptococci in dental caries was one of the reasons for selecting these microorganisms to be measured in the experiment.

The Author's experiment showed that the pH of the plaque became more acidic after the orthodontic intervention. The plaque became more acidic due to a quantitative increase of bacteria content and a quantitative increase of carbohydrate content of the plaque. The streptococci and the lactobacilli content both had a quantitative increase in the plaque. These two groups of bacteria are acid producing and acid tolerating in

character. The quantitative increase in carbohydrate content provide these bacteria with more substrate for degradation.

The data from the experiment showed that both the streptococci and the lactobacilli content of the plaque had a quantitative increase. It is quite conceivable that the inherent irregularities of fixed orthodontic appliances could, in general, provide additional surfaces for the collection and retention of food debris. These additional surfaces would contribute more area of environment suitable for the growth of bacteria. In this respect, Omisi and Kondo<sup>(48)</sup> found that placing a plastic plate on the palate increase the number of lactobacilli within a few days. They postulated that fermentation areas can lead to an increase in lactobacilli.

It is a well known fact that lactobacilli grows better in an acidic medium. It is possible that the increase in streptococci content will provide a more acidic plaque, which will be a better medium for the growth of lactobacilli. The increase in the carbohydrate content of the plaque will provide a more suitable environment for streptococci, which in turn provides a more suitable environment for the lactobacilli. Harrison<sup>(20)</sup> in a study of dental plaque from carious teeth always found acid producing and acid tolerating streptococci with lactobacilli.

A comparison was made of the lactobacilli and streptococci content of the plaque with the pH of the plaque. There was an increase in lactobacilli and streptococci content with an increase in acidity. There was no linear correlation of the increase in bacteria content and the pH as a

group, but the individual samples were positively related. The reason that the group did not show a linear relationship was due to the tremendous variation in increase of bacteria content with a small change in pH.

The orthodontic appliances tend to enhance bacterial growth by providing a more suitable environment for bacterial activity and thereby subjecting the oral environment to imbalance and possible disease. It is generally recognized that cariogenic bacteria are able to initiate the formation of dental plaque for it has been a consistent finding that animals harbouring cariogenic bacteria develop bacteria plaque whereas little or none accumulates in animals harbouring non-cariogenic bacteria. Keyes<sup>(26)</sup> 1960, Fitzgerald<sup>(27)</sup> and Keyes, 1961, Gibbons<sup>(28)</sup> et al. 1966. Carlsson<sup>(49)</sup> and Egelbery 1965 observed that sucrose stimulates plaque formation in man. It is possible that the increase of food debris in the area could aid the bacteria in producing dental plaque, and the new plaque could have higher carbohydrate content.

The modified anthrone test for the carbohydrate content of the plaque appears to be very valuable. The test determined the total carbohydrate content of the plaque with few limitations. The test is accurate to one millionth of a gram. The accuracy is not altered to any appreciable extent unless the reagent is over three days old.

It is important to note that there is a quantitative increase of the carbohydrate content of the plaque. It is obvious that there is an increase in total plaque after banding which in itself would increase the

total bacteria count. The increase in carbohydrate content could be due to an increase in dextran which is believed to be the matrix material for dental plaque according to Gibbons. The increase in carbohydrate content could also be due to an increase in unattached substrate, or both an increase in dextran and substrate.

The carbohydrate content of the plaque increased on the average by 40 per cent after the orthodontic intervention. Eastoe and Bowen 1967 showed that when a few drops of 0.1 M solution of sucrose was placed on the dental plaque, there was a drop in pH to 5.17 (S.D.  $\pm$  0.38) after 20 minutes. Whereas the absolute significance of the measured pH near tooth surface must be interpreted with caution, it is considered that these measurements provide clear evidence for the production of substantial quantities of acid at these sites and strongly suggests its participation in the development of the carious lesions.

It appears from the results of the experiment that the dental plaque must have an increase in carbohydrate content before there is an increase in acidity or bacteria content. The alteration of the oral environment is primarily that of increasing the food debris in the dental area due to fixed orthodontic appliances. The increase in bacteria and the increase of acidity of the plaque can increase the carious activity in the oral cavity. It appears that this chain of events could be retarded by instigating good oral hygiene in these patients. Proper oral hygiene would eliminate the excessive food debris, and the bacterial environment would

be favorable alter.

There was no linear relationship between the carbohydrate content and the bacteria content of the plaque. The change in each group was statistically significant with respect to measurement made before and after orthodontic intervention, but there was no linear relationship between the two due to the large variation of bacteria with a simultaneous small change in carbohydrate.

It is important to note that in this experiment the measurements were of the concentration of the carbohydrate and the concentration of the bacteria in the plaque. Packed organism (solid bacteria) at the bottom of a centrifuged tube show a concentration of  $2.3 \times 10^{14}$  /Mg.

This value is approximately the same as the plaque measurement after orthodontic intervention. The results would indicate that there is less other material and more bacteria and carbohydrate in carious plaque.

It is the opinion of the author that further research should be done on the dental plaque after orthodontic intervention. It is possible that the changes seen in the dental plaque after orthodontic intervention are the same as in carious plaque. The changes in the plaque of an orthodontic patient are relatively easy to measure, and the patient is reliable and the control of the patient during the experiment is assured.

A few of the characteristics that should be measured are the following; protein content, calcium content, phosphate content, and potassium content.

## CHAPTER VI

## SUMMARY

A comparison of the quantitative changes of the dental plaque of twelve patients, before and after orthodontic treatment, was made. The four microbial populations were measured by plating measured wet plaque samples on standard selective culture media. The pH of the plaque was measured in situ by an exploring glass electrode with an Antimony-Silver tip. The carbohydrate content of the plaque was quantitatively measured by using a modified anthrone-sulfuric acid test.

The following results were obtained:

- (1) The pH of the dental plaque became more acidic after the placement of orthodontic appliances.
- (2) The carbohydrate content of the plaque increased by 40% after the orthodontic intervention.
- (3) The Lactobacillus, Streptococcus mitis, Streptococcus salivarius, and total Streptococcus quantitative counts showed a numerical increase after the placement of orthodontic appliances.
- (4) The changes in the pH measurement, carbohydrate content, and microbial population of the dental plaque were all statistically significant.

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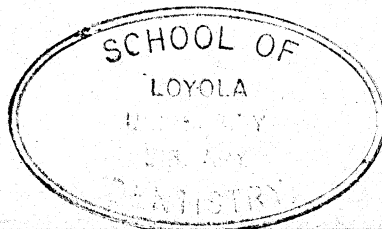
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Fig. 1 GLASS EXPLORING ELECTRODE



Fig. 2 CLOSE UP VIEW OF ANTIMONY-SILVER TIP

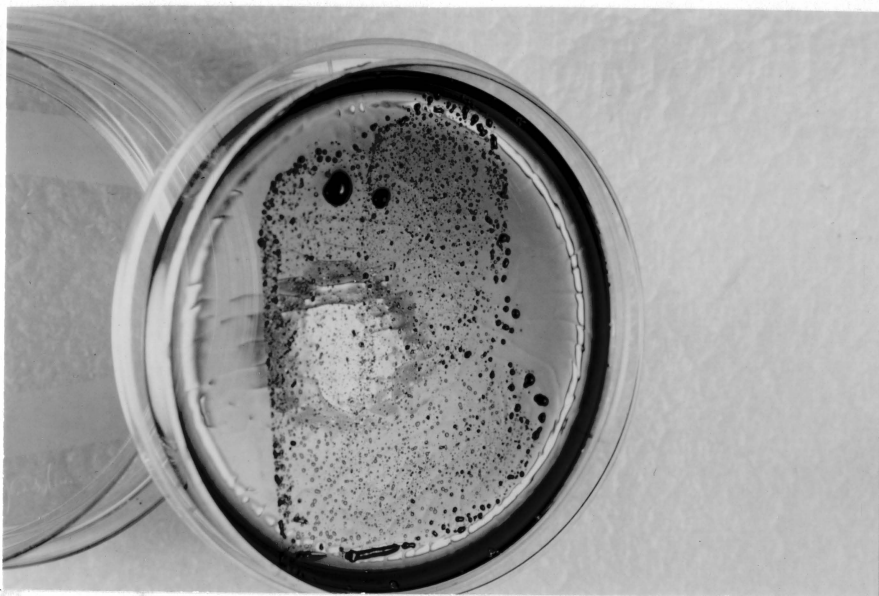


Fig. 3 STREPTOCOCCUS MITIS AND STREPTOCOCCUS SALIVARIUS ON MITIS-SALIVARIUS AGAR.

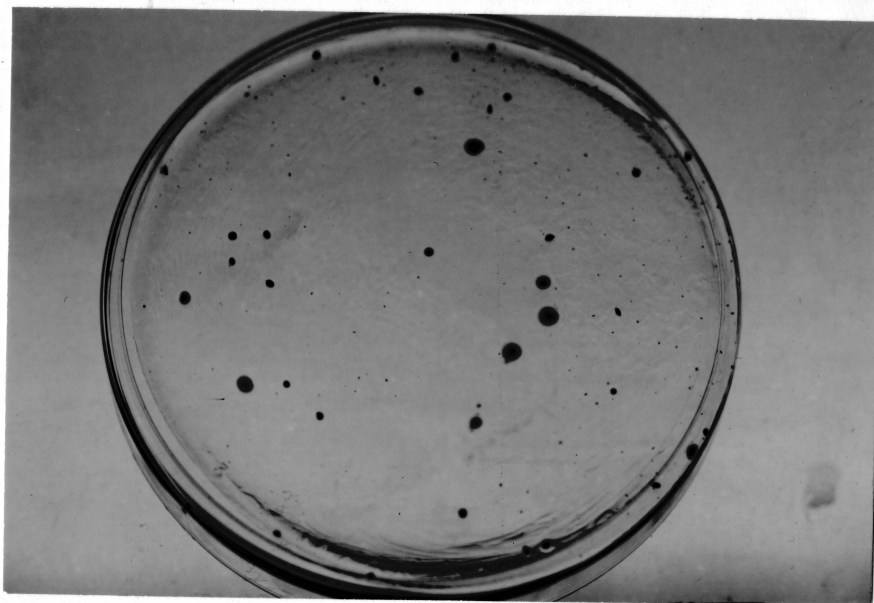


Fig. 4 LACTOBACILLUS ON LACTOBACILLI AGAR.

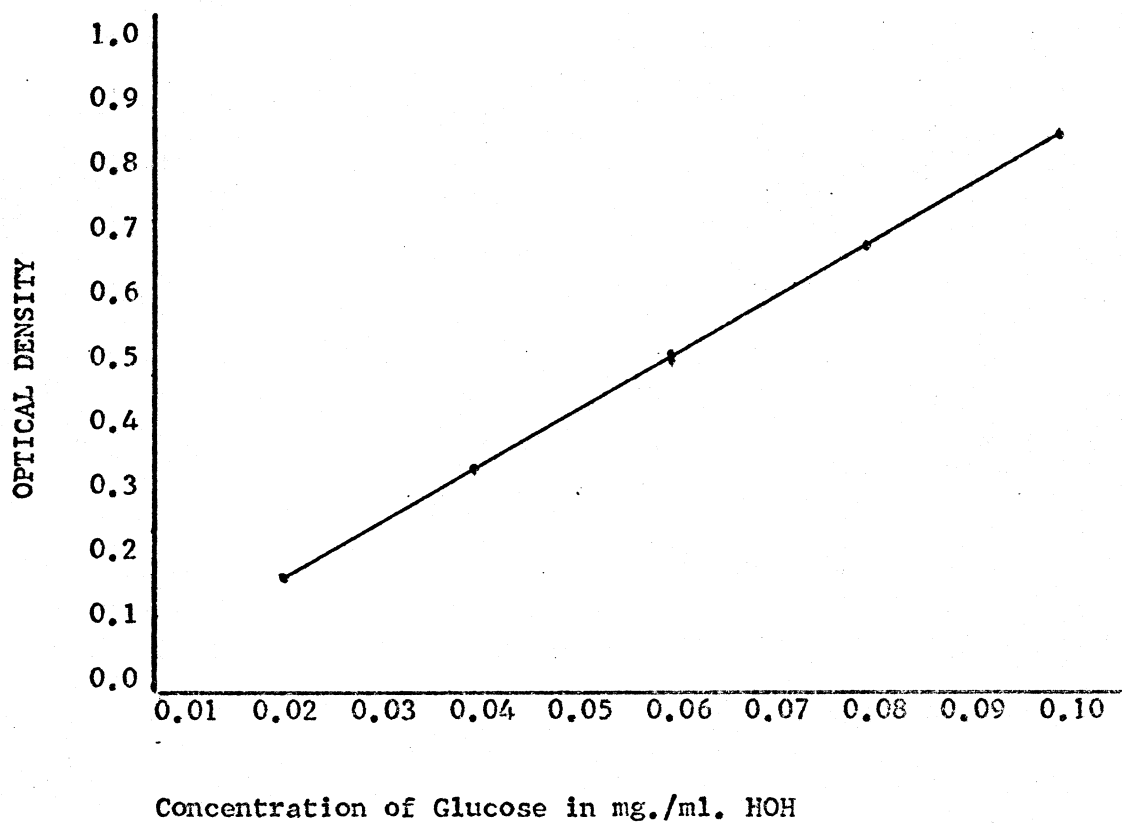


Fig. 5 Linear relationship of glucose in mg./ml. water to the solutions optical density.

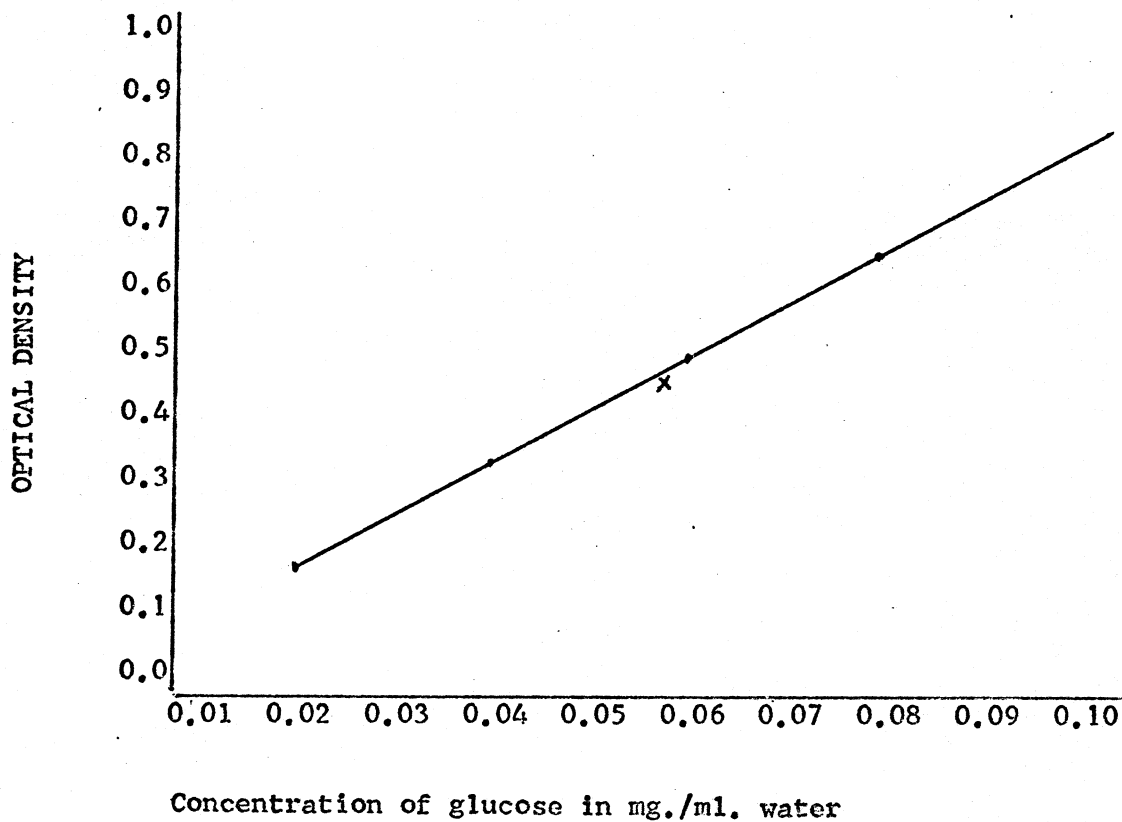


Fig. 6 Linear relationship of glucose in mg./ml. water to the optical density. A solution of 0.058 mg. protein/ml. water and 0.058 mg. carbohydrate/ml. water was checked, indicated by (x).

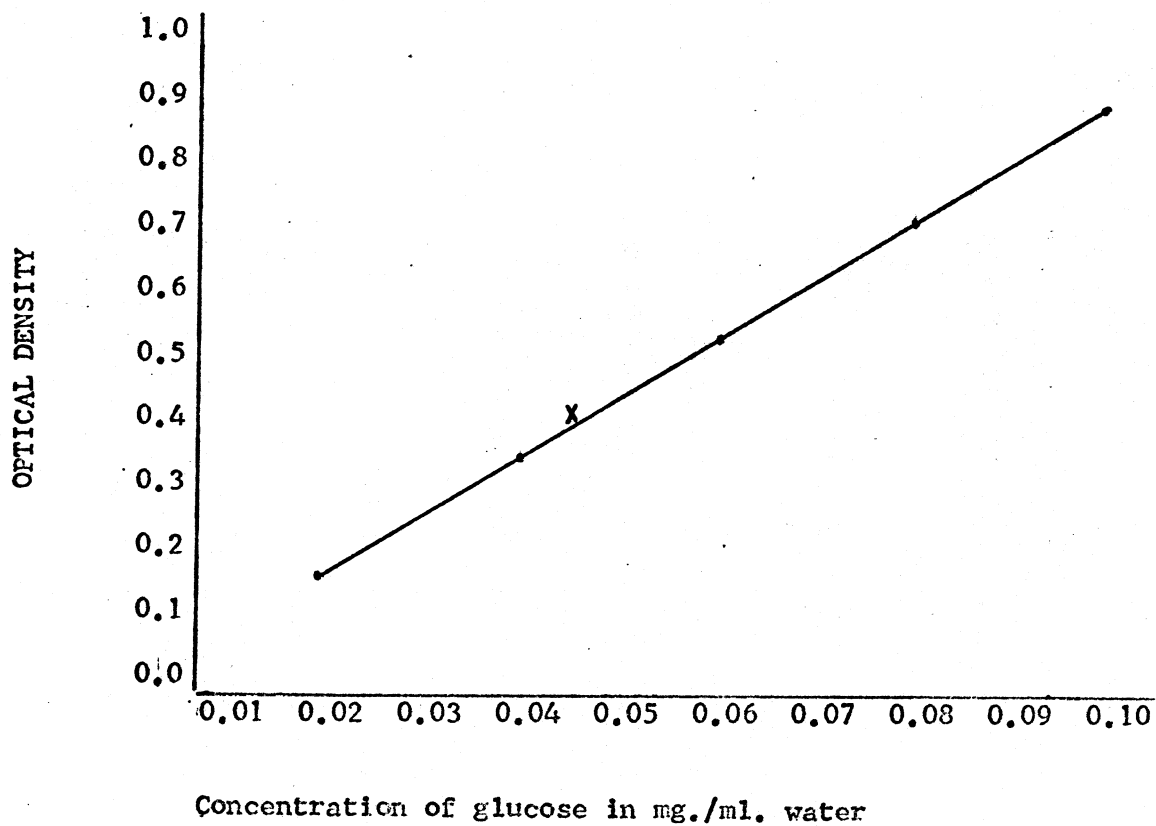


Fig. 7 Linear relationship of glucose in mg./ml. water to the optical density. A solution of 0.275 mg. protein/ml. water and 0.045 mg. carbohydrate/ml. water was checked, indicated by (x).



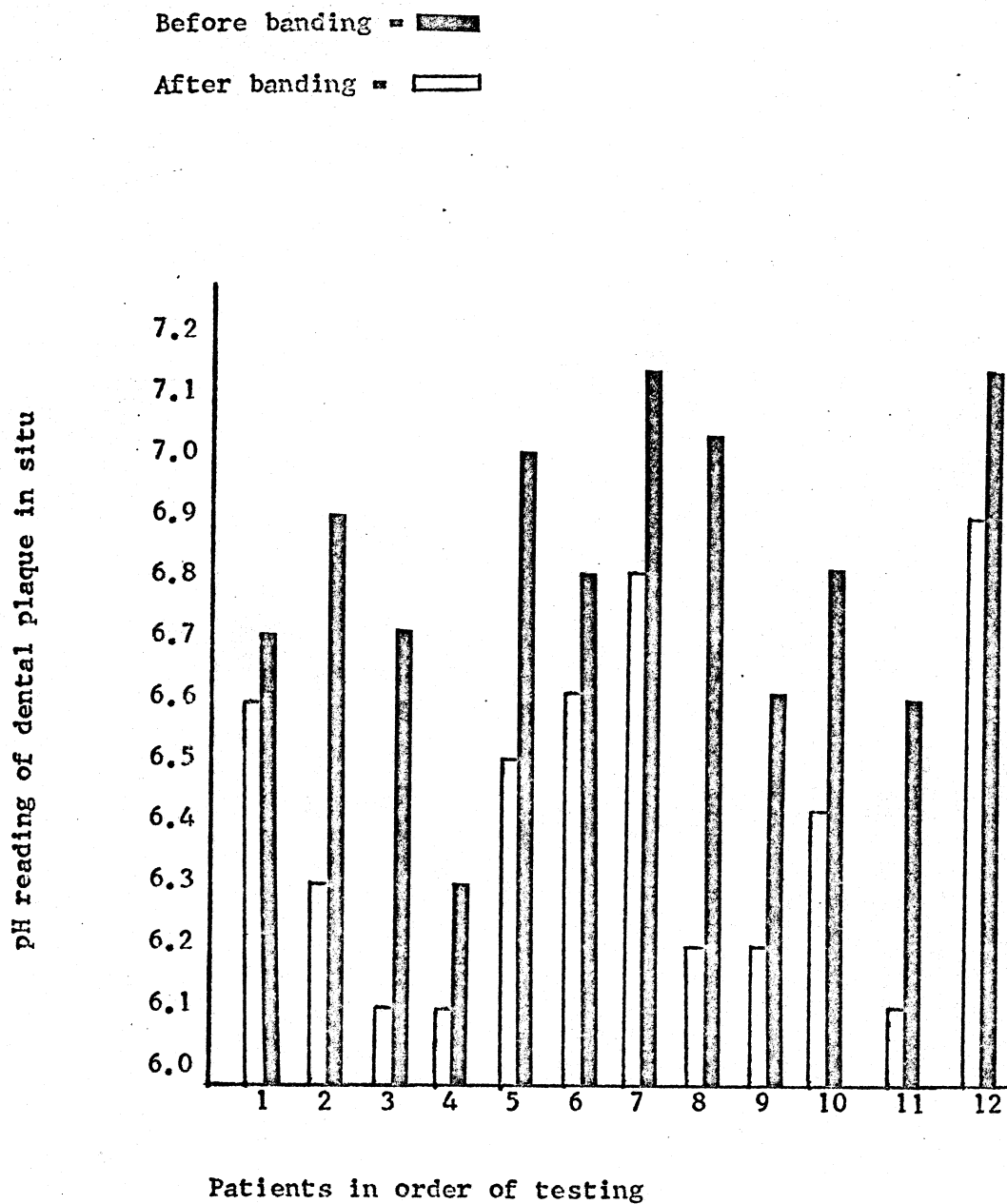


Fig. 8 pH reading of patients before and after banding (as based on Table 3).

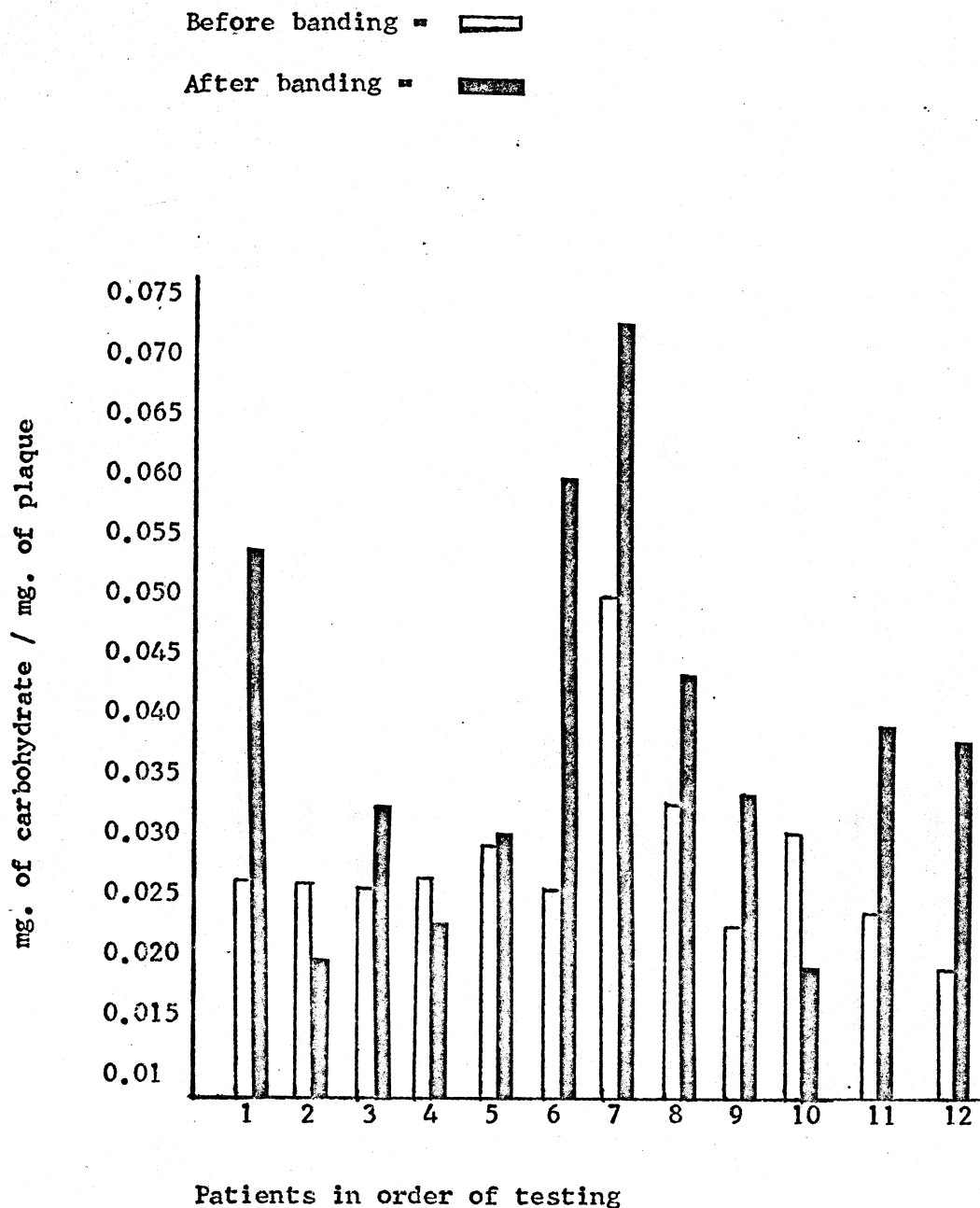


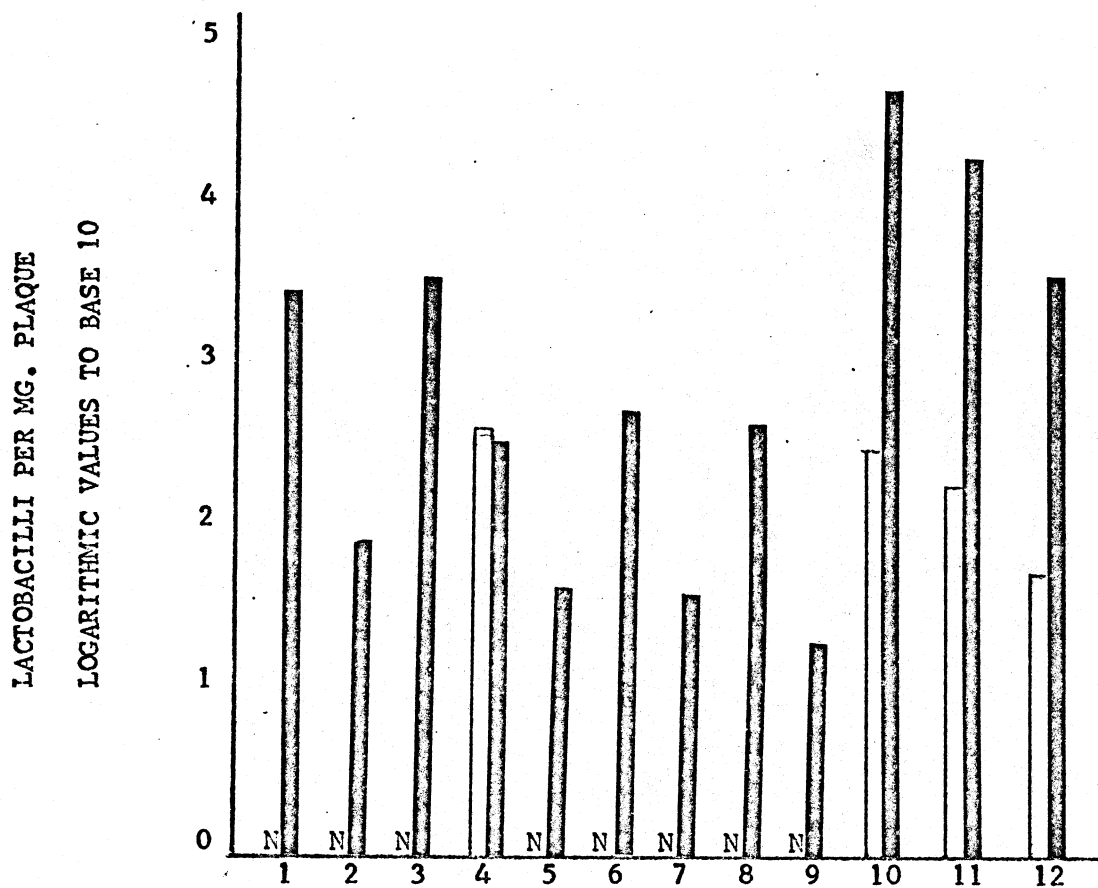


Fig. 9 The carbohydrate content of the dental plaque before and after banding (as based on Table 3).

Before banding = 

After banding = 

$300 \times N \geq 0$



Patients in order of testing

Fig. 10 The Lactobacillus counts logarithmically expressed (as based on Table 3).

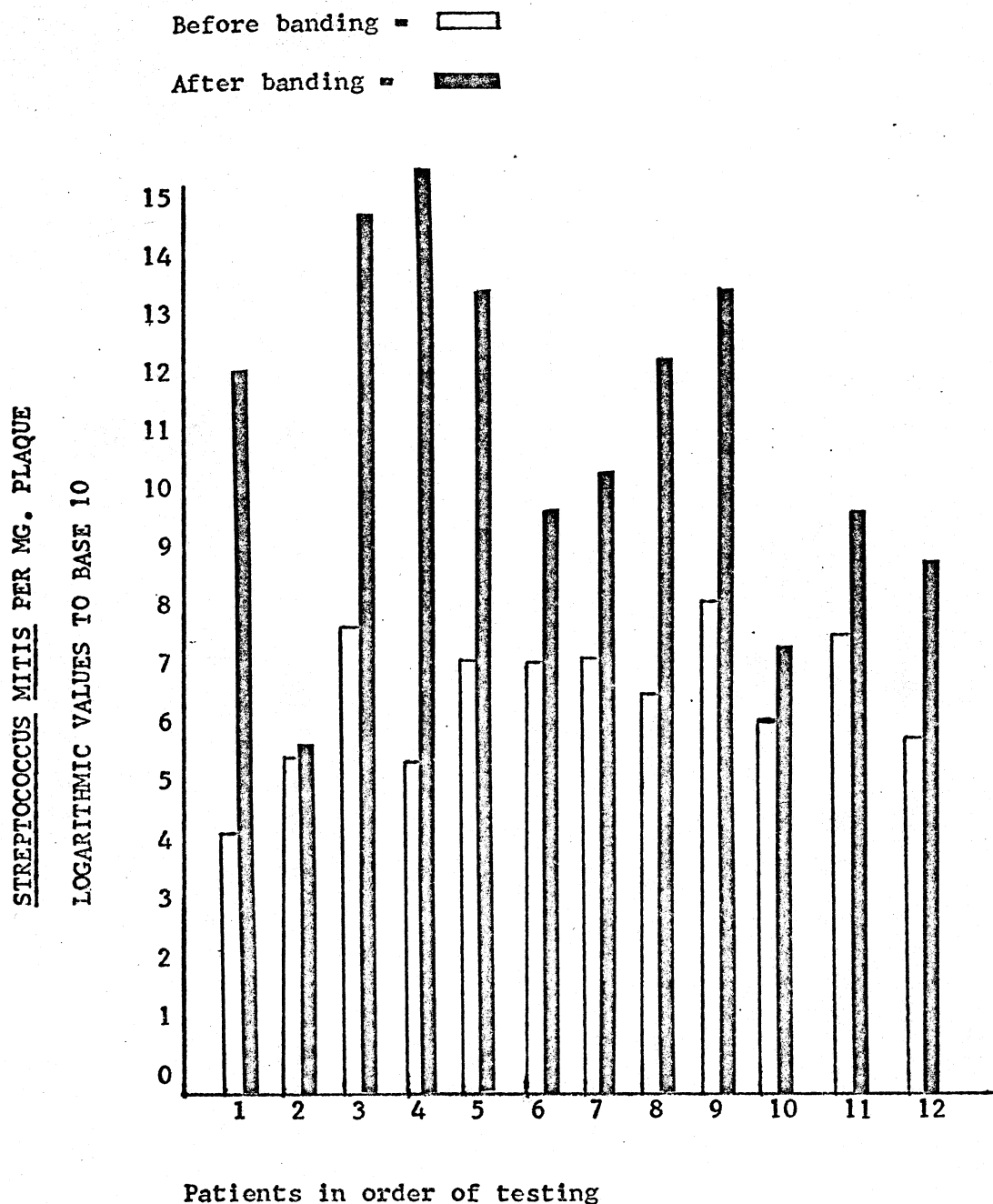


Fig. 11 The Streptococcus mitis counts logarithmically expressed (as based on Table 3).

STREPTOCOCCUS SALIVARIUS PER MG. PLAQUE  
LOGARITHMIC VALUES TO BASE 10

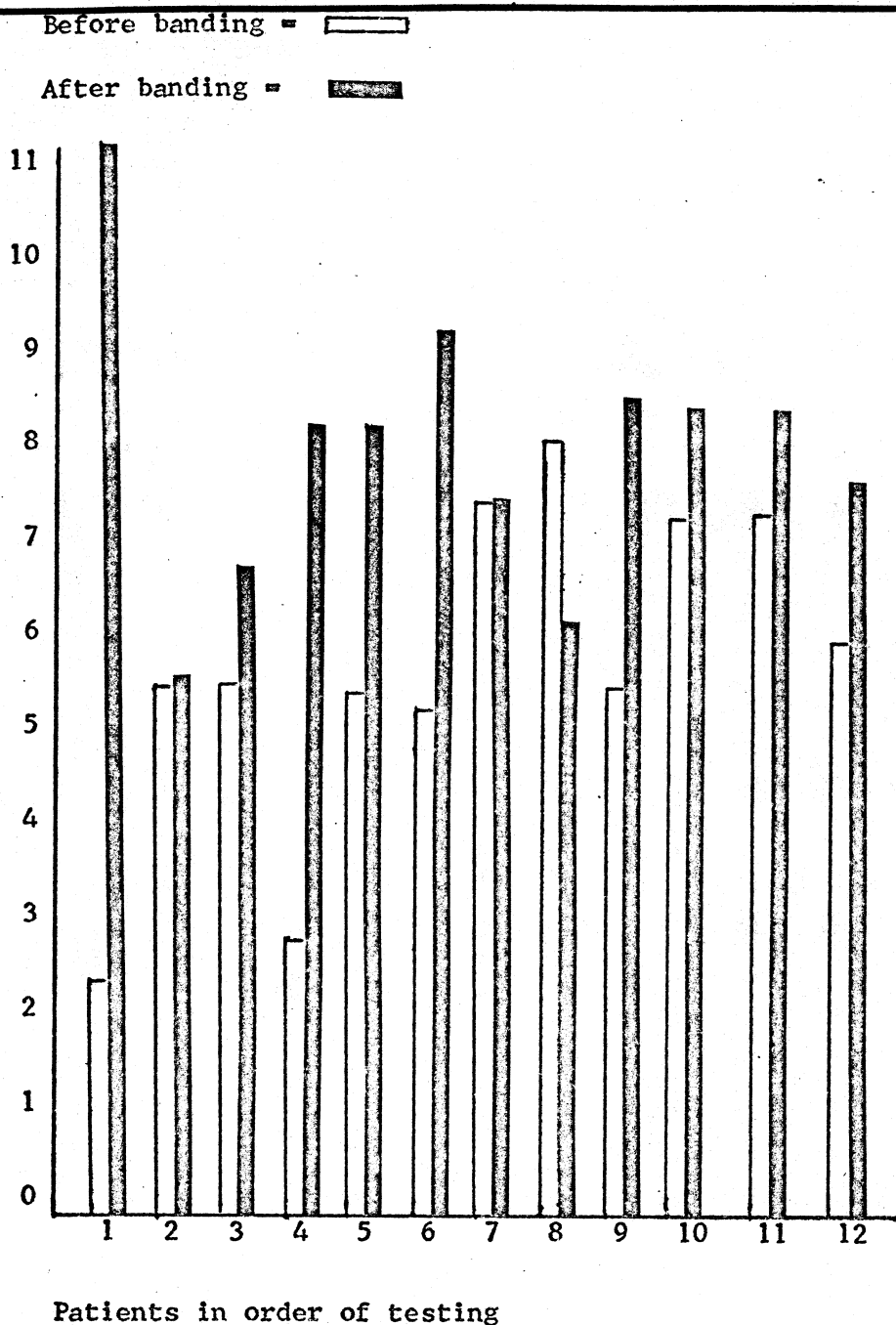


Fig. 12 The *Streptococcus salivarius* counts logarithmically expressed (as based on Table 3).

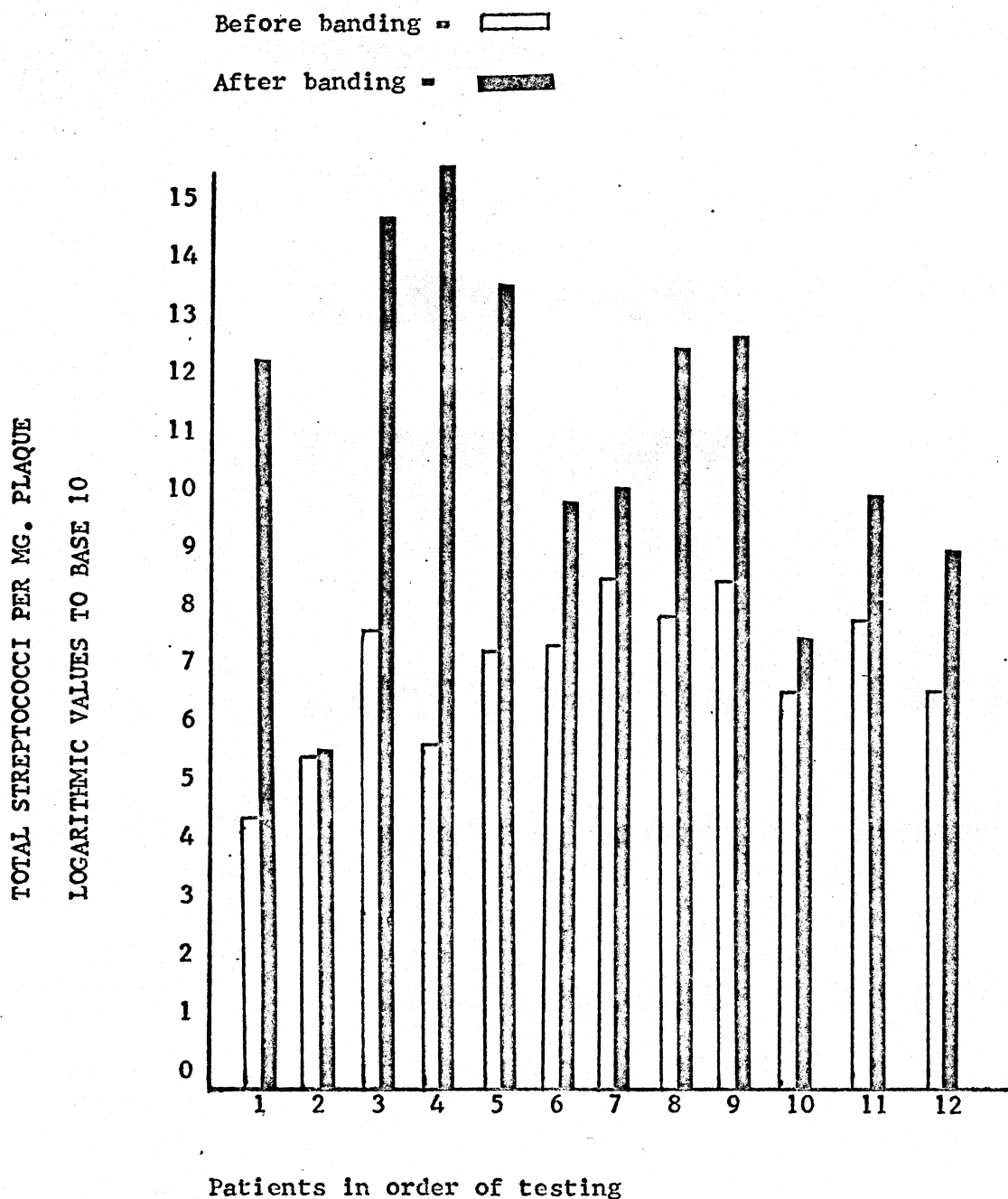


Fig. 13 The total Streptococcus counts logarithmically expressed (as based on Table 3).

TABLE 1

## MEAN PLAQUE CONTENT MEASUREMENT PER MG OF PLAQUE

	<u>BEFORE</u>	<u>AFTER</u>	<u>DIFFERENCE</u>
pH	6.8	6.4	0.4
Mg of Carbohydrate	0.0277	0.0377	0.0100
<u>Lactobacillus</u> Count	$4.35(10)^2$	$9.81(10)^4$	$9.80(10)^4$
<u>Streptococcus</u> <u>mitis</u> count	$2.28(10)^7$	$1.51(10)^{14}$	$1.50(10)^{14}$
<u>Streptococcus</u> <u>Salivarius</u> Count	$2.92(10)^7$	$1.81(10)^{10}$	$1.80(10)^{10}$

TABLE 2

## STATISTICAL ANALYSIS OF PLAQUE DIFFERENCES PER MG OF PLAQUE

	t	d	SE <sub>d</sub>	p
pH	8.36	0.418	0.05	0.01
Mg of Carbohydrate	2.35	0.00915	0.0039	0.05
<u>Lactobacillus</u> count	6.33	9380.2	1481.5	0.01
<u>Streptococcus</u> <u>mitis</u> count	7.89	151.6(10) <sup>12</sup>	1.91(10) <sup>13</sup>	0.01
<u>Streptococcus</u> <u>salivarius</u> count	6.8	1.79(10) <sup>10</sup>	2.63(10) <sup>9</sup>	0.01
Total streptococci	7.22	1.50(10) <sup>14</sup>	2.09(10) <sup>13</sup>	0.01



TABLE 3

Data (per mg. of plaque) collected 1 week before banding, and data collected 1 month after insertion of bands and arch wires.

<u>Patients</u>	<u>1</u>	<u>2</u>	<u>3</u>	
pH	6.70 6.60	6.9 6.3	6.70 6.1	Before After
mg of Carbohydrate	0.0266 0.0537	0.0267 0.0197	0.0256 0.0329	Before After
<u>Lactobacillus</u> count	N.S. 3.33(10) <sup>3</sup>	N.S. 9.0(10) <sup>1</sup>	N.S. 4.99(10) <sup>3</sup>	Before After
<u>Streptococcus</u> <u>mitis</u> count	2.18(10) <sup>4</sup> 1.17(10) <sup>12</sup>	3.07(10) <sup>5</sup> 4.20(10) <sup>5</sup>	4.28(10) <sup>7</sup> 6.49(10) <sup>14</sup>	Before After
<u>Streptococcus</u> <u>salivarius</u> count	2.54(10) <sup>2</sup> 2.15(10) <sup>11</sup>	4.20(10) <sup>4</sup> 4.50(10) <sup>5</sup>	3.06(10) <sup>5</sup> 6.66(10) <sup>6</sup>	Before After
Total Streptococci	2.20(10) <sup>4</sup> 1.38(10) <sup>12</sup>	3.49(10) <sup>5</sup> 4.65(10) <sup>5</sup>	4.31(10) <sup>7</sup> 6.49(10) <sup>14</sup>	Before After

TABLE 3 (cont'd)

Data (per mg. of plaque) collected 1 week before banding, and data collected 1 month after insertion of bands and arch wires.

<u>Patients</u>	<u>4</u>	<u>5</u>	<u>6</u>	
pH	6.30 6.10	7.00 6.52	6.80 6.60	Before After
mg of Carbohydrate	0.0262 0.0221	0.0272 0.0283	0.0248 0.0595	Before After
<u>Lactobacillus</u> count	4.68(10) <sup>2</sup> 4.50(10) <sup>2</sup>	N.S. 1.68(10) <sup>1</sup>	N.S. 6.60(10) <sup>2</sup>	Before After
<u>Streptococcus</u> <u>mitis</u> count	2.68(10) <sup>5</sup> 1.12(10) <sup>15</sup>	1.29(10) <sup>7</sup> 1.63(10) <sup>13</sup>	1.6(10) <sup>7</sup> 5.00(10) <sup>9</sup>	Before After
<u>Streptococcus</u> <u>salivarius</u> count	6.89(10) <sup>2</sup> 1.24(10) <sup>8</sup>	2.77(10) <sup>5</sup> 1.68(10) <sup>8</sup>	1.32(10) <sup>5</sup> 1.50(10) <sup>9</sup>	Before After
Total streptococci	2.69(10) <sup>5</sup> 1.12(10) <sup>15</sup>	1.31(10) <sup>7</sup> 1.63(10) <sup>13</sup>	1.61(10) <sup>7</sup> 6.5(10) <sup>9</sup>	Before After

TABLE 3 (cont'd)

Data (per mg. of plaque) collected 1 week before banding, and data collected 1 month after insertion of bands and arch wires.

<u>Patients</u>	<u>7</u>	<u>8</u>	<u>9</u>	
pH	7.10 6.80	7.00 6.20	6.60 6.20	Before After
mg of Carbohydrate	0.0504 0.0715	0.0310 0.0414	0.0216 0.0317	Before After
<u>Lactobacillus</u> count	N.S. 1.52(10) <sup>2</sup>	N.S. 5.33(10) <sup>2</sup>	N.S. 2.63(10) <sup>1</sup>	Before After
<u>Streptococcus</u> <u>mitis</u> count	2.63(10) <sup>7</sup> 2.32(10) <sup>10</sup>	4.63(10) <sup>6</sup> 1.55(10) <sup>12</sup>	1.22(10) <sup>8</sup> 3.15(10) <sup>13</sup>	Before After
<u>Streptococcus</u> <u>salivarius</u> count	2.56(10) <sup>8</sup> 2.72(10) <sup>8</sup>	6.38(10) <sup>7</sup> 2.84(10) <sup>6</sup>	1.53(10) <sup>5</sup> 2.63(10) <sup>8</sup>	Before After
Total streptococci	2.82(10) <sup>8</sup> 2.34(10) <sup>10</sup>	6.84(10) <sup>7</sup> 1.55(10) <sup>12</sup>	1.27(10) <sup>8</sup> 3.15(10) <sup>12</sup>	Before After

TABLE 3 (cont'd)

Data (per mg. of plaque) collected 1 week before banding, and data collected 1 month after insertion of bands and arch wires.

<u>Patients</u>	<u>10</u>	<u>11</u>	<u>12</u>	
pH	6.80 6.40	6.60 6.10	7.10 6.90	Before After
mg of Carbohydrate	0.0292 0.0165	0.0231 0.0382	0.0200 0.0368	Before After
<u>Lactobacillus</u> count	3.10(10) <sup>2</sup> 5.00(10) <sup>4</sup>	1.63(10) <sup>2</sup> 1.80(10) <sup>4</sup>	6.77(10) <sup>1</sup> 5.25(10) <sup>3</sup>	Before After
<u>Streptococcus</u> <u>mitis</u> count	1.55(10) <sup>6</sup> 1.92(10) <sup>7</sup>	4.57(10) <sup>7</sup> 3.19(10) <sup>9</sup>	8.71(10) <sup>5</sup> 4.43(10) <sup>8</sup>	Before After
<u>Streptococcus</u> <u>salivarius</u> count	1.27(10) <sup>7</sup> 1.28(10) <sup>8</sup>	1.55(10) <sup>7</sup> 1.86(10) <sup>8</sup>	8.94(10) <sup>5</sup> 4.59(10) <sup>7</sup>	Before After
Total streptococci	1.67(10) <sup>6</sup> 3.20(10) <sup>7</sup>	6.12(10) <sup>7</sup> 3.37(10) <sup>9</sup>	1.76(10) <sup>6</sup> 4.89(10) <sup>8</sup>	Before After

APPROVAL SHEET

The thesis submitted by Dr. Jack W. Balenseifen has been read and approved by three members of the Graduate School Faculty.

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

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

May 13, 1968  
Date

John V. Madonia DDS, Ph.D.  
Signature of Advisor