STUDIES ON ORAL HEALTH AS REFLECTED IN THE SALIVA WITH SPECIAL REFERENCE TO THE LOCAL AND SYSTEMIC USE OF CITRUS FRUITS, ORAL ACIDURIC MICROORGANISMS, DIASTATIC ACTIVITY AND pH

By

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INTRODUCTION

The problem of oral health has engaged the interest of investigators for a number of years. Dental pathology is not only receiving attention of the dentist but of the bacteriologist, the chemist, and the nutritionist as well.

One of the most important problems of preventive dentistry has been in regards to dental prophylactic measures. Studies of the efficacy of dentifrices in maintenance of oral health has resulted in development of a large number of commercial preparations, some of which have been accepted, and others which are seemingly misleading in their claims. It was one of the purposes of this investigation to determine the effects of several commercial tooth pastes and mouth washes on the microbial flora of the mouth, and to study the comparative effects of citrus fruit juices when used in the same capacity.

In view of conflicting reports concerning the relation of diet to oral health, a study was also made of the effects on the oral flora when citrus fruit was incorporated in the diet.

Studies of the etiology of dental caries has led a number of investigators to believe that oral lactobacilli are the cause of this disease. Confusing data prompted a more thorough consideration of this problem.

Numerous attempts have been made to find some chemical difference between the saliva of carious individuals and that of carious-immunes. Differences such as in pH, titratable acidity, total solids, and calcium and phosphorus content of saliva have been found to be so slight, or have shown such inconsistency, as to be of little value as measures of susceptibility. Review of the literature has shown that investigation of the diastatic activity of saliva and its possible relation to dental caries has apparently been neglected. It seemed advisable to study this property of saliva to determine if any possible relation existed between diastase content and decay.

II

HISTORICAL

Bacteriology of the Mouth: The microbiology of the mouth has been a subject of investigation for a number of years. The work of Miller (65) stands out as the stepping stone that has led to much of the subsequent research in oral bacteriology. His work is mainly of historical value, since bacteriological methods then were still in their infancy and a systematic study was impossible. Apparently the most exhaustve and comprehensive study of the oral flora has been that of Kligler (55), in which he determined the numbers and types of bacteria associated with carious and non-carious teeth. Howitt and Fleming (46) made a similar quantitative study of the flora under pyorrhetic conditions. They also studied the effects of diet on the numbers and types of oral bacteria, and found that the mouth flora was influenced more by food retention in the mouth than by any systemic effect produced through dietary changes.

The mouth at birth is generally believed to be sterile. It is not long before the mouth receives its first organisms, being derived as Gundel and Schwarz (27) maintained from the oral flora of the mother and other intimate contacts. According to a number of investigators, the streptococci and staphylococci are among the first bacteria to appear in the mouth of the new born infant. Within a few days Gram-positive rods begin to appear. Appleton (2) stated that Gram-positive

bacteria predominate through the first year of life. The flora then begins to vary as the individual grows older. Staphylococci can practically always be isolated from the adult mouth. The "viridans" type of streptococcus is almost always present, and it is also not uncommon to find the hemolytic variety. Dochez and Avery (16) have reported the frequent isolation of pneumococci of Group IV from the mouth. Micrococci, both Gram-positive and Gram-negative, true meningococci, diphtheroids, spirilla, and spirochaetes have also been reported as frequent inhabitants. Of the last two types mentioned, Borrelia vincenti is probably the most frequently found, and it is almost always associated with the fusiform bacillus. Bibby and Berry (6) using anaerobic methods isolated a large number of strains of filamentous bacteria from the mouth. They suggested the name Leptotrichia buccalis for the oral filaments most commonly isolated. Hall and Howitt (33) have reported the frequent occurrence in human saliva of a minute, obligate anaerobe which was Gram-negative, non-motile, non-sporulating, and gas-forming. The organism was designated Micrococcus gazogenes. Hall (32), in an attempt to isolate and identify sporulating anaerobes from the mouth, observed that such were not commonly found as spores in the saliva; and that when they were, it was usually impossible to detect the same species in subsequent samples from the same mouth.

Gram-positive, chain-forming bacilli are also found in the mouth, and although they have been considered largely

as saprophytic types, much attention has been given to certain members belonging to the lactobacillus group and their possible relation to certain dental diseases. These organisms, on the basis of morphological and cultural characteristics, have been classed by some workers with Lactobacillus acidophilus of the intestine. Morishita (66), studying aciduric organisms associated with dental caries, found high-acid tolerating organisms to be of almost constant occurrence in tooth enamel that was undergoing an active pathological process. They were also usually present in the saliva of persons with carious teeth, being relatively rare in saliva of non-carious mouths. The organisms isolated from teeth and saliva possessed the common characteristics of the Lactobacillus genus, but Morishita maintained that the group studied was markedly different in certain respects from L. acidophilus. Rosebury, Linton, and Buchbinder (76) made a comparative study of dental aciduric organisms and L. acidophilus and found no consistent difference between the groups when compared morphologically. The conclusion that dental aciduric bacteria and L. acidophilus of the intestine are not different has also been reached by Hadley, Bunting and Delves (31), Hadley and Bunting (30), and Howitt (45).

Although some investigators, Fleming (22), Bibby, Hine, and Clough (8), Clough, Bibby, and Berry (14), and Hill (43), have maintained that saliva has some bactericidal powers,

it nevertheless remains a favorable medium for a large number of microorganisms. Hanke (37) stated that saliva contains ordinarily from five million to fifty million bacteria per cc. Feirer and Leonard (19) found a greater number of bacteria to be contained in scrapings from gum margins than in relatively smaller amounts of fresh human stools.

The number of bacteria in the mouth is not a constant entity, and fluctuation in the oral flora from time to time is a common occurrence. Appleton (2) stated that in the well-cared for mouth the dominant flora is aerobic and facultative, while in the unhygienic mouth the flora becomes predominantly anaerobic. Bibby (5) studying the bacterial flora of different mouths found variations to be so great that no significant relationship could be established between the state of oral health and the oral flora. Throughout the day there is a periodic increase and decrease in the oral flora, in which the number of microorganisms is greatest upon rising in the morning, with a subsequent decrease following meals, and a gradual increase occurring between meals. Crowley and Rickert (15) found that the number of bacteria in the mouth decreased as much as 78% after a meal. In a study of bacterial counts of mouth washings during a seven-hour fast period, Feirer and Leonard (21) found an average percentage increase of about 300% over the initial count. Hine and Bibby (44) also found little constancy in various groups of

mouth bacteria observed from daily smears.

The fact that a large number of organisms, including potential pathogens, are normally present in the mouth suggests a possible relationship of the oral flora to the occurrence of various dental disorders.

Dentifrices: In studying oral hygiene one of the problems that has attracted attention of investigators has been the efficacy of dentifrices in mouth prophylaxis. It is generally agreed that the therapeutic value of a dentifrice is absolutely nil. The idea that caries may be prevented through the use of tooth pastes and mouth washes has met with little agreement, the only apparent effect gained by their use being by virtue of their cleansing action. Teuscher (80) believed the tooth-brush to be important in control of caries, stressing the importance of a clean mouth, and the use of a toothbrush for maintaining this condition. Marshall (59) stated that the role of dentifrices is insignificant in the treatment of caries. According to Appleton (2) the use of a non-medicated tooth paste and a mouth wash may reduce the number of bacteria by about 40%, but this decrease is of relatively short duration, the count returning to the original within a short time after prophylaxis. Incorporation of drugs or medicaments in dentifrices has been constantly opposed, argument against the use of such being based on the fact that concentrations effective for bacterial destruction would also destroy oral tissue. Antibacterial substances such as hexylresorcinol, sodium ricinoleate, and certain soap substitutes have been used in commercial tooth

pastes and mouth washes. Leonard and Feirer (57) examining forty-one different commercial tooth pastes found that none of the dentifrices tested effected any significant reduction in the bacterial content of mouth washings. In a subsequent publication the same authors (20) reported on the effectiveness of a group of alkyl resorcinols in oral antisepsis. Hexylresorcinol (S. T. 37) was found to be the most powerful antiseptic of the group studied, affecting practically complete disinfection of the gum margin in five minutes. Carroll (12) and Mead (63) found that the bacteriostatic action of sodium ricinoleate was far greater than that of hexylresorcinol. Gies (25) in a consideration of the dentifrice problem believed that the role of a dentifrice was mainly a physiological one. Contrary to the accepted idea that a dentifrice should be alkaline, Gies maintained that if neutralization of mouth acid is at all important such could be effectively done by brushing the teeth with an acid material. Benefits derived from the use of an acid dentifrice would be in the disintegration of mucin plaques and an increase in flow of an alkaline saliva.

Dental Caries: Dental caries, or tooth decay, is a disease that has been prevalent in man as far back as history can trace. The disease involves essentially the dissolution and disintegration of the enamel and dentine, and is generally believed to be caused by the action of acid-producing bacteria and their products.

Although a considerable amount of work has been done on the etiology of dental caries, the exact cause of the disease is still unknown. The theories may be classified, however, for convenience into two groups: (1) local conditions, (2) nutrition. The first school of thought maintained that decay is a local phenomenon, in which decalcification results through the production of organic acid by certain oral bacteria. Many attempts have been made to isolate a specific organism responsible for decay but reports have only been conflicting. Miller (65) maintained that the initial lesion could be produced by any of a number of different organisms. He was unable, however, to identify definitely any of these. Hartzell and Henrici (42) found oral streptococci of the "viridans" type to occur almost constantly in carious teeth, and believed that they played the chief role in caries. Anderson and Rettger (1) also observed a high incidence of oral streptococci and regarded them as possible important factors in caries. Clarke (13) believed that the carious lesion was produced by a new strain which he named Streptococcus mutans. Tunnicliff and Hammond (83) maintained that the rough phase of Streptococcus viridans was the invading organism in decay. Kligler (55) found Bacillus acidophilus, Cladothrix placoides, and Leptothrix buccalis to be prominent in carious enamel deposits, and observed that the first two possessed a marked decalcifying action on powdered tooth enamel "in vitro".

Within recent years considerable attention has been given to the possible relation of L. acidophilus to dental caries.

An intensive study of this phase of the problem has been made by Bunting and co-workers. They (9, 10,11,29,30,31) have concluded that L. acidophilus is a specific factor in decay, and have found approximately a 90% correlation between the presence of this organism and the presence of caries. They have also been able to demonstrate the presence of agglutinins for L. acidophilus in the blood of caries-free individuals, in the mouths of whom they were unable to implant known strains of the organism. They maintained that no direct relation existed between diet and caries, but that the amount of carbohydrate in the diet may become a factor only in so far as it might influence bacterial activity, especially the oral lactobacilli. McIntosh, James and Lazarus-Barlow (62) and Rodriguez (74) have likewise studied the relation of lactobacilli to dental caries and agreed with the above workers on the significance of oral lactobacilli to caries. Enright, Friesell and Trescher (18) examined 148 individuals, and found that "the only microorganism commonly found in the food debris, in direct contact with progressive caries of the enamel, that can tolerate and produce additional acid in an environment below pH 5.0, is a lactobacillus". They identified the organism as the Y type of L. acidophilus. These workers maintained that the use of a broth of pH 5.0 practically eliminated streptococci and oidium-like organisms. Tucker (82) found, however, that it was unusual to obtain L. acidophilus in pure culture from scrapings of teeth when a broth of pH 5.0 was used. Studying a group of 422 children,

he observed that aciduric streptococci were likewise contained in the mouths of practically all of the subjects examined "irrespective of the incidence of dental caries and irrespective of the ingestion of citrus fruit juice". Jay, cited in the work of Enright, Friesell and Trescher (18), also found that a broth of pH 5.0 was not differential for L. acidophilus, observing that streptococci, staphylococci and yeasts occurred with great regularity. Howitt and Fleming (46), in their quantitative study of the oral flora under different dietary conditions used anaerobic glucose veal-infusion agar plates of pH 4.8-5.0 to determine the types of microorganisms capable of growth in a highly acidified medium. They found that the number of organisms capable of growing on acid-agar was greater for individuals on a carbohydrate than on a protein diet. Members of the lactobacillus group were the chief organisms isolated, with a smaller percentage of streptococci and Gram-positive thread forms.

Hadley (29), realizing the crudeness of the qualitative broth-method, devised a quantitative method for the estimation of L. acidophilus in saliva. In addition to acid-broth cultivations she employed the streak method of inoculating plates for the determination of numbers of organisms in saliva samples from carious and non-carious individuals. The plating medium was essentially Kulp's tomato-peptone agar, which Hadley modified by increasing the agar content from 1.1% to 2%, and the acidity from pH 6.2 to 5.0. It was found that most oral streptococci were inhibited by this modified medium, and

only occasionally did colonies of micrococci and staphylococci appear. Quantitative estimation of L. acidophilus in the saliva of a group of 14 caries - susceptibles showed this organism to be invariably present and usually in large numbers. In 10 caries - immune subjects the presence of L. acidophilus was variable, and generally in small numbers when found. Bunting (9) maintained that the most consistent correlating factor observed in dental decay was the presence or absence of L. acidophilus in the mouth. Tucker (82), however, was unable to find any correlation between the incidence of dental caries and the occurrence of L. acidophilus in cultures prepared from tooth scrapings in acid broth of pH 5.0. L. acidophilus was found most frequently and most consistently in mouths of carious children, but this organism also occurred frequently and consistently in mouths of non-carious individuals.

Aciduric streptococci isolated by Tucker (82) were found to be almost as acidogenic as L. acidophilus. Isolating palisaded rods, streptococci and staphylococci from casein digest sodium oleate agar streaked with glucose beef infusion broth cultures from tooth scrapings, he inoculated fresh broth to determine the pH value after 2 - 3 days incubation. Cultures containing the rods generally ranged in pH from 3.6 to 4.2, while cultures of streptococci and staphylococci ranged from pH 4 to 4.6. Tucker concluded that L.acidophilus apparently is not an "obligate producer" of dental decay. Hadley, Bunting and Delves (31) found that their Group I

strains of oral aciduric rods produced an average acidity of pH 3.8 when incubated in glucose infusion broth for seven days, while strains of Group II and III lowered the pH to 4.1.

The second school of thought on the cause of dental caries believed that the predisposing factor in decay is faulty diet. Reports on the relation of nutrition to decay are many, but only a few are apparently important. Pederson (70), compared the teeth of Eskimo skulls 200 or more years old with teeth of living Greenlanders, and believed that the higher incidence of caries in the latter was attributable to modern civilized diet. Price (72) studied the relation of diet to the incidence of decay in certain African tribes and found that approximately 100% immunity to caries existed in those tribes adhering strictly to their native diet. As studies were continued in those groups living progressively farther away from the primitive habitat, and approaching modern civilization, Price observed a marked lowering of caries immunity. He found the only factor to change at the region where immunity was decreased was the diet. Osborn and Noriskin (68) observed this same relation to exist between diet and decay in the South African Bantu, in whom an increased incidence of caries was shown to be associated with the incorporation of European articles of food in their diet. Ker (52), Klein and Palmer (54), Price (71) and Waugh (85) have reported further evidence to support the belief that the adoption of so-called civilized foods has been responsible for the increase in incidence of decay in primitive peoples.

Rosebury (75) stated that civilization has played an important role in the cause of decay, and in the attainment of its present distribution. According to Kirk (53), statistics show that 85 to 95% of civilized people possess carious teeth or were affected at some time during life.

Schiotz (77) found 99 1/2% of 25,000 school children examined to have defective teeth, and was convinced that a diet deficient in vitamins and minerals was the cause of the trouble. Read and Knowles (73) observed that the general diet of children with extensive caries was deficient in fats, proteins, and green vegetables. McBeath and Zucker (60), McCollum (61), and Mellanby (64) have stressed the importance of vitamin D in caries control, while Hanke and co-workers (34,35,36,40,41) have focussed attention on the significance of vitamin C. The latter found that the addition of a pint of orange juice and the juice of one lemon to a daily diet otherwise adequate furnished "something" that was capable of arresting approximately 50% of caries. When citrus juice intake was reduced to three ounces a day for one year, decay again became rampant. Webber (86) found that vitamins A, C and D had no influence at all in preventing the onset of caries in rats when these dietary factors were fed as cod liver oil and orange juice. Fosick and Hansen (23) mentioned that a high carbohydrate diet may be an essential factor in decay. Belding (4) found that the carbohydrates of modern diet that were fermented in the mouth with the greatest

rapidity and with the largest amount of acid were mainly wheat and oats. He believed these materials to be the "nutritional factors" in the cause of dental decay. The isolation of an organism, Streptococcus odontyliticus, was reported by Belding, which he concluded to be the chief cause of decay, and which organism is very much favored by the modern diet. Hadley (29) observed that by reducing the carbohydrate in the diet the incidence of oral L.acidophilus could be decreased.

Simmonds (78) has discussed the relation of diet to dental caries, and offered the following suggestions:

"1. If an individual is susceptible to tooth decay, he should reduce his intake of all sweet foods to a minimum. In this way he will keep the flora of his mouth low in those organisms known to be acid formers. Starches and fats should be his main energy foods.

"2. In addition to keeping the intake of sweet foods low, each person should plan his diet so that all factors, including vitamin D, are present in abundance."

Saliva: Considerable attention has been given to the study of physico-chemical properties of saliva in an attempt to find some difference that might explain caries activity. Hubbell (48) found blood serum calcium, inorganic acid-soluble phosphorus, CO_2 capacity and pH to be the same in both carious and non-carious groups. A difference in the CO_2 capacity and titratable alkalinity of the saliva, however, was correlated with dental caries. Marshall (58) likewise

observed a relationship between titratable alkalinity of the saliva and caries susceptibility. Mull, Bill, and Kinny (67) observed that differences in the titratable acidity of salivas of both carious and non-carious pregnant women were too small to be of diagnostic importance. Most observations on pH of the blood and saliva of carious and non-carious individuals have shown such little differences that they became practically valueless as criteria of individual susceptibility. Although Karshan, Krasnow and Krejici (51) found a relationship between pH and caries, they believed titratable alkalinity to be more reliable. Hanke (39) stated that about 3% of our adult population has well-formed teeth and occlusion, and are free of caries and gingivitis. He maintained that this small group of individuals secrete a constantly alkaline and well buffered saliva.

It is apparent from a review of the literature that the significance of ptyalin has received very little attention as a possible factor in dental caries. Gore(26) has shown that salivary ptyalin is capable of hydrolysing the complex carbohydrate moiety of mucin into simpler sugar, which is thus made available for fermentation to lactic acid by acidogenic bacteria present in practically all mouths. He found that the amount of carbohydrate combined with the protein in mucin varied greatly, and that the time required for its hydrolysis to fermentable sugar was in direct proportion to the concentration of ptyalin in the saliva. Examining samples of saliva from caries-susceptibles and caries-immunes, Gore observed

that the carbohydrate content was always higher in carious than in non-carious individuals, and that when the carbohydrate intake was increased diastatic activity increased. Hubbell (48) analysed saliva of 15 non-carious and 17 carious children and found no consistent differences in diastatic activity between the two groups.

Relationship of Oral Health to General Health: The knowledge that dental infection may bring about various systemic changes has been a great influence in modern dentistry. However, it has only been recently, as Haden (28) stated, that dental practice has given attention to the importance of focal infection.

According to Appleton (2), the majority of primary foci are situated in the head, including as such the following conditions: tonsillitis, adenoid infections, sinus infections, otitis media, mastoiditis, pyorrhea alveolaris, and infections of the dental pulp and of the periapical tissues. Billings, Coleman and Hibbs (8) have summarized as follows the relative frequency with which the various primary foci are responsible for secondary infections:

Number of Times Each Focus was Considered a Probable Source of Infection

Tonsil	336
Teeth	136
Sinus	12
Bronchi	5
Uterus and tubes	12
Prostate and genito-urinary tract	24
Gall-bladder	3
Enterocolitis	2
Appendix	1
Middle ear	1

Haden (28) maintained that most foci of dental infection are the ultimate results of dental decay and pyorrhea, and that systemic disease may be caused by dental infection either through extension of the latter into neighboring tissues or by metastasis to other regions of the body.

In a study of the treatment of paradental disease by surgery, Sweigert, Gobar and Bartlett (79) observed a close relationship between paradentosis and systemic disease. Surgical treatment of paradentosis was found to relieve the symptoms of about 75% of the patients. Palmer and Kempf (69) reported a number of cases of Streptococcus viridans bacteremia following the extraction of teeth. Haden (28) stated that chronic ulcerative colitis may be due to focal infection, and Bargen (3) has shown that a specific non-hemolytic streptococcus isolated from foci especially about the teeth is the cause of this disease. Thoma (81) mentioned some of the more special diseases that may originate from oral infections and included the following: (1) tonsillitis and pharyngitis, which may result from the discharge of pus into the mouth, (2) ear infections, occurring subsequent to metastasis through the Eustachian tube or circulatory system, and (3) cysts, which may be formed in the bone from chronic abscesses.

III

STUDIES OF THE EFFECT OF LOCAL APPLICATION OF CITRUS JUICES AND VARIOUS MOUTH PROPHYLAXES ON THE ORAL FLORA

Studies of the effect of various dentifrices and methods of mouth prophylaxis on microbial flora of the mouth have shown that disinfection of the oral cavity is practically impossible. This is not surprising when it is considered that the mouth harbors a large and heterogeneous number of microorganisms, the complete removal of which could only be accomplished by the use of substances of such strength that would be injurious to oral tissues.

In view of past work done on dentifrices the present study was undertaken to determine the relative effectiveness of citrus juices when used as oral prophylaxes, and to compare their action on microbial growth with that of some of the more popular commercial dentifrices.

Experimental

Materials: Quantitative determinations of the microorganisms present in the saliva of nine normal individuals were made before and after each had used the following materials as mouth prophylaxes: (1) sterile distilled water, (2) orange juice, (3) grapefruit juice, (4) Product A (tooth paste and mouth wash containing hexylresorcinol), (5) Product B (tooth paste and mouth wash containing sodium ricinoleate), and (6) Product C (liquid dentifrice containing

sodium alkyl sulfate).

The flow of saliva was stimulated by chewing sterile paraffin, and approximately 25 cc. of saliva were collected from each subject in sterile sputum bottles just before lunch, after lunch, immediately after prophylaxis, and two hours later. Each individual repeated this procedure three times at two-day intervals for each material tested. The saliva samples were always plated out as soon as collected and the three counts averaged.

Tooth brushes of identical make and stiffness (medium) were used, and Charter's method was employed for brushing the teeth. This method is essentially a wedging one, in which the bristles of the brush are kept in contact with the teeth while a slight vibrating or wiggling movement is applied. The teeth were brushed with each material for two minutes, followed by a rinse of one minute with the respective mouth wash. When water and the citrus juices were used fresh test material was added to the brush several times during the brushing interval. The commercial liquid dentifrice (Product C) was applied to the brush as directed on the label, while the tooth pastes tested (Products A and B) were squeezed along the whole length of the bristles. Thirty cc. of the respective mouth wash were used to rinse the mouth after brushing.

In preliminary tests considerable discomfort and irritation of the mucous membrane was experience with Product

A mouth wash wash which was used in full strength as directed. In full strength this product contained a 1:1000 dilution of hexylresorcinol. A 1:4000 dilution of the rinse was finally adopted with still some degree of discomfort. Product B mouth wash, containing 1% sodium ricinoleate, was used full strength, and it likewise was not without an irritating effect upon oral tissue. Product C, which contained 2% sodium alkyl sulfate as an active ingredient, existed on the market only as a liquid dentifrice. To make the tests with this material comparable to tests with other dentifrices studied, a 10% solution of the dentifrice was used as a rinse.

Citrus juices used in the investigation were obtained from fruit grown in Florida and obtained on the open market. Freshly extracted juice was used for all tests, a lever-type squeezer operated by hand serving for extraction. During extraction care was taken not to exert too great a pressure, so as to eliminate as much as possible skin oil from the juice.

Immediately after use each tooth brush was sterilized by immersion into a chlorine solution containing approximately 600 p.p.m. of available chlorine. Sterility tests made on the brushes at weekly intervals showed this solution to be of proper strength for complete sterilization within 10 minutes.

Analysis of Materials: Because of the significance attached by some investigators to L. acidophilus (18,31,49,74) and other aciduric organisms (13,42,83) as etiologic factors

in dental caries, a quantitative study of these types seemed warranted. Likewise, since such organisms as streptococci and staphylococci may be constantly found in the mouth both in health and in disease, a study of these types was also undertaken. All samples of saliva were diluted with sterile water, dilutions of 1:100,000 and 1:1,000,000 being found adequate for all counting purposes. Platings were made in these two dilutions in the following media; (1) nutrient agar, to determine total numbers of the general saprophytic types, (2) neopeptone tomato juice agar (pH 6.6), for aciduric organisms, (3) blood agar, for those organisms requiring animal protein for growth. Veal infusion agar (pH 7.4) was used as the blood agar base, 5% sterile defibrinated horse blood being added aseptically to the medium just before use. Nutrient agar plates were incubated for 48 hours at 37°C.; tomato juice and blood agar plates were placed in an atmosphere of 10% CO_2 and incubated for 5 days at 37°C.

Evaluation of Data: To determine the relative effectiveness of the various materials tested averages of the individual counts obtained immediately after lunch were taken as a 100% base, and the percentage decrease after prophylaxis computed therefrom on each medium. The percentage increase in counts two hours after prophylaxis was similarly determined using the averages of counts immediately following prophylaxis as the 100% base.

Results

The effectiveness of the various materials in the actual removal of microorganisms from the mouth will be apparent from the figures given in Table I. It will be observed that Products B and C effected the greatest percentage decrease in microorganisms; the least percentage removal being obtained with orange juice and distilled water. When the percentage decreases on all culture media were averaged (Table II, Figure 1) it was found that the results with water were the same as with orange juice, while the decrease with grapefruit juice was almost as great as with Product A, which contained hexylresorcinol as an active ingredient.

Although Products B and C were the most effective in actual removal of organisms, they were not so effective in inhibiting further microbial activity during the two hours immediately following prophylaxis (Table III). When the percentage decreases on all culture media just after prophylaxis were averaged and used as a 100% base for determining the average percentage increase two hours later, it was observed that the citrus juices were the most effective in inhibiting bacterial growth (Table IV, Figure 2). Although water brought about as great a decrease after prophylaxis as did orange juice (Table II) it will be seen from Table IV that the percentage increase with water two hours later was 98.7% greater than with orange juice. The observation that citrus juices exert some inhibitory action on bacteria is again shown in a comparison of results obtained with grapefruit

TABLE I

Percentage Decrease in Bacterial Counts Per CC. of Human Saliva After Various Mouth Prophylaxes

Prophylactic Agents	Decrease Due to Prophylaxis*		
	Medium (agar)		
	Nutrient	Tomato Juice	Blood
	%	%	%
Water	45.1	53.0	42.1
Orange juice	46.7	50.1	43.3
Grapefruit juice	60.9	48.6	57.9
Product A	50.9	61.1	64.9
Product B	86.7	89.9	91.1
Product C	79.2	87.5	90.3

* Counts after lunch before prophylaxis as 100% base.

Product A - hexylresorcinol tooth paste and mouth wash (1/4000).

Product B - sodium ricinoleate tooth paste and mouth wash (1%).

Product C - liquid dentifrice, active ingredient 2% sodium alkyl sulfate; mouth wash (10%).

TABLE II

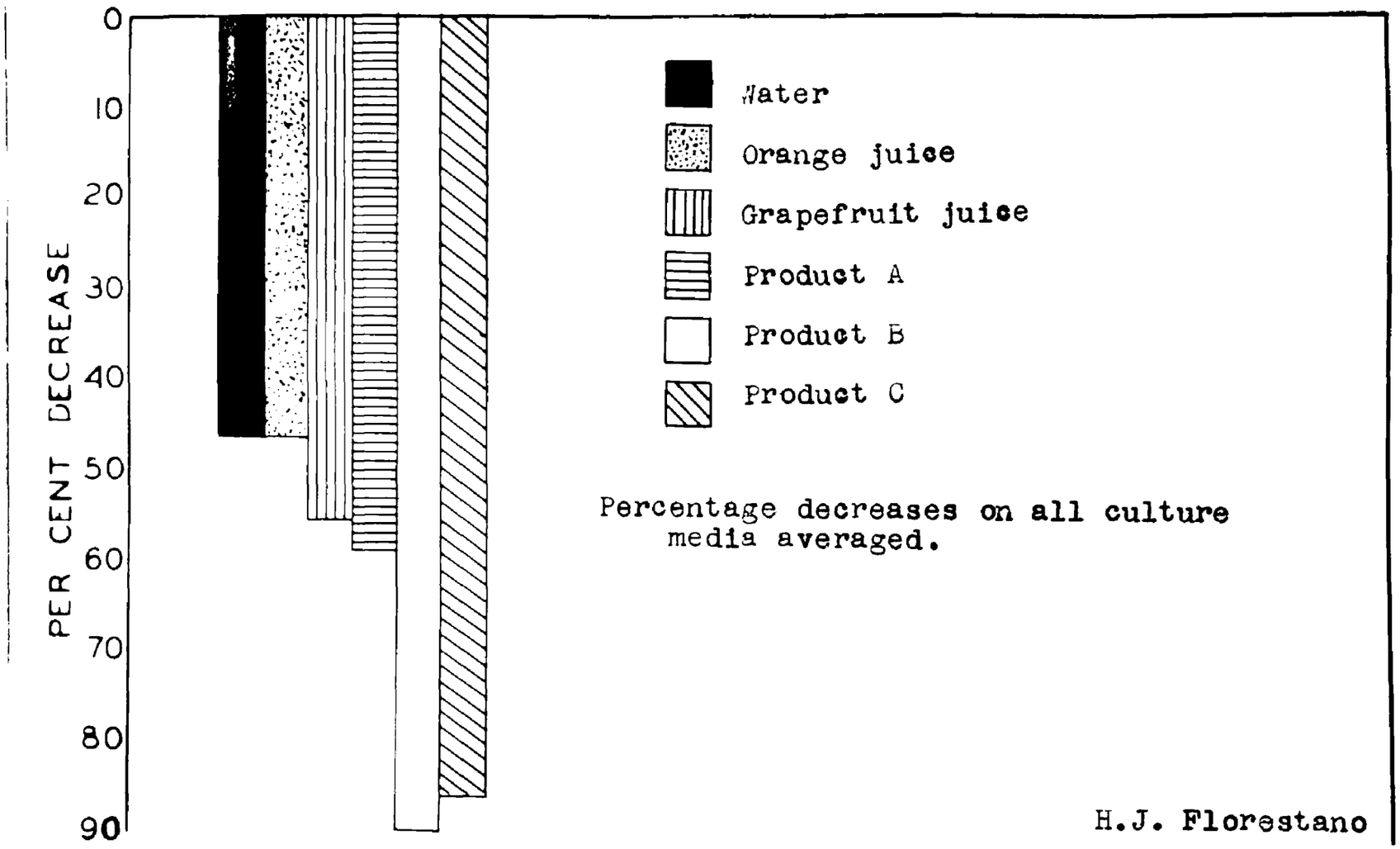
Percentage Decrease in Bacterial Counts Per CC. of Human Saliva After Various Mouth Prophylaxes

(The percentage decreases on all culture media are averaged)

	Prophylactic Agent					
	Water	Orange Juice	Grapefruit Juice	Product A	Product B	Product C
Decrease due to prophylaxis (per cent)*	46.7	46.7	55.8	59	89.2	85.7

*Counts after lunch before prophylaxis as 100% base.

FIGURE 1: Percentage Decrease in Bacterial Counts Per cc. of Saliva After Prophylaxis.



Percentage decreases on all culture media averaged.

H.J. Florestano

TABLE III

Percentage Increase in Bacterial Counts Per CC. of Human Saliva Two Hours After Various Mouth Prophylaxes

Prophylactic Agents	Increase Two Hours After Prophylaxis*		
	Medium (agar)		
	Nutrient	Tomato Juice	Blood
	%	%	%
Water	267.3	362.1	176.2
Orange juice	164.3	219.3	125.8
Grapefruit juice	188.9	198.8	227.6
Product A	288.8	262.3	389.2
Product B	635.1	793.4	632.7
Product C	103.5	360.1	530.2

*Counts after prophylaxis as 100% base.

TABLE IV

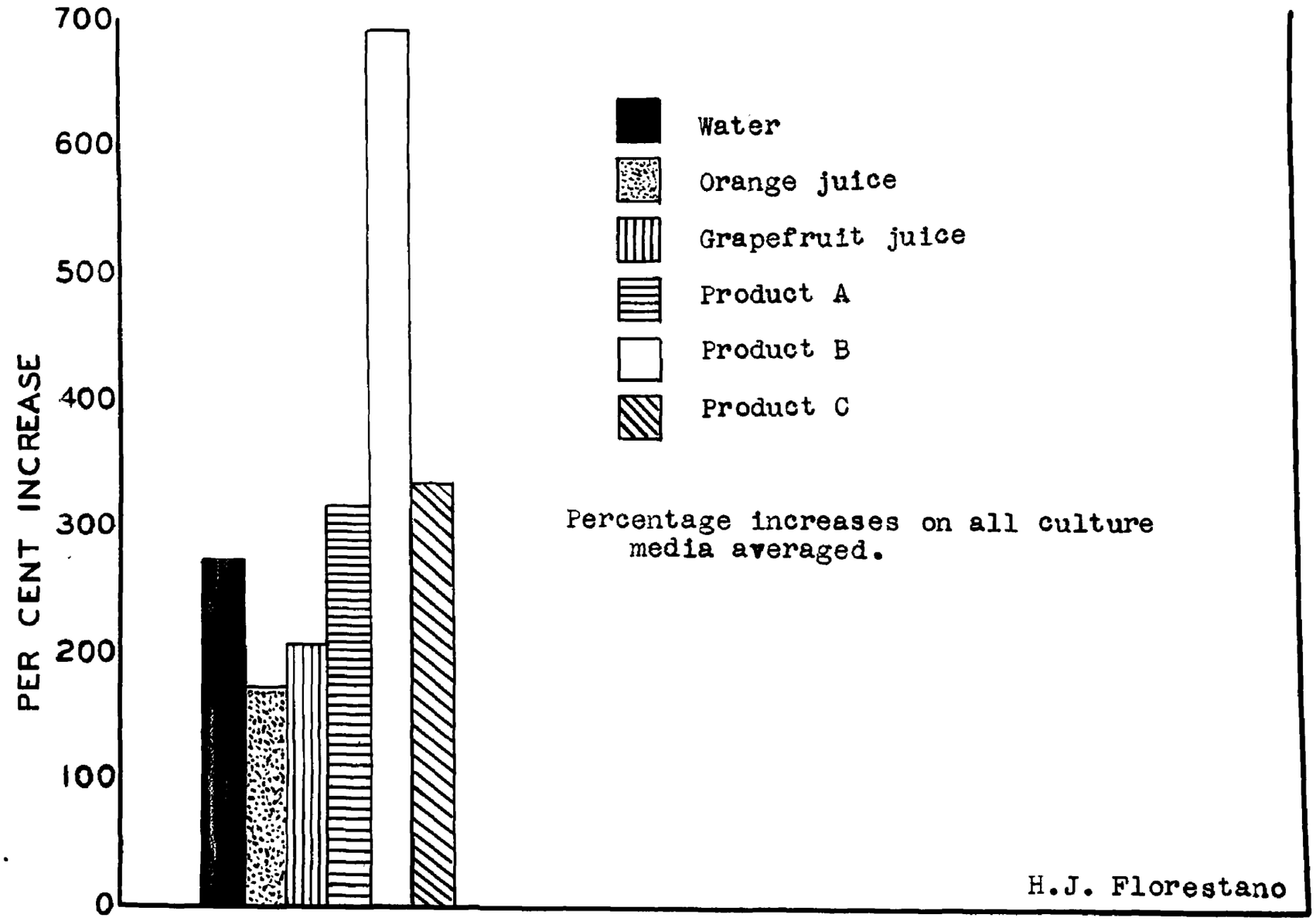
Percentage Increase in Bacterial Counts Per CC. of Human Saliva Two Hours After Various Mouth Prophylaxes

(The percentage increases on all culture media are averaged)

	Prophylactic Agents					
	Water	Orange Juice	Grapefruit Juice	Product A	Product B	Product C
Increase two Hours after prophylaxis (per cent)*	268.5	169.8	205.1	313.4	687.1	331.3

*Counts after prophylaxis as 100% base.

FIGURE 2: Pèrcentage Increase in Bacterial Counts Per cc. of Saliva Two Hours After Prophylaxis.



juice and Product A. Both materials effected practically the same percentage removal of microorganisms (Table II), yet the increase in counts with Product A two hours after prophylaxis was 108.3% more than with grapefruit juice (Table IV). The marked increase in counts with Products B and C will also be observed in Table IV.

Discussion

Study of the oral cavity has shown that the mouth is more or less naturally the habitat of a large number and variety of microorganisms. The number and types not only vary in different individuals, but there is a difference from day to day in the same individual.

Disinfection of the oral cavity by means of medicated dentifrices and mouth washes has as yet been unattainable. Even when the bacterial count is lowered by artificial aids laboratory tests have shown that this decrease lasts but for only a short period. Aside from the use of artificial means of prophylaxis the maintenance of oral health is normally assisted by the functioning of the different parts of the mouth and by deglutition. A marked influence of eating on the microbial content of the mouth has been reported by Crowley and Rickert (15) with whom our results have agreed. In some cases we have observed as high as an 88% decrease in the oral flora immediately following a meal.

The efficacy of the incorporation of bactericidal substances in tooth pastes and mouth washes is still debated.

Concentrations sufficient to kill bacteria apparently have deleterious effects on the oral tissue. If a minimum concentration of anti-bacterial agent is used there is then to be considered the influence of the saliva upon further dilution of the agent. It would seem a valuable aid in maintaining oral health if at least some inhibition of bacterial growth in the mouth could be accomplished. This apparently has been accomplished with orange and grapefruit juice, when used both as a dentifrice and mouth wash. The citrus juices not only proved effective in their inhibitory action, but also, no oral discomfort was experienced by any of the subjects. On the other hand, the commercial products not only failed to check microbial activity in the mouth, but two in particular proved quite irritating to the oral mucous membrane.

The efficacy of Products A, B and C in the removal of microorganisms from the mouth during prophylaxis was apparently due to the presence of a surface tension reducing agent in each of the materials. The role played by surface tension in oral antisepsis and the mechanism involved have been reported on by Feirer and Leonard (20).

Comparison of results obtained with Products A and B presented an interesting situation. Recalling that Product A contained hexylresorcinol, and Product B sodium ricinoleate, the percentage decrease due to prophylaxis with Product B was more than 1 1/2 times as great as with Product A (Tables I and II), despite the fact that hexylresorcinol possesses

a higher phenol coefficient. However, in the two-hour interval following prophylaxis (Tables III and IV) Product A maintained a lower percentage increase than did Product B. Carroll (12) and Mead (63) likewise found that sodium ricinoleate effected a greater decrease in the bacterial count of the mouth than did hexylresorcinol. Contrary to the author's results, however, the above workers found that the counts remained lower for a longer time when sodium ricinoleate was used rather than when hexylresorcinol was used. Mead believed that the anti-bacterial action of sodium ricinoleate was due to its "unusual cleansing powers and peptizing action".

Gies (25) has stressed the importance of the use of an acid dentifrice for the satisfactory removal of bacterial plaques. He believed that although neutralization of mouth acid should be sought for in prophylaxis, this would necessarily follow by virtue of an increase in flow of alkaline saliva as the result of brushing the teeth with a favorable acid agent. Gies maintained that organic acid, such as is present in fruits like orange and lemon, has this ability to markedly stimulate the flow of a "highly alkaline saliva". Hanke (38) in a study of the relation of the buffer value of saliva to dental caries found that in caries-susceptibles the saliva was usually acid and poorly buffered, whereas the saliva of individuals immune to caries was alkaline and highly buffered. Results (17) have shown that the local application of citrus fruit juices apparently stimulates a flow of saliva that is

highly alkaline and well-buffered. Although the increase in buffer capacity lasted for a short time only, a real change in the saliva was nevertheless produced. It is believed that consistent use of citrus products may effect a more permanent change not only in the saliva but in the oral flora as well.

IV

STUDIES OF THE EFFECT OF INGESTION OF CITRUS FRUIT ON THE ORAL FLORA

In the study of the effects of the local application of citrus fruit juices on the oral flora it was apparent that orange and grapefruit juices exerted a definite inhibitory action on the microorganisms of the mouth. Following these observations a study was undertaken to determine the influence of the actual ingestion of citrus fruit on the numbers and types of oral bacteria.

Experimental

Materials: Two groups of individuals were used for the tests: one to serve as a control, and the other to be fed fresh grapefruit in addition to the daily diet. Inmates of the District of Columbia Penal Farm, Lorton, Virginia were selected for study. The selection of this group of men proved favorable not only from the standpoint of convenience but also from a nutritional one. The men were available at all times for examination, and the distance between the institution and the laboratory was such that saliva could be obtained and analyses begun within a relatively short time after collection. Any dietary or nutritional variations that might possibly have affected the results were believed to be taken care of by choosing men who had been on the regular prison diet for at least one year and who would remain under term for the duration of the study.

Cooperation in the investigation was entirely voluntary on the part of the inmates. The ages of individuals selected ranged from 19 to 58 years, both white and negro men being included. Data were obtained on 131 men, 64 of whom served as the control group, and 67 as the test group. The men in both groups received the regular prison rations, but the test group was fed, in addition, one-half of a grapefruit three times a day, the fruit being served with each meal. All fruit used in the study was grown in Florida, and purchased on the open market.

Collection of Saliva Samples: Examinations were made on samples of saliva obtained at regular intervals. Flow of saliva was stimulated by chewing sterile paraffin, and approximately 25 cc. of saliva were collected from each subject into sterile sputum bottles. The saliva samples were always collected at approximately 10:00 A.M. on the respective test days, and packed in an iced container for transportation to the laboratory. An interval of about three hours occurred between the time of sampling and the time of bacteriological analyses.

Analyses of Materials: An initial quantitative examination of the oral flora of each individual of both groups was made previous to the feeding of grapefruit to the test group. These data served for future interpretation of results. Immediately following the primary estimations of numbers and types of bacteria the test group then began

to receive its daily quota of grapefruit. Samples of saliva were subsequently collected once a month from each member of both groups. Analyses extended over a period of 4 months.

Dilutions and Media: Dilution and plating of saliva samples were carried out according to methods given in Section III.

Results

Data on the bacteriological examinations of 131 of 139 men used during this study are presented below. Eight of the men obtained paroles or were forced to drop out because of prison restrictions before some of the tests were completed. Therefore the results on these eight individuals were incomplete and are not included.

No initial bacterial counts were obtained for numbers 69, 70 and 71 of the test group and numbers 119, 131, 160-168 of the control group. Those men of the test group had been on the grapefruit diet for one month before the first analyses of saliva were made. Each number (Tables V and VI) represents the average of the counts obtained on plain nutrient, tomato juice and blood agars for that individual for each examination.

In Table V it may be observed that of the 65 individuals on whom initial counts were obtained, 54, or 83%, showed a decrease in counts after one month on the grapefruit diet. In Table VI it may be noted that of 54

TABLE V

Effect of Ingestion of Grapefruit on the Bacterial Content of the Mouth

Case No.	Average Total Counts of Bacteria Per CC. of Saliva* After Following Interval on Grapefruit Diet: Initial	1 month	2 months	3 months
	Millions	Millions	Millions	Millions
1	15.2	13.7	16.7	23.0
2	32.3	12.1	11.9	28.1
3	59.3	5.2	38.9	38.8
4	33.6	2.7	14.6	38.2
5	208.7	71.3	63.7	59.9
6	77.0	31.5	14.6	17.2
7	95.4	32.2	37.4	34.6
8	10.7	40.2	15.6	21.9
9	61.2	50.0	32.5	-----
10	116.0	57.2	52.7	-----
11	32.3	16.2	11.6	10.1
12	70.3	51.6	79.7	26.1
13	30.3	28.0	23.3	13.8
14	66.5	35.2	38.6	17.2
15	59.7	34.4	34.0	21.8
16	121.3	64.7	84.0	87.7
17	55.8	57.5	5.9	12.0
18	36.7	52.0	61.7	57.7
19	116.3	104.7	45.5	29.2
20	34.0	17.9	38.6	33.5
21	38.1	17.5	26.6	33.4
22	53.8	20.3	7.4	10.5
23	43.7	25.2	37.7	48.1

Test Group (Continued)

Case No.	Initial	1 month	2 months	3 months
24	53.0	22.5	43.7	41.1
25	57.7	17.4	48.5	28.0
26	20.8	6.3	23.4	36.0
27	99.7	35.2	46.6	-----
28	39.4	7.0	18.0	18.5
29	52.4	10.9	15.8	32.0
30	109.7	104.3	117.7	82.7
31	41.0	31.1	29.3	31.6
32	14.5	17.9	11.4	7.5
33	84.7	82.7	47.8	37.7
34	110.3	52.1	22.8	-----
35	92.3	95.0	68.3	70.7
36	22.7	0.9	28.8	13.9
37	43.3	29.3	47.7	36.9
38	48.8	41.8	76.0	30.9
39	86.7	30.3	42.3	-----
40	31.9	20.7	55.0	6.2
41	114.3	61.2	22.3	46.5
42	74.3	30.7	71.0	-----
43	20.0	19.5	38.6	29.2
44	75.0	31.6	26.8	30.2
45	137.3	42.4	90.7	119.7
46	26.6	19.4	46.9	41.7
48	17.4	12.4	12.9	16.7
49	20.1	27.5	30.1	34.7
50	28.4	52.3	65.3	44.1
51	19.8	19.9	14.7	9.3
52	101.6	83.8	21.7	67.7

Test Group (Continued)				
Case No.	Initial	1 month	2 months	3 months
53	40.1	73.0	20.7	23.9
54	38.9	57.1	22.6	27.5
55	42.9	9.8	23.1	50.0
57	117.7	37.1	22.8	-----
58	104.0	26.0	82.3	69.3
59	143.7	38.8	80.3	62.7
60	31.7	9.2	17.7	18.2
61	68.1	15.4	36.4	46.6
62	61.7	33.1	27.5	50.6
63	68.0	46.6	43.2	40.8
64	57.1	20.3	32.1	12.4
65	54.3	52.2	57.0	52.1
67	28.1	40.8	22.2	24.0
68	73.1	31.4	52.2	34.6
69	-----	34.7	59.3	53.0
71	-----	46.5	49.3	46.7

* Each figure represents average of counts on 3 media; plain nutrient, tomato juice, and blood agars.

TABLE VI

Results of Bacteriological Examinations of Saliva of Control Group

(Receiving no grapefruit)

Case No.	Average Total Counts of Bacteria Per CC. of Saliva*			
		After Following Interval		
	Initial	1 Month	2 Months	3 Months
	Millions	Millions	Millions	Millions
101	93.3	42.0	59.7	42.8
102	21.7	61.0	57.0	66.7
103	25.8	63.5	45.3	92.7
104	25.8	72.4	30.3	22.7
105	79.3	97.0	91.3	-----
106	56.8	51.7	61.5	56.3
107	39.5	67.7	110.3	70.7
108	71.3	101.0	88.3	75.0
109	18.0	9.9	37.4	25.1
110	60.2	69.7	100.7	151.7
111	4.4	52.5	38.7	21.4
112	65.1	30.1	61.0	62.3
113	40.8	64.3	73.7	36.1
114	105.3	122.7	88.0	-----
115	42.9	27.7	52.1	58.6
116	40.1	76.7	73.0	77.7
117	46.2	81.7	127.0	89.0
118	26.4	66.0	41.4	64.0
119	-----	58.7	39.1	59.7
120	76.0	10.8	18.9	30.8
122	51.4	81.7	118.0	103.3
124	53.0	54.1	30.2	37.9

Control Group (continued)

Case No.	Initial	1 month	2 months	3 months
125	72.0	152.7	98.0	49.9
126	57.3	78.7	71.7	83.7
127	58.0	68.6	72.3	31.7
128	57.6	54.7	42.1	31.4
129	34.5	53.3	94.3	83.3
130	44.0	56.7	50.7	45.7
131	-----	94.7	55.3	40.8
132	71.3	81.7	90.3	97.3
133	60.3	85.0	77.3	92.0
134	50.9	71.3	61.0	43.7
135	24.3	21.9	22.5	25.4
136	23.9	32.3	42.0	22.1
137	29.6	40.7	56.7	34.1
138	89.7	87.3	110.3	104.0
139	21.5	20.5	42.3	23.7
140	63.3	51.0	50.9	43.0
141	58.5	101.0	53.1	41.1
142	69.4	63.9	71.7	96.3
143	20.4	-----	44.7	25.4
144	46.8	81.0	103.0	71.7
145	96.3	66.7	152.7	80.7
146	36.3	49.0	65.7	50.0
147	17.5	29.2	99.2	33.2
148	55.3	84.7	35.3	56.3
149	24.8	55.0	91.7	53.0
150	104.1	102.7	103.0	-----

Control Group (continued)

Case No.	Initial	1 month	2 months	3 months
151	64.4	8.9	35.2	76.3
152	77.1	54.3	59.0	74.3
153	96.8	42.4	70.7	35.3
155	52.6	35.7	67.0	25.2
156	37.0	19.9	47.5	28.4
157	81.1	65.0	48.4	57.7
158	71.2	63.0	107.7	88.7
159	48.8	85.3	57.3	24.6
160	-----	36.6	48.7	15.3
161	-----	57.7	28.3	12.1
162	-----	68.3	56.7	53.3
163	-----	42.9	26.2	50.6
164	-----	135.0	85.3	55.3
166	-----	38.3	56.9	43.2
167	-----	113.7	64.3	84.7
168	-----	96.0	32.2	50.3

* Each figure represents average of counts on 3 media; plain nutrient, tomato juice, and blood agars.

TABLE VII

Effect of Ingestion of Grapefruit on the Bacterial Content of the Mouth.
Each Figure Represents the Average of all Counts of all Individuals
in Each Respective Group

Medium (agar)	Total Numbers of Bacteria Per CC. of Saliva							
	Initial Count		After Following Interval on Grapefruit Diet: 1 Month		2 Months		3 Months	
	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group	Control Group	Test Group
Plain nutrient	41,259,000	48,238,000	54,184,000	26,356,000	46,469,000	29,681,000	43,653,000	28,344,000
Tomato Juice	20,685,000	33,780,000	45,438,000	13,831,000	47,229,000	30,935,000	36,081,000	20,961,000
Blood	96,948,000	104,655,000	91,408,000	67,728,000	101,840,000	58,439,000	83,615,000	61,719,000

men in the control group only 20, or 37%, showed any decrease in count upon analysis one month after the initial examination. At the second month, 75% of the men examined in the test group showed a decrease in counts when compared to the initial, while 26% in the control group gave counts lower than the initial. At the end of the third month on the grapefruit diet it was found that 81% of the test group still had counts lower than those obtained at the start of the experiment, while only 39% of men examined in the control group showed a decrease in counts over the initial.

In Table VII the average count of all individuals in the respective groups are given for each medium employed. The average counts of the test group are markedly lower than those of the controls.

Discussion

Comparison of the quantitative bacteriological findings of the mouth as reflected in the saliva of test and control groups showed that a definite decrease occurred in bacterial counts in those men receiving grapefruit in addition to the regular prison diet.

It was found that after one month 83% of the test individuals and 37% of the controls had bacterial counts lower than the initial. At the second month 75% of the test men and 26% of the men in the control group showed counts lower than the initial. The lower percentage of decreases in counts of both groups at the second month may

possibly be attributed to an epidemic of influenza at the prison during this period.

A decrease in count below the initial was maintained by 81% of the test individuals after 3 months on the grapefruit diet as compared to 39% of the controls. This is highly significant and indicates that the bacterial contamination of the mouth may be altered by daily ingestion of grapefruit.

Marked decreases in average bacterial counts occurred in individuals receiving grapefruit in addition to their regular daily diet. Decreases of 45%, 38% and 41% in counts below the initial occurred on plain nutrient agar after 1, 2, and 3 months on grapefruit, respectively. In the control group, however, increases of 31%, 13% and 6% over the intial counts on plain nutrient agar resulted at these respective monthly periods. On blood agar, decreases of 35%, 44% and 41% below the initial occurred in the test group after 1, 2, and 3 months on grapefruit, respectively. Blood agar supports the growth of a number of potential pathogens. Their presence in the mouth should not be overlooked as possible agents in oral disease. The marked reduction in bacterial counts obtained on blood agar thus becomes highly significant. Slight decreases of 6% and 14% in counts below the initial on blood agar occurred in the control group at the first and third monthly examinations, respectively, while a 5% increase resulted at the second month. Results obtained on tomato

juice agar for the test group were not as consistent as those on the other media used. Decreases in counts of 59%, 9%, and 38% below the initial occurred after 1, 2, and 3 months, respectively. On the other hand, marked increases in the average counts obtained on tomato juice agar for the control individuals were presented at each monthly examination. Increases of 120%, 128% and 74% over the initial counts occurred after the first, second and third month, respectively. The growth of oral aciduric microorganisms was very much favored by tomato juice agar. It is believed by some workers that these organisms play a role in dental decay. If this theory is correct, the inhibition of growth of these microorganisms may possibly be of aid in caries treatment. In this respect, the decrease in counts of aciduric microorganisms presented by test individuals becomes of importance.

Through the cooperation of Dr. Frank G. Klune, medical director, and Dr. George W. Bogikes, resident dentist, of the Penal Farm at Lorton, physical and dental examinations of the men were made at the start of the investigation and at regular intervals throughout. All individuals on the grapefruit diet showed considerable improvement in general health. The dental examination indicated marked improvement in the tone of oral tissues, and several severe cases of bleeding gums were observed to have been checked in individuals receiving grapefruit.

Certain dietary factors supplied by grapefruit may have had some influence on both the physical and dental conditions of test individuals. It was apparent that ingestion of grapefruit had influenced general systemic body reactions. It is not definitely known, however, whether the change in oral flora was produced through the effect on the general system, or as a result of local environmental changes produced in the mouth. King, cited by Gies (25), maintained that bacteria grew in an alkaline medium, and that an acid substance would inhibit their growth. Black, also cited by Gies (25), stated that certain acids placed in the mouth tend to inhibit microbial action. These views would seem to indicate that local environmental changes produced by the ingestion of grapefruit may have been responsible for the decrease obtained in bacterial counts of the mouth.

Howitt and Fleming (46) and Howitt, Fleming and Simonton (47) believed that oral bacteria were influenced more by food remains in the mouth and by mouth hygiene than by general systemic reactions. In the study dealing with the local application of citrus fruit juices a definite inhibitory action on microbial growth was observed (Section III). A corresponding inhibitory action was manifested when grapefruit was received with the daily diet. Similar

results obtained with both methods of administration would seem to indicate that citrus fruits possess some factor, or factors, inhibitory to oral bacteria.

It seems probable that reduction in bacterial numbers of the oral flora was due more to local influences of citrus fruit and fruit juices than to systemic involvement that may have resulted through their use.

V.

THE POSSIBLE RELATIONSHIP BETWEEN (A) THE PRESENCE OF ORAL ACIDURIC BACTERIA, AND (B) DIASTATIC ACTIVITY AND H-ION CONCENTRATION OF SALIVA AND INCIDENCE OF DENTAL CARIES

Marked differences of opinion as to the significance of bacteria in dental caries are found in the literature. It appears that in the majority of cases too few observations may be the reason. Accordingly, a relatively large number of individuals was studied in an attempt to determine the relation between the presence of aciduric microorganisms and the incidence of caries. The incidence of aciduric bacteria in the mouths of carious and non-carious individuals was determined using a combination of the qualitative broth-method and the quantitative method described by Hadley (29).

A number of investigations have been made on the physico-chemical properties of saliva in an attempt to find some difference between non-carious and carious individuals. Study of the diastatic activity of saliva was undertaken to see if any correlation could be made between ptyalin content and caries activity. The H-ion concentration of saliva is also still receiving the attention of investigators and in this work determinations were made of pH values of saliva of various individuals.

Experimental

A. Possible Relationship Between the Presence of Oral Aciduric Bacteria and Incidence of Dental Caries.

Materials: A total of 166 individuals were included in this study. Each individual was given a thorough dental

examination and his case history recorded. Of the total number, 76 were non-carious and 90 were carious. The individuals were divided into the following 8 groups according to their dental condition:

I. Non-carious.

Group A, those with all teeth intact, and exhibiting no open cavities nor any fillings (28 individuals).

Group B, those with previous extractions, but no existing cavities nor fillings (29 individuals).

Group C, those with no open cavities, but teeth filled (19 individuals).

II. Carious.

Group D, those with 1 open cavity; designated as first degree caries (19 individuals).

Group E, those with 2 open cavities; second degree caries (10 individuals).

Group F, those with 3 open cavities; third degree caries (5 individuals).

Group G, those with 4 to 10 open cavities; fourth degree caries (28 individuals).

Group H, those with 10 or more open cavities; rampant caries. (28 individuals).

Collection of Materials: The determinations of the numbers of aciduric bacteria in the mouths of carious and non-carious

individuals were made from samples of saliva obtained from the respective subjects. Saliva samples were collected between 10:00 and 12:00 A.M. Stimulation of the flow of saliva was induced by chewing sterile paraffin, and approximately 25 cc. of saliva were collected from each subject into sterile sputum bottles. As soon as samples were obtained they were packed in an iced container, transported to the laboratory and analyses begun within three hours.

Media: The medium used for the enumeration of micro-organisms was essentially Kulp's (56) tomato juice peptonized milk, peptone agar, with the modifications described by Hadley (29). Kulp's original formula was as follows: to 400 cc. of tomato juice filtered from a good grade of canned tomatoes were added 10 grams of Difco peptone and 10 grams of Difco peptonized milk. This mixture was heated gently to dissolve the peptone and then adjusted to pH 6.0-6.2. A second mixture was prepared by adding 11 grams of agar to 600 cc. of distilled water and autoclaved to dissolve the agar. The two mixtures were then mixed together while hot, filtered through absorbent cotton, dispensed into bottles, and sterilized at 120°C. for 8 minutes.

The only modifications of Kulp's medium made by Hadley were (1) the use of 2% agar instead of 1.1% and (2) adjustment of the reaction to pH 5.0 instead of 6.2 with lactic acid. Hadley maintained that the mixture of tomato juice, peptonized milk, peptone, agar and water possessed an initial reaction of

about pH 5.4 to which she added 1 N lactic acid for the final reaction of pH 5.0. The present author did not find the addition of lactic acid necessary in every case. Some lots of media would have an initial reaction of about pH 4.8, necessitating final adjustment with alkali, while other mixtures would have an initial pH of 5.3-5.5, in which case 1 N lactic acid was used for final adjustment. In other cases no adjustment was needed. The variations in initial reaction were probably due to differences in the quality of tomatoes used, although the same brand was employed throughout the study.

In adjusting the reaction of the medium before sterilization it was necessary to adjust to a pH of 5.2, since it was observed that autoclaving for 8 minutes at 120°C. almost invariably lowered the reaction by 0.2 pH.

Qualitative acid-broth tests were carried out in a medium made according to Kulp's formula for the tomato peptone agar medium minus the agar and adjusted to pH 5.0. This broth was found to give a much better growth when inoculated with saliva than the glucose beef infusion broth (pH 5.0) of previous workers (18),(29),(82).

Analyses of Materials: Samples of saliva were diluted with sterile water. Bacterial counts were obtained on the acid-tomato medium by plating out dilutions of 1:10, 1:100, 1:1,000; and 1:10,000 and 1 cc. of saliva direct. Plates were incubated at 37°C. for 4 days.

The tomato juice agar did not prove quite as specific for L. acidophilus as Hadley (29) maintained, streptococci, staphylococci and yeast appearing with great regularity. Therefore, in the determination of aciduric types the total number for each saliva sample was obtained, and then Gram-staining resorted to for the evaluation of the incidence of the various types that were capable of growth in a medium of pH 5.0. Gram-positive, non-spore-forming rods appearing singly or in chains were regarded as lactobacilli.

Each sample of saliva was inoculated into the acid-tomato broth at the same time acid-tomato agar plates were poured. The qualitative broth tests were made by inoculating 10-12 cc. of the acid-tomato broth with 0.5 cc. of saliva. After 2 days incubation at 37°C. the cultures were removed and Gram stains prepared to determine the types supported by the medium. Following incubation of both tests, a comparison was made of the types of organisms in each.

As the bacteriological investigation of non-carious and carious individuals progressed a study was made of the acidogenic properties of various types of microorganisms capable of growth on the acid-tomato agar. Gram-stains were made of a number of representative colonies of each type to determine cell morphology and Gram reaction. The colonies were then picked and inoculated into 1% dextrose beef infusion broth of pH 6.6, and incubated for 48 hours at 37°C.

After incubation Gram-stains were made of the broth cultures as a check on the stain prepared from the original colony. Two loopsful of each culture were again inoculated into 15 cc. of 1% dextrose beef infusion broth (pH 6.6) in triplicate and incubated at 37°C. At the end of 48 hours, 72 hours and one week a tube of each respective culture was removed and the pH determined by means of a glass electrode pH meter. In this manner the acidogenic powers of 74 cultures of bacteria isolated from the saliva of various individuals were tested. Included were Gram-positive rods (lactobacilli), streptococci, staphylococci and several yeasts.

B. Possible Relationship Between Diastatic Activity and H-ion Concentration of Saliva and Incidence of Dental Caries

Materials: Samples of saliva collected from individuals examined in the study on the relationship of aciduric bacteria to dental decay (Section V A) were used in this work. The diastatic activity of each sample was determined by means of the starch-iodine test:

Starch Solution: A 1% solution of starch was prepared by dissolving soluble starch powder (Baker's Analyzed chemicals) in distilled water. The solution was boiled for 15 minutes, cooled to room temperature, and then restored to the original volume by addition of distilled water.

Iodine Solution: A 2% solution of potassium iodide was prepared in distilled water, and the solution colored a deep

yellow by the addition of 10 mgms. of iodine crystals (resublimed).

The starch and iodine solutions were never more than 2 days old, and when not in use were kept at 4-5°C.

Analyses of Materials: Conditions for the tests were carefully standardized. All solutions were thoroughly agitated before use. Saliva samples were shaken for 1 minute, and passed through filter paper before testing. With a standard pipette, 20 cc. of starch solution were measured into an 8" x 1" test tube and placed in a water-bath at 37°C. When the starch solution reached the temperature of the bath 5 drops of the filtered saliva were introduced into the starch solution, and the diastatic index determined by the spot-plate method. The end of the time interval required for complete hydrolysis of the starch was determined as that point at which the mixture of starch and iodine turned from a light brown to yellow.

In addition to the study of the diastatic activity of saliva, determinations were also made of the H-ion concentration of various samples. The pH values were obtained electrometrically, using a glass electrode pH meter.

Results

A. Possible Relationship Between the Presence of Oral Aciduric Bacteria and Incidence of Dental Caries: Data obtained from the quantitative and qualitative examinations of saliva

of non-carious and carious individuals are shown in Tables VIII to XVI.

Of the 76 non-carious individuals 69, or 91%, were found positive for lactobacilli as evidenced by the plate count on acid-agar (pH 5.0). The 7 non-carious individuals who were lactobacilli-negative were re-examined. Two (cases 117 and 130) showed the presence of these organisms in the saliva, and continued to give positive cultures for a period of 3 months when analyses were discontinued. Of the 90 individuals exhibiting caries, 88, or 98% were lactobacilli-positive.

It will be noticed from the tables that the incidence of aciduric streptococci was relatively high in both the non-carious and carious groups. Streptococci were found in the saliva of 40, or 53%, of the non-carious individuals, and in 47, or 52%, of carious individuals. Staphylococci appeared in saliva of both groups, but with somewhat less regularity than the streptococci. Staphylococci were present in 12, or 16%, of the non-carious individuals, while 12, or 13% of the carious individuals were positive. The presence of yeast in saliva of both groups was not uncommon. Of the non-carious individuals, 16, or 21%, were yeast-positive, while of the carious individuals 25, or 28% were positive.

Of the 28 individuals with rampant caries (Table XV) 96% were lactobacilli-positive. It is interesting to note

that of the 28 non-carious individuals in Group A (Table VIII) 96% were also positive for lactobacilli. Only one individual in each of the two groups failed to show the presence of lactobacilli when saliva was plated out on acid-agar. Inoculation of saliva of these two individuals into acid-broth likewise resulted in negative cultures for lactobacilli.

The individual lactobacilli counts of the non-carious individuals varied from 2 organisms per cc. of saliva to 2,370,000. The average count of the 76 individuals was 84,000 per cc. The range of variation for the carious groups was between 1 and 1,540,000 lactobacilli per cc. of saliva, the average count for the 90 individuals being 207,000 per cc. Although the percentage incidence of streptococci and yeast showed no marked difference between the non-carious and carious individuals, it will be seen from Table XVI that these types appeared in greater numbers in saliva of carious individuals than in the non-carious.

Data on the acidogenic properties of 74 strains of aciduric organisms isolated from saliva were obtained. Of 39 strains of lactobacilli examined, 25 produced a pH ranging from 4.0 to 3.6 in glucose broth after 7 days incubation. The remaining 14 strains produded an acidity between pH 4.9 and 4.1. Of 22 strains of streptococci, 12 produced a pH ranging from 4.0 to 3.4. Only 3 of the 8 strains of staphylococci tested were capable of producing an acidity between pH 4.0 and 3.7. The acidogenic powers in glucose broth of oral yeast were relatively slight as compared to acid production

by aciduric rods and cocci. The lowest pH produced by any of the 5 yeasts tested was pH 4.17.

B. Possible Relationship Between Diastatic Activity and H-ion Concentration of Saliva and Incidence of Dental Caries: The diastatic index for each individual is also shown in Tables VIII to XV. In general the time required for starch hydrolysis was greater with non-carious than with carious individuals. The average time for complete diastatic action of the 76 non-carious individuals was 9.2 minutes, while for the 90 carious individuals it was 4.0 minutes. It may be observed in Table XVI that for the 28 immune individuals in Group A the average diastatic index was 9.3 minutes. For the 28 individuals showing rampant caries (Group H) the average time was 3.6 minutes. Thus an increase in diastatic activity was accompanied by an increase in the incidence of dental caries.

Results of determinations of the H-ion concentration of saliva of various individuals examined are given in Tables VIII to XVI. Variations in the pH of saliva appear to be unrelated to the incidence of caries. The respective averages of the eight groups (Table XVI) show that the pH values for the non-carious groups (A, B, and C) were relatively high (average pH 7.48, 7.33, and 7.38, respectively), with the lowest pH value of 7.09. This occurred in group F, which was made up of individuals with third degree caries.

TABLE VIII

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Non-Carious Individuals in Group A, i.e., All Teeth Present, No Cavities Nor Fillings

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
38	106,000	82,000	24,000	0	0	+	+	-	-	10.5	6.72
42	3,300	3,000	0	0	300	+	-	-	+	10.0	7.10
75	9,400	5,400	0	0	4,000	+	-	-	+	28.5	8.00
96	10,300	9,700	600	0	0	+	+	-	-	2.5	
102	53,000	53,000	0	0	0	+	-	-	-	2.5	
124	810	740	70	0	0	+	-	-	-	4.5	
138	8,100	8,000	100	0	0	+	+	-	-	4.5	
139	1,040,000	1,040,000	0	0	0	+	-	-	-	3.0	
140	180	180	0	0	0	+	-	-	-	4.5	
148	3,200	2,300	900	0	0	+	+	-	-	4.0	
149	40,000	40,000	0	0	0	+	-	-	-	8.5	7.38
150	2,370,000	2,370,000	0	0	0	+	-	-	-	10.0	7.42
151	1,900	1,300	600	0	0	+	+	+	-	26.0	7.31
152	3,500	3,000	500	0	0	+	+	+	-	5.5	7.29
153	2,700	0	300	2,400	0	-	+	+	-	4.5	7.78
154	1,450	1,000	210	240	0	+	+	+	-	5.5	7.64
155	8,500	500	3,000	5,000	0	+	+	+	-	6.5	7.55
156	85,000	40,000	35,000	10,000	0	+	+	+	-	7.5	7.74
157	46,000	46,000	0	0	0	+	-	-	-	5.0	7.80
158	145,000	95,000	36,000	14,000	0	+	+	+	-	6.5	7.46

TABLE VIII (Continued)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
159	3,800	3,600	200	0	0	+	-	-	-	12.5	7.46
160	267,000	107,000	110,000	50,000	0	+	+	+	-	15.5	7.55
161	3,130	630	1,500	1,000	0	+	+	+	-	16.5	7.71
162	7,400	7,400	0	0	0	+	-	-	-	16.0	7.31
163	600	580	0	0	20	+	-	-	+	7.5	7.47
164	7,000	6,600	0	0	400	+	-	-	+	7.5	7.45
165	25,000	25,000	0	0	0	+	-	-	-	11.0	7.55
178	110	110	0	0	0	+	-	-	-	13.5	7.40

TABLE IX

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Non-Carious Individuals in Group B, i.e., Several Teeth Missing, but No Cavities Nor Fillings

Case No.	No. of Teeth Missing	Number of Bacteria Per CC. of Saliva (pH 5.0 Agar)					Growth in Tomato Broth (pH 5.0)				Diastatic Index mins.	pH
		Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Lacto-bacilli	Strep.	Staph.	Yeast		
12	4	45,000	35,000	0	0	10,000	-	±	-		21.5	7.52
41	14	550,000	480,000	70,000	0	0	+	+	-	-	7.5	7.00
62	3	3	2	0	0	1	-	+	-	-	24.5	7;40
63	4	2,400	2,100	300	0	0	+	-	-	-	7.5	7.56
65	4	56,000	51,000	0	0	5,000	+	-	-	+	31.0	7.51
66	8	0	0	0	0	0	-	-	-	-	20.0	7.15
67	3	226	17	0	0	209	+	+	-	-	14.0	7.30
71	5	753	29	724	0	0	+	-	-	+	11.5	6.90
73	7	400,000	330,000	70,000	0	0	+	-	-	-	7.5	7.65
79	2	29,000	29,000	0	0	0	+	-	-	-	4.5	
83	2	177,000	169,000	0	0	8,000	+	+	+	±	2.0	
86	6	100	100	0	0	0	+	-	-	-	2.5	
88	2	150	0	0	150	0	-	+	+	-	3.5	
93	7	28,000	7,000	0	20,000	1,000	+	+	+	-	6.0	
94	24	9,800	4,700	0	5,100	0	+	+	+	-	26.0	
95	4	5,000	2,700	2,300	0	0	+	+	-	-	5.0	
97	7	930	930	0	0	0	+	+	+	-	7.5	
104	2	3,000	2,600	400	0	0	+	-	-	-	8.5	
107	2	36,000	36,000	0	0	0	+	-	-	-	4.5	
111	6	28,600	18,600	10,000	0	0	+	+	-	-	3.5	
114	11	1,500	500	1,000	0	0	+	+	-	-	2.5	

TABLE IX (Continued)

Case No.	No. of Teeth Missing	Number of Bacteria Per CC. of Saliva (pH 5.0 Agar) Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Broth (pH 5.0) Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
115	11	7,000	6,200	800	0	0	+	+	-	-	2.5	
117	2	1,900	0	1,900	0	0	-	+	-	-	6.0	
120	3	50,000	40,000	10,000	0	0	+	+	-	-	3.0	
126	5	600	100	500	0	0	+	+	-	-	4.5	
128	6	1,100,000	1,000,000	100,000	0	0	+	+	-	-	4.0	
130	1	540	0	0	540	0	+	-	+	-	27.5	
136	1	6,000	4,000	2,000	0	0	+	+	-	-	2.5	
146	8	7,500	7,000	500	0	0	+	+	-	-	13.0	

TABLE X

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Non-Carious Individuals in Group C, i.e., No Open Cavities, but Some Teeth Filled

Case No.	No. of Teeth Filled	Number of Bacteria Per CC. of Saliva (pH 5.0 Agar)					Growth in Tomato Broth (pH 5.0)				Diastatic Index	pH
		Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Lacto-bacilli	Strep.	Staph.	Yeast	mins.	
1		2,000	1,800	200	0	0	+	-	-	-	6.5	7.10
5		27,000	17,000	10,000	0	0	+	+	-	-	4.5	7.03
7	4	17,000	6,000	500	500	10,000	+	+	-	+	8.5	7.79
8	4	1,000	1,000	0	0	0	+	-	-	-	9.5	7.62
9	8	2,000	2,000	0	0	0	+	-	-	-	7.0	7.90
11	3	27,000	22,000	5,000	0	0	+	+	-	-	13.5	7.74
13	3	17,300	14,000	0	0	3,300	+	-	+	+	11.0	6.76
23	6	1,500	1,500	0	0	0	+	-	-	-	8.5	7.00
31	3	7,400	5,400	2,000	0	0	+	+	-	-	7.5	6.85
33	2	115	95	20	0	0	+	-	-	-	9.5	7.15
51	2	73	0	4	0	69	+	+	-	+	6.5	7.95
52	4	74	74	0	0	0	+	-	-	+	6.5	8.00
56	1	315	0	25	0	290	-	+	-	+	14.0	7.50
57	6	50,000	50,000	0	0	0	+	-	-	-	12.5	7.25
60	3	262	204	0	0	58	+	-	-	+	5.0	7.45
68	2	120,000	120,000	0	0	0	+	-	-	-	2.5	7.35
72	3	68,100	57,000	11,000	0	100	+	+	-	-	4.5	7.30
76	4	170,000	170,000	0	0	0	+	-	-	-	9.5	7.00
77	11	200,000	175,000	25,000	0	0	+	+	-	-	12.0	7.40

TABLE XI

Results of Bacteriological Analyses, Diastatic Activity and pH Measurement of Saliva of Carious Individuals in Group D, i.e., One Cavity (First Degree Caries)

Case No.	Number of Bacteria Per CC. of Saliva (pH 5.0 Agar)					Growth in Tomato Broth (pH 5.0)				Diastatic Index mins.	pH
	Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Lacto-bacilli	Strep.	Staph.	Yeast		
44	150,000	79,000	71,000	0	0	+	+	-	-	4.0	7.45
49	1,080,000	1,080,000	0	0	0	+	-	-	-	5.0	7.00
61	60,000	52,000	6,500	0	1,500	+	+	-	+	5.0	7.48
78	133,000	133,000	0	0	0	+	-	-	-	9.5	
80	40,000	40,000	0	0	0	+	+	+	-	2.5	
84	137,000	137,000	0	0	0	+	-	-	-	3.5	
89	12,600	10,000	0	2,600	0	+	+	+	-	13.0	
92	11,500	11,200	0	300	0	+	+	+	-	4.5	
103	300,000	291,000	3,000	6,000	0	+	+	+	-	3.5	
106	41,000	24,000	17,000	0	0	+	+	+	-	5.5	
116	500,000	400,000	100,000	0	0	+	+	-	-	3.0	
119	1,000,000	965,000	0	0	35,000	+	-	-	+	3.0	
122	450	350	100	0	0	+	+	-	-	3.5	
123	1,500	1,500	0	0	0	+	-	-	-	3.0	
131	440,000	440,000	0	0	0	+	-	-	-	4.5	
132	23,800	17,200	6,600	0	0	+	+	-	-	3.5	
141	5,500	5,500	0	0	0	+	-	-	-	1.5	
144	41,500	4,500	27,800	8,000	1,200	+	+	+	+	3.5	
147	70	70	0	0	0	+	-	-	-	1.5	

TABLE XII

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Carious Individuals in Group E, i.e., Two Cavities (Second Degree Caries)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
30	135,000	119,000	16,000	0	0	+	+	-	-	5.0	7.10
48	2	1	0	0	1	-	-	-	-	8.0	7.30
87	4,500	600	1,400	2,500	0	+	+	+	-	2.5	
90	126,000	120,000	6,000	0	0	+	+	-	-	11.0	
108	1,100,000	800,000	300,000	0	0	+	+	+	-	2.5	
118	148,000	130,800	17,000	0	200	+	+	-	+	3.5	
121	58,000	52,000	6,000	0	0	+	+	-	-	4.5	
134	1,500	1,500	0	0	0	+	-	-	-	1.5	
143	42,000	42,000	0	0	0	+	-	-	-	3.5	
145	0	0	0	0	0	-	-	-	-	1.5	

TABLE XIII

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Carious Individuals in Group F, i.e., Three Cavities (Third Degree Caries)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
10	340,000	282,000	38,000	0	20,000					3.5	7.54
53	1,400,000	1,120,000	280,000	0	0	+	+	-	-	3.5	6.62
59	350,000	350,000	0	0	0	+	-	-	-	8.5	7.10
135	18,500	18,000	500	0	0	+	+	-	-	1.5	
137	42,000	42,000	0	0	0	+	-	-	-	4.0	

TABLE XIV

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Carious Individuals in Group G, i.e., Four to Ten Cavities (Fourth Degree Caries)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
2	239,000	205,000	30,000	0	4,000	+	+	-	+	4.0	7.02
14	382,000	290,000	10,000	0	82,000	+	+	-	-	1.5	7.42
18	955,000	785,000	115,000	0	55,000	+	+	-	-	4.5	7.08
21	56,000	43,000	3,000	10,000	0	+	+	-	-	6.0	7.88
24	16	16	0	0	0	-	-	-	-	4.5	7.00
26	426	44	0	20	362	-	-	-	+	7.5	7.20
27	42,000	28,000	14,000	0	0	+	+	-	-	5.0	7.35
28	118,000	105,000	13,000	0	0	+	+	-	-	3.0	6.92
34	262,000	202,000	60,000	0	0	+	+	-	-	2.0	7.00
35	2,000	2,000	0	0	0	+	-	-	-	2.5	7.40
40	46,000	45,000	1,000	0	0	+	+	-	-	2.5	7.62
43	22,700	10,000	12,700	0	0	+	+	-	-	3.5	6.98
45	19,400	14,900	2,000	0	2,500	+	+	-	-	5.0	7.70
47	63,000	42,000	20,000	1,000	1,000	+	+	-	+	5.0	6.55
50	20,500	14,300	6,200	0	0	+	+	-	-	5.0	6.91
55	25,200	25,200	0	0	0	+	-	-	-	3.5	7.47
64	16,000	15,000	1,000	0	0	+	+	-	-	4.5	7.86
70	73,000	69,000	0	0	4,000	+	-	-	-	4.5	6.85
74	96,020	96,000	0	0	20	+	-	-	-	7.0	7.10
98	228,000	208,000	20,000	0	0	+	+	+	-	5.0	

TABLE XIV (Continued)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
101	2,800	2,200	600	0	0	+	+	+	-	3.5	
105	48,000	48,000	0	0	0	+	+	-	-	3.0	
112	46,000	42,000	4,000	0	0	+	-	-	-	3.5	
113	41,000	41,000	0	0	0	+	-	-	-	2.0	
125	200,000	100,000	100,000	0	0	+	+	-	-	10.0	
127	370,000	370,000	0	0	0	+	-	-	-	2.5	
133	100,000	100,000	0	0	0	+	-	-	+	2.5	
142	2,500	2,500	0	0	0	+	-	-	-	2.0	

TABLE XV

Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Carious Individuals in Group H,i.e.,Ten or More Cavities (Rampant Caries)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
3	730,000	650,000	50,000	0	30,000	+	+	-	+	1.5	6.84
4	178,000	113,000	65,000	0	0					1.5	7.65
6	89,000	74,000	5,000	10,000	0	+	+	+	-	2.5	7.13
15	341,000	289,000	35,000	15,000	2,000	+	+	+	-	3.5	7.10
16	316,000	300,000	13,000	0	3,000	+	+	-	+	1.5	7.15
17	172,000	170,000	0	0	2,000	+	-	-	-	2.5	7.02
19	590,000	280,000	280,000	0	30,000	+	+	-	+	5.0	7.95
20	44,000	42,000	0	0	2,000	+	-	-	-	4.0	7.80
22	300,000	100,000	200,000	0	0	+	+	-	-	6.5	7.87
29	166	166	0	0	0	-	-	-	-	2.5	7.10
32	15,000	15,000	0	0	0	+	-	-	-	3.5	7.40
37	1,000,000	900,000	100,000	0	0	+	+	-	-	1.5	7.25
46	30,000	30,000	0	0	0	+	-	-	-	5.5	5,80
58	855	855	0	0	0	+	-	-	-	4.5	7.51
69	1,540,000	1,540,000	0	0	0	+	-	-	-	4.5	7.00
129	600,000	570,000	0	0	30,000	+	-	-	+	7.0	
166	140	10	130	0	0	+	+	-	-	4.0	7.42
167	192,000	192,000	0	0	0	+	-	-	-	3.0	7.55
168	400,000	172,000	195,000	30,000	3,000	+	+	+	+	3.5	7.37

TABLE XV (Continued)

Case No.	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lacto-bacilli	Strep.	Staph.	Yeast	Growth in Tomato Juice Broth: Lacto-bacilli	Strep.	Staph.	Yeast	Diastatic Index mins.	pH
169	136,500	136,000	0	0	500	+	-	-	+	6.0	7.11
170	229,000	136,000	93,000	0	0	+	+	-	-	5.0	7.17
171	0	0	0	0	0	-	-	-	-	1.5	7.41
172	340	340	0	0	0	+	-	-	-	2.5	7.23
173	8,100	8,100	0	0	0	+	-	-	-	3.0	7.46
174	932,000	810,000	70,000	40,000	12,000	+	+	+	+	1.5	6,97
175	4,000	4,000	0	0	0	+	-	-	-	5.5	7.62
176	97,000	75,000	20,000	0	2,000	+	+	-	+	3.0	7.27
177	270,000	270,000	0	0	0	+	-	-	-	5.5	7.42

TABLE XVI

Summary of Results of Bacteriological Analyses, Diastatic Activity and pH Measurements of Saliva of Non-Carious and Carious Individuals

(All figures represent average values of respective groups)

Group*	Number of Microorganisms Per CC. of Saliva Plated on Tomato Juice Agar: Total Count	Lactobacilli	Strep.	Staph.	Yeast	Diastatic Index**	pH
A	151,870	141,140	7,610	2,950	170	9.3	7.48
B	87,840	76,780	9,330	890	840	9.7	7.33
C	37,440	33,850	2,830	30	730	8.4	7.38
D	209,360	194,280	12,210	890	1,980	4.4	7;31
E	161,500	126,590	34,640	250	20	4.4	7.20
F	430,100	362,400	63,700	0	4,000	4.2	7.09
G	124,200	103,760	14,730	390	5,320	4.1	7.23
H	293,390	245,620	40,220	3,390	4,160	3.6	7.28

*, A - all teeth present; no cavities nor fillings
B - several teeth missing, but no cavities nor fillings
C - no open cavities, but some teeth filled
D - 1 open cavity (first degree caries)
E - 2 open cavities (second degree caries)
F - 3 open cavities (third degree caries)
G - 4 - 10 open cavities (fourth degree caries)
H - 10 or more open cavities (rampant caries)

** time and minutes for starch hydrolysis.

On the other hand, group G, composed of cases with fourth degree caries, had an average value of 7.23, while the individuals with rampant caries comprising group H had an average value of 7.28. Such differences in H-ion concentration of saliva of carious and non-carious individuals are apparently too small to be of any significance in dental caries.

Discussion

The importance of L. acidophilus in dental caries has been stressed by a number of workers (10), (18), (29), (30), (74). Bunting (10) stated that the most constant differential between carious and non-carious individuals was that of the relative numbers of L. acidophilus in the mouth. A 90% positive correlation was observed. Hadley (29) found that when L. acidophilus was present in mouths of non-carious individuals the number of organisms present was very small in comparison with that in carious individuals. In the present investigation, examinations of saliva of 76 non-carious and 90 carious individuals showed that oral lactobacilli appeared with almost the same frequency in both types of individuals. It will be observed from data presented in Tables VIII, IX, and X that a number of the non-carious individuals had lactobacilli counts as great, and in some cases greater than those of carious individuals (Tables XI to XV). It is noteworthy that of the 166 individuals examined the highest lactobacilli count was obtained from a

non-carious case (No. 150, Table VIII), in whom 2,370,000 organisms per cc. of saliva were found. The greatest number of lactobacilli shown by any of the carious individuals was 1,540,000 organisms per cc. of saliva (No. 69, Table XV). It will be further observed in Table XV that numbers 29, 58, 166 and 172 presented relatively low lactobacilli counts despite the fact that caries was rampant in these individuals. Number 171, also with rampant caries, was negative for lactobacilli in both acid-agar and acid-broth.

Enright, Friesell and Trescher (18) maintained that a lactobacillus was the only organism commonly found in food debris "that can tolerate and produce additional acid in an environment below pH 5.0". Hadley (29) reported tomato juice peptone agar of pH 5.0 to be specific for L. acidophilus, and that this medium inhibited growth of most oral streptococci. She maintained that when an occasional aciduric strain did appear differentiation from L. acidophilus could be made on the basis of colonial morphology, the streptococci being of pin-point size. Data presented in this study have failed to show that L. acidophilus is practically the only organism capable of growth at pH 5.0 in tomato agar. Although oral lactobacilli grew most consistently, streptococci, staphylococci and yeast appeared with considerable frequency. Most streptococci grew in pinpoint colonies, but there were a number of instances in which aciduric strains grew in larger colonies, approximating the size of the "small-colony" phase

of lactobacilli. The fact that Hadley used a streak method for plating saliva may have been responsible for her missing streptococci, because of possible overgrowth by other organisms or to misinterpretation of colony morphology. In the investigation reported here these discrepancies were removed by using the "pour-plate" method and the system of Gram-staining.

Earlier work on studies of the incidence of L. acidophilus in non-carious and carious individuals was carried out by qualitative methods only, in which the presence of the organism was determined by its growth in acid glucose-broth cultures. The fallacy in such a method for estimation of the number of microorganisms in a given sample can readily be seen when it is considered that a large number of bacteria are just as apt to result from a small inoculum as from a large one. When this method was used in conjunction with the quantitative method it did aid in correlation of results. The use of acid-broth did not prove as specific for lactobacilli as maintained by other investigators, and a close correlation was found to occur between the growth of various aciduric microorganisms in acid-broth and in acid-agar.

It is of interest to note that streptococci occurred with practically the same regularity in non-carious and carious individuals, 53% of non-carious cases and 52% of carious cases being positive for streptococci, respectively. A definite

role has been ascribed by certain workers (13), (42), (83), (84) to streptococci as etiological agents in caries. Kligler (55) reported a high incidence of streptococci in carious processes. Data presented here apparently do not indicate that streptococci are determining facotrs in dental caries.

Anderson and Rettger (1) observed a relatively high incidence of yeast in carious patients and were able to correlate the presence of yeast with progressive caries. The results in Table XVI show that in general the numbers of yeast for the various individuals studied were largest in groups F, G and H, that is, groups containing the greatest degree of caries. Fosdick, Hansen and Wessinger (24) found a symbiosis to exist between yeast and L. acidophilus, in which a greater acid production was effected with both organisms together than with either alone. In view of work by the above investigators (1), (24), it seems that if microorganisms are the cause of dental caries, more than one organism may be involved, either in the initiation of the lesion or in its progressive development.

Study of the acidogenic properties of various types of oral aciduric bacteria indicated that streptococci and staphylococci may produce as much acid as lactobacilli. Certain strains of oral streptococci were capable of even greater acid production than either lactobacilli or staphylococci. The lowest pH value obtained with glucose broth

cultures containing lactobacilli was 3.65, for cultures of staphylococci the lowest value was 3.78, while certain strains of streptococci were capable of reducing the pH to 3.40. The values for staphylococci and streptococci were much lower than those obtained by Tucker (82), but results with lactobacilli were in agreement with those reported both by Tucker, and Enright, Friesell and Trescher (18). Considering the acidogenic properties of these types of bacteria it would seem possible that any one may, of itself, be the cause of enamel decalcification. Rodriguez, cited by Enright, Friesell and Trescher (18), believed that "massiveness of oral invasion" was the determining factor in enamel decalcification, and that enamel was protected against slight lactobacilli invasions by a healthy oral fluid. This theory fails to explain why a number of individuals of group A (Table VIII) have never exhibited any caries although possessing relatively large numbers of lactobacilli in their mouths.

Proponents of the germ-theory of decay have apparently placed too much weight upon the significance of bacteria as determining factors in the etiology of caries. From the bacteriological data shown in Tables VIII to XVI it is possible that aciduric bacteria may be merely secondary in the cause of the disease.

Results obtained from studies on the ptyalin content of saliva seem to indicate that a close relationship exists between diastatic activity and the development of caries. The negative results obtained by Hubbell (48) undoubtedly may be explained by the fact that too small a number of cases were studied. From the results obtained it is believed that diastatic activity of saliva may serve as an index of caries susceptibility. The conclusion reached by certain investigators that oral lactobacilli bear a diagnostic relationship to caries is hardly justifiable on the basis of data presented in this paper. It is apparent that numbers of aciduric bacteria are not important in themselves. These organisms require simple sugars for acid production, and it is possible that in this respect diastatic activity may be an essential factor. By virtue of the action of ptyalin on complex carbohydrate, simpler sugars are formed and thus made available for acid fermentation by aciduric organisms that are almost invariably present regardless of the absence or presence of decay. This theory may aid in explaining why Jay (49) has been able to control lactobacilli counts in some cases by restricting sugar alone, while, with other individuals, both sugar and starch had to be restricted to control the growth of lactobacilli.

The data on H-ion concentration of saliva show that variations in pH were too small to be significant. Over-

lapping of pH values occurred in a number of cases irrespective of the degree of caries. These results are in accord with those of other workers (48), (59) (67).

VI

SUMMARY AND CONCLUSIONS

The effect on the oral flora of the local application of citrus fruit juices was studied and compared to that of several commercial dentifrices. Six different materials were included in the study: (1) water, (2) orange juice, (3) grapefruit juice, (4) a commercial tooth paste and mouth wash containing hexylresorcinol, (5) a commercial tooth paste and mouth wash containing sodium ricinoleate, and (6) a commercial liquid dentifrice containing sodium alkyl sulfate.

Two of the commercial dentifrices, the one containing sodium ricinoleate and the other sodium alkyl sulfate, proved the most effective in actual removal of microorganisms from the mouth. These two dentrifices were least effective in inhibiting bacterial growth during a two-hour interval after prophylaxis. Local application of citrus fruit juices was found to produce the greatest inhibitory action of all materials tested.

Eating grapefruit as a part of the daily diet produced a marked decrease in bacterial numbers in the oral flora. The average bacterial counts of the mouth were reduced 47% in the test group after the individuals comprising this group had received one-half of a grapefruit three times a day for one month. Monthly examination of saliva showed that a reduction was maintained as long as

individuals received grapefruit in addition to the daily diet.

Results of the examination of 76 non-carious and 90 carious individuals showed that oral lactobacilli appeared with almost the same frequency in both types. A relatively high incidence of streptococci was presented in mouths of both non-carious and carious individuals, and cultures were found to be as acidogenic as lactobacilli. Staphylococci were found in saliva of both groups of individuals, but appeared with less regularity than either lactobacilli or streptococci. Yeast occurred most frequently and in largest numbers in individuals with the greatest degree of caries.

If microorganisms play a role in dental caries aciduric streptococci should be considered as important in the disease as lactobacilli.

A close correlation was found between ptyalin content of saliva and the degree of caries. Results indicate that with increase in degree of caries there is a corresponding increase in diastatic activity. The average diastatic activity of individuals showing rampant caries was found to be 2 1/2 times as great as that of non-carious individuals.

No relationship was observed between H-ion concentration and the degree of caries-variations were too small to be of significance.

The difference between the diastatic activity of the saliva of non-carious and carious individuals was very striking, and it is suggested that diastatic activity may be an index for caries susceptibility.

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